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Antiarthritic Activity of *Achyranthes Aspera* on Formaldehyde - Induced Arthritis in Rats

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Abstract

AIM: To determine the ameliorative potential of aqueous extract of Achyranthesaspera(AEAA) against arthritis using swiss albino mice and Wistar rats, and its possible mechanism of action.

METHODS: Swiss albino mice (25-30 g) and Wistar rats (150-180 g) under standard controlled conditions (24 ± 2°C, 50-70 humidity and 12 h light/dark cycle). The groups were divided into 6 groups (n = 6/group) and assigned as control, negative control, standard and, formaldehyde supplemented with two different test dose groups of A. aspera for 4 weeks. Arthritis induced by subplantar administration of 0.1 ml formaldehyde (2% v/v) into the left hind paw in all groups except normal control. Arthritis was assessed using serum Hb, ESR, paw volume, joint diameter, radiological and histopathological investigation.

RESULTS: Oral administration of AEAA shown a significant ($p < 0.01$) dose-dependent protection against formaldehyde induced arthritis. At 21st day, A.asperashown an inhibition of paw volume in the different doses of 250 mg/kg and 500 mg/kg were found to be 30% and, 38.33% respectively. At 14th day the joint swelling was found to be 27.2% and 36.36 respectively. Diclofenac (10 mg/kg) had an effect of 36.61% inhibition of arthritis and joint swelling at 21st and 14th day.

CONCLUSION: Thus, the present study revealed that the aqueous extract of A. aspera offered significant protection against arthritis and joint inflammation.

Introduction

Rheumatoid arthritis (RA) is a chronic systemic inflammatory autoimmune disease [1], [2]. Although many medicines are prescribed for the treatment of RA, they are known to produce various side effects. So, there is still a need to seek therapeutic agents with lower side effects that can be used for long-term administration [3], [4]. *Achyranthesaspera* belongs to the family Amaranthaceae; it is an annual shrub found distributed throughout the tropical and subtropical regions. In the recent past, there has been a tremendous increase in the use of plant-based health products in developing as well as developed countries resulting in an exponential growth of herbal products globally. It is commonly found in India, Baluchistan, Sri Lanka, tropical Asia, Africa, Australia, and America [4], [5], [6]. Herbal drugs constitute a major part of all traditional systems of medicines [5]. The plant is a popular folk remedy in traditional system of medicine throughout the tropical Asian and African countries. The World Health Organization has attempted to identify all medicinal plants used globally and listed more than 20,000 species [6]. The plant is highly esteemed by traditional healers and used in treatment of asthma [5], [6], spermicidal activity [7], post-coital antifertility activity [8], anti-parasitic activity [9], hypoglycaemic activity [10], hepatoprotective activity

[11], anti-inflammatory [12], nephroprotective [13], anti-depressant [14], bronchoprotective [15], antiallergic [16]. Hence, the present study is made for the evaluation of aqueous extract of AA for anti-arthritic activity in rats fed with formaldehyde. In this study, we assessed the effects of *Achyranthesaspera* against rheumatoid arthritis.

Material and Methods

Collection and identification of the plant materials

The leaves of *Achyranthesaspera* collected from Tirumala Forest, Tirupathi, Chittoor District, Andhra Pradesh, India. Botanical identification was carried out at the Department of Botany, Sri Venkateswara University, Tirupathi. Where, voucher specimen No.SKCCP/2015/110a was deposited in the museum of the department of Pharmacognosy Sri Krishna Chaithanya College of pharmacy, Madanapalle, Andhra Pradesh, India

Drugs and Chemicals

Serum kits were obtained from Span Diagnostics Ltd. Surat, India. Diclofenac sodium was supplied from Ajanta Pharma Limited, Maharashtra, India. Formaldehyde, purchased from Sigma Aldrich Chemical, India. Normal saline purchased from bio-aids scientific, Bangalore, India. All other chemicals were of analytical grade procured from reputed Indian manufacturers.

Experimental animals

All the experiments were carried out using Swiss Albino mice (25-30 g) and Wister rats (150-180 g). The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed in a temperature of 24 ± 2°C and relative humidity of 30–70%. A 12 hrs day: 12 hrs night cycle was followed. All animals were allowed free access to water and fed. Ethical clearance was obtained from the Institutional Animal Ethical Committee (IAEC) of Sri Krishna Chaithanya College of Pharmacy, Madanapalle, Andhra Pradesh.

Preparation of the extracts

The collected leaves were dried completely and powder with a mechanical grinder. The powder was passed through sieve no. 60 to get uniform powdered. The 250 gm of dried powder of *Achyranthesaspera* leaves was defatted with Petroleum ether. The defatted powder material (marc) thus obtained was successively extracted with aqueous solvent by maceration. The Maceration process involves the separation of medicinally active portions of the crude drugs. The drug material is taken in a stoppered container and immersed in the bulk of the solvents in the ratio of 1: 2 (Drug & Solvent) and allowed to stand for 7 days in room temperature with frequent shaking of every 30 minutes up to 6 hours on each day. The solvent was separated by filtration and concentrated under reduced pressure. The resulting semisolid mass was vacuum dried, and the percentage yield was calculated [17].

Acute toxicity study

The acute toxicity study was carried out to extract of *Achyranthesaspera*as per OECD 423 Guidelines. Swiss albino mice with weight ranging (25-30 g) were taken for the experiment. The animals were made into a group of 3 each, dose of aqueous extract was given according to the bodyweight (mg/kg), starting dose of 5 mg/kg was given to the first group of animal, no death was occurring and higher doses were given to the next group of animals up to 5000 mg/kg. The animals were observed for a further 14 days for any signs for delayed toxicity [18].

The time at which signs of toxicity appear/disappear was observed systematically and recorded for each animal.

Formaldehyde induced arthritis

Group 1: Normal control (1% v/v tween 80, p.o. for 28 days).

Group 2: Negative Control (Formaldehyde 0.1 ml 2% v/v by Sub-plantar region).

Group 3: Formaldehyde 0.1 ml 2% v/v + Standard (Diclofenac 10 mg/kg p.o. for 28 days).

Group 4: Formaldehyde 0.1 ml 2% v/v + Low dose of AEAA (125 mg/kg p.o. for 28 days).

Group 5: Formaldehyde 0.1 ml 2% v/v + Medium dose of AEAA (250 mg/kg p.o. for 28 days).

Group 6: Formaldehyde 0.1 ml 2% v/v + High dose of AEAA (500 mg/kg p.o. for 28 days).

Animals were divided into six groups (n = 6). Group I received the vehicle (2 ml/kg, 1% v/v tween 80) and served as the normal control. Group II received formaldehyde, served as a negative control. Group III received the standard drug diclofenac (10 mg/kg body weight), groups IV, V and VI received AEAA in doses of (125, 250 and 500 mg/kg body weight), respectively. Thirty minutes after oral administration of vehicle/drugs, arthritis was induced by subplantaradministration of 0.1 ml formaldehyde (2% v/v) into the left hind paw of all the animals except normal control. This was designated as day 1.

Vehicle/drug treatment was continued for the duration of 28 more days. Formaldehyde (0.1 ml 2% v/v) was again injected into the same paw on the third day [19], [20].

Paw volume and paw thickness were measured at 0 days, 7th day, 14th day, 21st day and 28th day by using Plethysmometerand vernier calibre, respectively. The body weights of the animals were measured by digital balance to access the exact dose & course of the disease at the initial day after induction, 14^{th} day and the end of 28^{th} day.

% inhibition of paw oedema concerning untreated groups was calculated using the following formula:

$$i= [1-(\Delta V_{treated}/\Delta V_{untreated})]x\ 100$$

Where,

i= % inhibition of paw edema

$\Delta V_{treated}$= mean change in paw volume of treated rat

$\Delta V_{untreated}$= mean change in paw volume of untreated rat

Method for collection of a blood sample

On the 28^{th} day, the blood (2 ml) collected by retro-orbital cavity under the influence of ether anaesthesia. Some of the collected blood was used to perform Hematological studies to estimate Hb gm/dl, & ESR mm/hr. The remaining blood was used to centrifuge at 3000 rpm at room temperature, and collected serum was used to perform the RA factor.

Radiography

Radiographic evaluation was performed based on radiographs and coned down views of lower limbs. Radiographs were taken with GE 500 mA, 40 kvp and 4 MAS.

Histopathological analysis

A portion of the bones was immediately kept in 10% formalin to fix the tissue after isolation. The bones were washed in running tap water, decalcified by placing in formic acid and dehydrated in the descending grades of alcohol and finally cleared in xylene. The tissues were embedded in molten paraffin wax.

Hard paraffin wax was melted and poured into square-shaped blocks. The knee joints were then dropped into the liquid paraffin quickly and allowed to cool. The blocks were cut using microtome to get sections of thickness 10 µm. The section was dried completely before staining. Eosin an acidic stain and hematoxylin a basic stain was used for staining and observed under an electronic microscope for histopathological changes [21].

Statistical analysis

The statistical significance was assessed by using one-way analysis of variance (ANOVA) and followed by Dunnet`s comparison test. All data are presented as mean ± SEM, and $p < 0.01$ was considered significant.

Results

Preliminary phytochemical screening

The preliminary phytochemical analysis of aqueous extracts of *Achyranthesaspera* revealed the presence of Alkaloids, Carbohydrates, glycosides phenolic compound, flavonoids, tannin and proteins (Table 1).

Table 1. Preliminary phytochemical screening of aqueous extract of Achyranthesaspera

Sl. No	Constituents	Observation in AEAA
1.	Alkaloids	Positive
2.	Carbohydrates	Positive
3.	Glycosides	Positive
4.	Phenolic compounds	Positive
5.	Flavonoids	Positive
6.	Tannin and Proteins	Positive

Blood and serum analysis

As a result of inflammation induced by formaldehyde, the levels of Hb mg/dl & ESR mm/hr were increased in all arthritis rats as compared to negative control rats. After extract treatment, the levels of these haematological parameters were significant ($p < 0.01$) decreased in group V &VI rats as compared to negative control rats except Group IV revealed non-significant.

The dose of 500 mg/kg aqueous extract-treated group prevented haematological changes to a greater extent than the Diclofenac sodium (10 mg/kg). However, treated groups III, V & VI serum show RA factor negative results as compared to the negative control, which shows positive. The group IV shows the RA factor positive, the group V & VI proving its anti-arthritic efficacy.

Effect of AEAA on joint swelling (paw volume) in formaldehyde induced arthritis

An increase in paw volume was seen in all animals throughout the observation period. Maximum paw volume was observed on day 21, after which there was a gradual decrease except in the negative control and AEAA (125 mg/kg) treated groups, which showed an increase in paw volume from Day 1 to day 28.

Although drug-treated groups showed a decrease in joint swelling as compared to the negative

control, the difference was significant ($p < 0.01$) in Group III, V, VI on all observation day. AEAA at a dose of 125 mg/kg (Group IV) produced a nonsignificant reduction in paw volume on all observation day.

Radiological studies

The radiographic features of the rat joints in formaldehyde induced arthritic model, as shown in Figure 1. In formaldehyde induced arthritis rats (group II), soft tissue swelling along with a narrowing of the joint spaces were observed which implies the bony destruction in arthritic condition [22]. The standard drug Diclofenac sodium treated groups have prevented this bony destruction and also there is decreased swelling of the joint. The AEAA 250 & 500 mg/kg treatment for 28 days have shown significant prevention against bony destruction by showing less soft tissue swelling and narrowing of joint spaces when compared with the negative control.

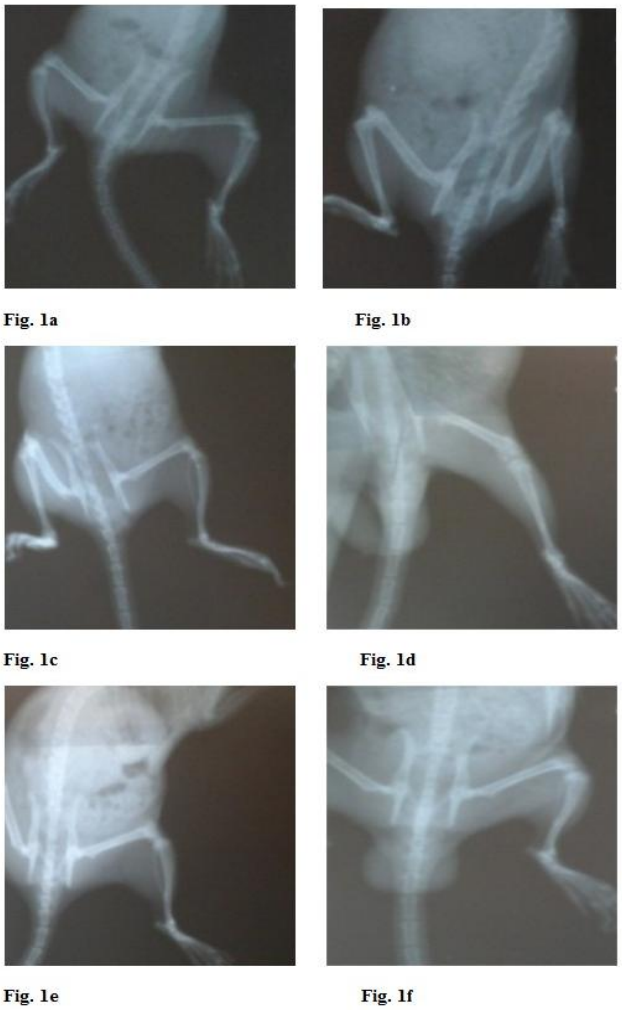

Figure 1: Radiography of the rat joints in formaldehyde induced arthritic model; A) Normal Control (DMSO 2 ml/kg); B) Negative control; C) Standard (Diclofenac 10 mg/kg); D) AEAA 125 mg/kg; E) AEAA 250 mg/kg; F) AEAA 500 mg/kg

Effect of AEAA on joint diameter in formaldehyde induced arthritis

Administration of formaldehyde 0.1 ml 2% v/v produced an increase in the joint diameter of all the animals, which was persistent throughout the observation period (Figure 2). Maximum joint swelling was observed on day 14, after which there was a gradual decrease except in the negative control and AEAA (125 mg/kg) treated groups, which showed an increase in joint diameter from Day 1 to day 28. At the highest dose (500 mg/kg), AEAA was more efficacious as equal to Diclofenac in reducing the joint swelling.

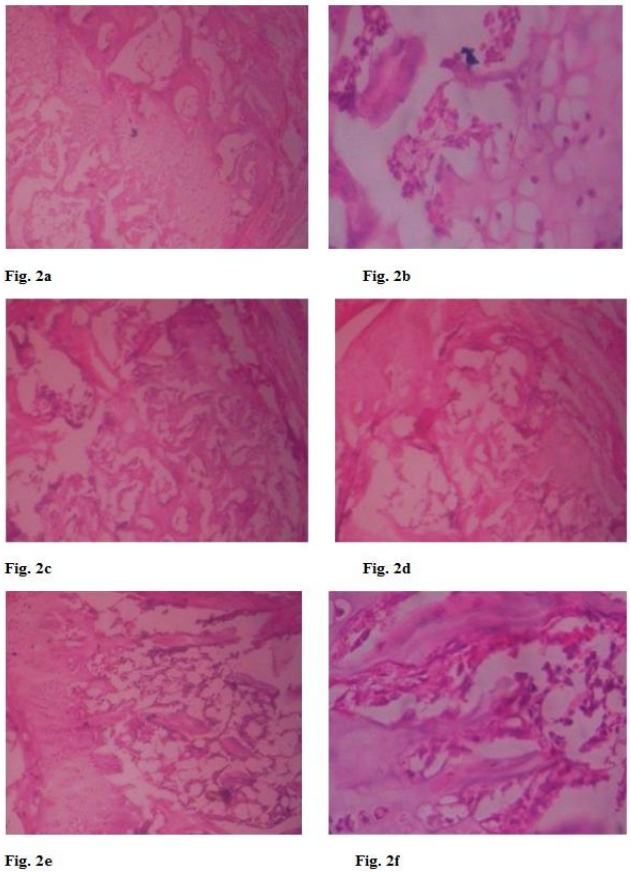

Figure 2: Histological examination of experimental rats; A) Normal Control (DMSO 2 ml/kg); B) Negative control; C) Standard (Diclofenac 10 mg/kg); D) AEAA 125 mg/kg; E) AEAA 250 mg/kg; F) AEAA 500 mg/kg

Histopathological studies

Histological studies of ankle joint reports confirm that there is severe bone erosion with the presence of neutrophil infiltration and pannus formation in the negative control group, as shown in Figure 2b. The treatment groups AEAA 250 mg/kg and 500 mg/kg revealed a reduction in pannus formation and bone resorption, joint inflammation with reduced neutrophil infiltration. Among the two-dose group, 500 mg/kg proved a dose-dependent action that is comparable to that of standard diclofenac group and 125 mg/kg slide shows a moderate level of cells and mild effect on inflammation.

Achyranthes aspera not only reduced the progression of inflammation and arthritis in experimental animals but also improved several symptoms associated with the disease. This study highlights the potential of *Achyranthes aspera* as a reliable treatment for arthritis substantiating the claims of the *plant drugs.*

Discussion

In our study, the AEAA exhibited significant anti-arthritic activity in a dose-dependent manner. In the present study, we showed that 250 & 500 mg/kg AEAA could significantly inhibit the progression of rheumatoid arthritis in treating animals. However, standard drug and aqueous extract significantly suppressed the swelling of the paws in the chronic phase, which may be due to the suppression of inflammatory mediator released due to induction of formaldehyde. Though the actual mechanism of suppressing inflammation is not known, it can be correlated with the presence of phenolic compound and flavonoids in suppressing the inflammation and antioxidant activity [23]. Numerous studies have suggested a role of oxidative stress in the pathogenesis of rheumatoid arthritis [24].

Table 2: Effect of aqueous extract of Achyranthesasperaon Hb, ESR & RA factor

Groups	Treatment & Dose	Hb mg/dl	ESR mm/hr	RA factor
1	Normal Control	16.8 + 0.35	11.9 + 0.54	Negative
2	Negative Control	9.58 + 1.04	23.25 + 1.49	Positive
3	Diclofenac (10 mg/kg)	14.56 + 0.81**	8.96 + .42**	Negative
4	AEAA 125 mg/kg	10.64 + 0.7^{ns}	24.32 + 0.31^{ns}	Positive
5	AEAA 250 mg/kg	12.86 + 0.42**	7.73 + .28**	Negative
6	AEAA 500 mg/kg	15.71 + 0.28**	10.44 + 0.57**	Negative

Therefore; It was assumed that the reported and well-established antioxidant properties of AEAA and its ability to block the COX2 pathway during the progression of inflammation justify the usage of the plant extract in the treatment of rheumatoid arthritis [25], [26].

Table 3: Effect of AEAA on joint swelling (Paw volume)

Groups	Treatment & Dose	Paw volume in ml					% inhibition of paw volume on 21st day
		1st day	7th day	14th day	21st day	28th day	
1	Normal Control	0.04 ± 0.00	0.04 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.04 ± 0.00	-
2	Negative Control	0.26 ± 0.01	0.46 ± 0.01	0.55 ± 0.02	0.6 ± 0.02	0.67 ± 0.01	0
3	Diclofenac (10 mg/kg)	0.20 ± .02**	0.31 ± 0.03**	0.34 ± 0.01**	0.38 ± 0.02**	0.33 ± 0.01**	36.6
4	AEAA 125 mg/kg	0.25 ± 0.02^{ns}	0.42 ± 0.02^{ns}	0.52 ± 0.02^{ns}	0.54 ± 0.02^{ns}	0.65 ± 0.01^{ns}	10
5	AEAA 250 mg/kg	0.21 ± 0.00**	0.37 ± 0.01**	0.38 ± 0.00**	0.42 ± 0.02**	0.38 ± 0.02**	30
6	AEAA 500 mg/kg	0.18 ± 0.00**	0.30 ± 0.02**	0.35 ± 0.01**	0.37 ± 0.01**	0.31 ± 0.02**	38.33

As shown in Table 2, assessment of the levels of Hb and ESR provides an excellent and simple tool to measure the anti-arthritic activity [27] of the target drug. The activities of these parameters were significantly decreased and increased in arthritic rats. These are good indicators, considered to be features of formaldehyde arthritis [28], [29]. There was a significant reduction in the paw volume and joint diameter in formaldehyde induced arthritic rats. The Shruti et al. study also revealed cardinal signs of the chronic inflammatory reactions like redness, swelling, arthralgia and immobility of affected joints were significantly less in the drug-treated animal than the negative control [30] (Table 3).

Radiographic changes in RA conditions are useful diagnostic measures which indicate the severity of the disease [31]. Soft tissue swelling is the earlier radiographic sign, whereas prominent radiographic changes like bony erosions and narrowing of joint spaces can be observed only in the development stages (final stages) of arthritis [31] (Table 4).

Table 4: Effect of AEAA on the joint diameter

Groups	Treatment & Dose	Joint diameter in mm					% inhibition of joint diameter 14th day
		1st day	7th day	14th day	21st day	28th day	
1	Normal Control	0.42 ± 0.02	0.42 ± 0.02	0.42 ± 0.02	0.42 ± 0.01	0.43 ± 0.01	-
2	Negative Control	0.55 ± 0.02	0.69 ± 0.01	0.77 ± 0.01	0.81 ± 0.02	0.89 ± 0.02	0
3	Diclofenac (10 mg/kg)	0.40 ± 0.03	0.42 ± 0.02	0.51 ± 0.03	0.47 ± 0.02	0.45 ± 0.02	36.61
4	AEAA 125 mg/kg	0.58 ± 0.02	0.66 ± 0.02	0.74 ± 0.01	0.78 ± 0.02	0.86 ± 0.01	3.89
5	AEAA 250 mg/kg	0.46 ± 0.01	0.48 ± 0.01	0.56 ± 0.01	0.55 ± 0.03	0.50 ± 0.03	27.2
6	AEAA 500 mg/kg	0.38 ± 0.01	0.42 ± 0.02	0.49 ± 0.02	0.45 ± 0.02	0.45 ± 0.02	36.36

Radiography result shows that the extract 500 mg/kg of *Achyranthesaspera* prevents joint destruction, swelling & narrowing of joint space. Haematological parameters RA factor results also showed a significant improvement of the arthritic condition. The pathogenesis or reasons for the development of arthritis following injection of formaldehyde are not fully understood. As shown in Figure 2, the treatment groups AEAA 250 mg/kg and 500 mg/kg showed a reduction in pannus formation and bone resorption, joint inflammation with reduced neutrophil infiltration. Formaldehyde induced arthritis is one of the most widely used models as it has been shown to share several clinical features with human arthritis [32]. Changes in haematological parameters were observed due to arthritic condition [33].

In conclusion, the present experimental findings of pharmacological, radiological, histological and hematological parameters observed from the current investigation, it is concluded that at the doses of 250mg/kg and 500 mg/kg AEAA possesses potentially useful anti-arthritic activity since it gives a positive result in controlling inflammation in formaldehyde induced arthritis model in rats. The high dose of AEAA reflected highly beneficial and treatment of inflammatory disorders.

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Basic Science

Loss Path Influence on the MRI Radio Frequency Pulse Sequence: A Theoretical Evidence

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Abstract

The RF pulse is initiated from either the loop or loopless MRI antenna. It has shown an increased advancement in recent times. Somehow, the concept has proven successful in the MR imaging procedure. Using the fundamental theories of the MRI concept, mathematical experimentation was carried out analytically to investigate the Loss Path Concept (LPC). The LPC was proposed to be one of the defects responsible for poor/blurred medical imaging of certain parts of the body. The LPC results obtain in this mathematical experimentation was found to be -56 dB and 6.7 dB. Theoretically, the LPC can be resolved mathematically by incorporating the molecular boundaries of the tissues. Practically, LPC can be resolved by introducing a detachable RF strip detector to synchronise-particles across different molecular boundaries and prevent patients from excess exposure to RF radiation.

Introduction

Safe medical imaging is gradually advancing beyond mere visual interpretations of the interior parts of the human body into an improved technologically initiated process. This process includes improving the mathematical codes of the imaging device [1]. Mathematical codes are basic mathematical principles or algorithm used to initiate, construct or improve devices. These codes are rooted in sound physics principles to describe the functionality of devices. The operational techniques of the MRI machine are traceable to salient mathematical codes, e.g. the Bloch NMR. The mathematical codes of the MRI had been queried or faulted severally [2], [3]. Solutions had been inferred to the ab-initio mathematical codes of the MRI machines via recent inclusion of polynomial function [4], [5] to resolve imaging issues. Therefore, mathematical codes can be used to probe into the complexity behind the functionality of the MRI machine. For example, aside from the known abnormalities in the MRI machine, i.e. signal-to-noise, excess heat on patients, increased radiation loss e.t.c., there are subtler issues about MRI that should be critically analysed. One of such salient challenge is the amount of signal attenuation recorded during radio propagation and reception. In this research, the signal attenuation anomaly is referred to as the loss path concept (LPC). LPC defines the performance (i.e. system frequency and link budget) of the radio propagation from the MRI antenna [6]. The MRI antenna transports energy into protons through resonance in the form of RF pulse. When the RF pulse is truncated, the excited protons return-back to its ab-initio state and emit RF signals. These signals are processed by the RF coil to generate tissue image on any output device, e.g. a computer. The image generation at the RF coil depends on the relaxation

time of hydrogen protons in different tissues (as shown in Figure 1).

Figure 1: MRI RF coils (adapted from http://198.173.87.9/mri/mri_litzcagepg.htm)

Different medical resonance imaging processes [7], [8], [9] depend on the functionality of the MR antenna and the RF coils. These devices help to improve the acquisition and reconstruction of signal attenuation (as shown in Figure 2, A and B). Therefore, an antenna of large-quality factor and gain is required to provide a highly uniform electromagnetic field for high-resolution imaging.

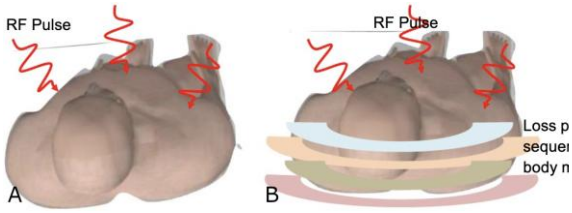

Figure 2: A) RF pulse from the MRI chamber; B) Loss Path in the RF Pulse Sequence

The constructive and destructive interference of electromagnetic fields generated by each part of the coil leads to low imaging [10]. This challenge has been reduced by introducing TEM resonators to control the distribution of the magnetic field [10]. It also assists meta/nano materials to focus on the RF magnetic field [11]. While solving the uniform distribution of the electromagnetic field, there exists the basic challenge of signal attenuation which leads to Loss Path Concept (LPC). LPC is as a result of continuous negligence of the Bio Savart law via the inadequate sustainability of the quality factor. Like 'shadow fading' in wireless capsule endoscopy [12], the Loss Path Concept (LPC) in the MRI is prominent when scanning deep tissues or cancerous growth of the human body. The RF attenuation when scanning deep tissues is a subtle concept. This concept was explained in subsequent sections of this paper. The relaxations of the excited protons of deep tissues are haphazardly different from general body imaging. This may be largely due to molecular interactions or boundaries of the deep tissues. Traditionally, the meta-material which act as the lens is used to focus on excess RF magnetic field to the patient to obtain clearer imaging. Hence, the generalisation of the MRI procedure for body tissues should include the safety of the patient using the MRI machine.

The operational tendency for MRI technicians to subject patients to excessive RF radiation may be hazardous in the long-run [32]. This may lead to the weakening of body tissues and ultimately lead to organ malfunction e.t.c. Though none of these symptoms has been reported clinically, however, the essence of this research is to seek proactive measure – using a mathematical approach to probe the MRI technique. The LPC concept has been applied in some medical technologies, such as medical implant communication [19] and wireless capsule endoscopy [22]. In this paper, we developed a Hamiltonian to incorporate the shortcoming of the Bloch NMR model. The effects of electromagnetic signatures from the MRI antenna on the radio frequency (RF) are expected to excite both the selection technique and conservation of excess loss of energy during signalling. This concept was considered in the calculation. Also, the equations for the LPC were derived, and the simulated LPC effect during specific RF selection was reported.

Material and Methods

The flow properties or fluid dynamics of the modified time-independent Bloch NMR flow equation under the influence of the RF magnetic field is given as [4], [5].

$$v^2 \frac{\partial^2 M_y}{\partial x^2} + v\left(\frac{1}{T_1} + \frac{1}{T_2}\right)\frac{\partial M_y}{\partial x} + \left(\frac{1}{T_1 T_2} + \gamma^2 {B_1}^2\right) M_y - \frac{\gamma B_1 M_o}{T_1} = 0 \quad (1)$$

v Is the flow velocity. γ Is the gyromagnetic ratio, M_y are the transverse magnetisation, M_z is the longitudinal magnetisation, M_o is the equilibrium magnetisation,x is the distance along the x-axis when time is not zero, B_1 external magnetic field, T_1 is the longitudinal and T_2 transverse relaxation times.

The RF magnetic field is derived from antenna coils whose frequency is consistently higher than an amateur radio. The electromagnetic field from the antenna coils is governed by the Maxwell equations written below.

$$\nabla . E = \frac{\rho}{\varepsilon_0} \quad (2)$$

$$\nabla \times E = -\frac{\partial B}{\partial t} \quad (3)$$

$$\nabla \times B = \mu_0 J + \mu_0 \varepsilon_0 \frac{\partial E}{\partial t} \quad (4)$$

$$\nabla . B = 0 \quad (5)$$

Equation (2) represents the Coulomb's law where E is the electric field, ρ is the charge density, ε_0 is the permittivity of free space. Permittivity describes the ability of materials to transmit an electric field. Equation (3) represents the Faraday's law where B is the magnetic induction. The negative sign can be justified using the Lenz law. Equation (4) is the Ampere's law where μ_0 is the permeability of free space. Permeability is the ability of a material to support the formation of a magnetic field within itself in response to an applied magnetic field. J is the current density. Equation (5) represents the Gauss's law. The phenomena of both fields are the idea behind this paper.

Enhanced Flow Parameters of the Bloch NMR Model

We propose a generic Hamiltonian to incorporate the Bloch NMR and Molecular boundary dynamics as shown below

$$H_T = H_{Bloch} + H_{Molecular} \quad (6)$$

H_{Bloch} is represented by the flow magnetisation equation derived by Awojoyogbe et al., [13]. The first step of this paper is to simplify the motion of the magnetisation vector in an externally polarised alternating magnetic field [26], [27], [28]. Secondly, the useful results are transferred to the rotating frame which mandates equation [1] not to be equal to zero. It is written as:

$$H_{Bloch} = v^2 \frac{\partial^2 M_y}{\partial x^2} + v\left(\frac{1}{T_1} + \frac{1}{T_2}\right)\frac{\partial M_y}{\partial x} + \left(\frac{1}{T_1 T_2} + \gamma^2 {B_1}^2\right) M_y - \frac{\gamma B_1 M_0}{T_1} \quad (7)$$

The molecular boundary dynamics can also be expressed as:

$$H_{Molecular} = \frac{\partial E}{\partial x_i}\frac{\partial E}{\partial x_j} \quad (8)$$

All parameters maintain its original interpretations, i.e. E is the energy absorbed in the tissue. Along with the i^{th} site, the elastic model for macromolecular interactions is predominant because of body fluid, therefore, $E = \sum_i^N V(x_i) + \sum_{i=2}^N \frac{1}{2} k(x_i - x_{i-1})^2$, Along with j^{th} the site, the energy E coincide with the energy levels worked out by Emetere [14] for NMR studies. $V(x_i)$ Is the potential across compartmental boundaries, x_i is the distance along in the i^{th} site in the x-axis, x_j is the distance along with the j^{th} site in the x-axis, N is the highest number of proton around scanned body mass, k is the elasticity of the blood vessel. The elasticity of the arteries or blood vessel determines the blood flow rate in the body [15]. If the arteries are narrow, less blood can flow. In clinical practice, this feature is described by elasticity index.

Therefore the energy absorbed in the tissue is important for analysing both the microscopic and macroscopic imaging processes. Hence, the absorbed energy was represented by the ground energy level worked out by Emetere [14]

$$E = 1 - m \,.\omega_1 M_y \mathrm{T}_1 \quad (9)$$

Here m is the magnetic moment. Assume $m \,.\omega_1 M_y \mathrm{T}_1 \gg 1$, i.e. when the external magnetic field is large; equation (8) can be further developed into equations (10 and 11).

$$\frac{\partial E}{\partial x_j} = \mu . \omega_1 \mathrm{T}_1 \frac{\partial M_y}{\partial x} \quad (10)$$

$$\frac{\partial E}{\partial x_i} = \sum_i^N \frac{\partial V(x_i)}{\partial x} + \sum_{i=2}^N \frac{1}{2} k(x_i - x_{i-1})^2 \quad (11)$$

This method is usually done in the matrix form, which is not the approach used in this section. The total Hamiltonian can be written as

$$H_T = v^2 \frac{\partial^2 M_y}{\partial x^2} + v\left(\frac{1}{T_1} + \frac{1}{T_2} + \mu . \omega_1 \mathrm{T}_1\right)\frac{\partial M_y}{\partial x} + \sum_i^N \frac{\partial V(x_i)}{\partial x} + \left(\frac{1}{T_1 T_2} + \gamma^2 {B_1}^2\right) M_y - \frac{\gamma B_1 M_0}{T_1} \quad (12)$$

We assume $\sum_{i=2}^N \frac{1}{2} k(x_i - x_{i-1})^2 = 0$ for other tissues of the body, i.e. non- blood vessels because of its negligible elasticity. Applying the Schrödinger, i.e. $H\psi = E\psi$, equation [12] transforms into

$$v^2 M_y \frac{\partial^2 \psi}{\partial x^2} + \left[v\left(\frac{1}{T_1} + \frac{1}{T_2} + \mu . \omega_1 \mathrm{T}_1\right) M_y + \sum_i^N V(x_i)\right]\frac{\partial \psi}{\partial x} + \left[\left(\frac{1}{T_1 T_2} + \gamma^2 {B_1}^2\right) M_y - E - \frac{\gamma B_1 M_0}{T_1}\right]\psi = 0 \quad (13)$$

ψ Have been calculated by Emetere [14], [25] in a generalised form as $\psi = Aexp(i\omega t)$. To analyse the time-independent domain, $t = \frac{xr}{\omega}$, where x is the circumference of the base sector of the coil, r is the radial component. Therefore,

$$\psi = Aexp(ixr) \quad (14)$$

Substituting equation (14) into equation (13) yields two sets of governing equations

$$v^2 r^2 M_y + \left(\frac{1}{T_1 T_2} + \gamma^2 {B_1}^2\right) M_y - E - \frac{\gamma B_1 M_0}{T_1} = 0 \quad (15a)$$

$$v\left(\frac{1}{T_1} + \frac{1}{T_2} + \mu . \omega_1 \mathrm{T}_1\right) M_y + \sum_i^N V(x_i) = 0$$

(15b)

The governing equations yield the following solutions

$$M_y = \frac{ET_1T_2 + \gamma B_1 M_o T_2}{v^2 r^2 T_1 T_2 + 1 + \gamma^2 {B_1}^2 T_1 T_2)} \quad (15c)$$

$$M_y = \frac{T_1 T_2 \sum_i^N V(x_i)}{(\tau + \mu . \omega_1 {T_1}^2 T_2) v} \quad (15d)$$

Where $\tau = T_1 + T_2$. If a low relaxation of the excited proton is considered, then $v^2 r^2 T_1 T_2 \ll 1$ and $ET_1T_2 \ll 1$. Equation (15c) becomes,

$$M_y = \frac{\gamma B_1 T_2 M_o}{1 + \gamma^2 {B_1}^2 T_1 T_2} \quad (15e)$$

Equation (15e) is the exact solution of the continuous wave nuclear magnetic resonance (CW NMR) and is expressed in the laboratory frame as

$$M_{xo} = \frac{-\sin(\omega t)\gamma B_1 T_2 M_o}{1 + \gamma^2 {B_1}^2 T_1 T_2} \quad (16a)$$

$$M_{yo} = \frac{\cos(\omega t)\gamma B_1 T_2 M_o}{1 + \gamma^2 {B_1}^2 T_1 T_2} \quad (16b)$$

This result had been reported by numerous researchers (16, 17), i.e. showing the validity of our approach. Also, if we consider a high relaxation of the excited proton, then $\mu . \omega_1 {T_1}^2 T_2 \gg \tau$, equation (15d) yields a new exact solution of the CW NMR i.e.

$$M_y = \frac{\sum_i^N V(x_i)}{\mu . \omega_1 T_1 v} \quad (17)$$

The exact solution of CW NMR in a laboratory frame can also be written as

$$M_{xo} = \frac{-\sin(\omega t) \sum_i^N V(x_i)}{\mu . \omega_1 T_1 v} \quad (18)$$

$$M_{yo} = \frac{\cos(\omega t) \sum_i^N V(x_i)}{\mu . \omega_1 T_1 v} \quad (19)$$

The processes highlighted in equations (16a and 16b) and equations (18 and 19) are driven by the concept discussed in the succeeding section.

Results

The time-independent Schrödinger equation was modelled to open up the proton's dynamics initiated by the MRI antenna. The time-independent Schrödinger equation is given as

$$i\hbar \frac{\partial}{\partial t}\psi - \frac{\hbar^2}{2m}\nabla\psi + V\psi = 0 \quad (20)$$

The Lagrangian density which shows the functionality between the transmitting and receiving antenna coil (equation [24]) is given as

$$\mathcal{L}_1 = \frac{1}{2}\left[\left|\frac{\partial \psi}{\partial t}\right|^2 - \frac{\hbar^2}{2m}|\nabla\psi|^2 - V|\psi|^2\right] \quad (21)$$

The minimum coupling rule to describe the interaction of ψ with the electromagnetic field was applied to get

$$\frac{\partial}{\partial t} \longmapsto \frac{\partial}{\partial t} + ieV, \qquad \nabla \longmapsto \nabla - ieA \quad \text{where}$$

$V = V(r, \theta) = V_o + E_o(\frac{a^2}{r} - r)$

Where V_o is a constant on the surface of the faraday loop of the MRI antenna, E_o is the field, r is the radius of the antenna, 'a' is the radius of the RF circular loop. Here, it is assumed that the shape of the antenna is loop-like.

Equation [25] transforms into

$$\mathcal{L}_1 = \frac{1}{2}\left[\left|\frac{\partial \psi}{\partial t} + ie\psi\phi\right|^2 - \frac{\hbar^2}{2m}|\nabla\psi - ieA\psi|^2 - V|\psi|^2\right] \quad (22)$$

The circular conductor is accounted for where $r = x$

$$\mathcal{L}_1 = \frac{1}{2}\left[\left|\frac{\partial \psi}{\partial t} + ie\psi V_o + iE_o e\psi\left(\frac{a^2}{x} - x\right)\cos\omega t\right|^2 - \frac{\hbar^2}{2m}|\nabla\psi - ieA\psi|^2 - V|\psi|^2\right] \quad (23)$$

Applying the solution of the standing wave $\psi(x,t) = e^{iS(x,t)}T(x,t)$ in equation [4]

Where E, B: $\mathbb{R}^3 X\ \mathbb{R} \to \mathbb{R}$, the lagrangian density takes the form

$$\mathcal{L}_1 = \frac{1}{2}\left\{E_{rt}^2 - |E_z|^2 - \left[\frac{\hbar^2}{2m}|B_r - eA|^2 + |B_z + V_o e|^2 - \left(\left|B_z - E_o e\left(\frac{a^2}{x} - x\right)\right|^2 - |B_z|\right) + 2E_o V_o e^2\right]E_r^2\right\} \quad (24)$$

Considering the Lagrangian density of the particle electromagnetic field E-H field of the circular MRI antenna-coil,

$$\mathcal{L}_o = \frac{1}{8\pi}(|E_1|^2 - |E_2|^2 - |H_1|^2 - |H_2|^2) \quad (25)$$

Where the values of electric and magnetic were adapted from Glenn (18) and restructured into the circular MRI loop antenna

$$E_1(a,z) = (\beta E_r(a,z)e_r + E_z(a,z)e_z)e^{-j\beta r}\sin\theta \quad (26)$$

$$E_2(a,z) = (\beta E_r(a,z)e_{r1} + E_z(a,z)e_{z1})e^{-j\beta r}\cos\theta \quad (27)$$

$$H_1(a,z) = (\beta B_r(a,z)f_r + B_z(a,z)f_z)e^{-j\beta r}\sin\theta \quad (28)$$

$$H_2(a,z) = (\beta B_r(a,z)f_{r1} + B_z(a,z)f_{z1})e^{-j\beta r}\cos\theta \quad (29)$$

Where $e_r = e_{r1} = \frac{\xi m}{4\pi r}$ and $e_z = e_{z1} = \frac{\xi mj}{4\pi z^2}$; $f_r = f_{r1} = \frac{\mu_o mj}{4\pi r^2}$ and $f_z = f_{z1} = \frac{\mu_o m}{4\pi z^3}$

Beyond the mere introduction of boundary conditions to expatiate on the dynamics of equations [26-29], its 'real-time' applications include reducing computational challenges (when writing the mathematical codes (1)) and micro-analysis of the selection technique.

The boundary conditions for equation [26] are

$$\begin{cases} E_1(a,0) = E_\alpha(z) \\ E_1(\infty,z) = 0 \\ E_1(a,x) = E_\alpha(z).\alpha \\ E_1(a,\infty) = 0 \end{cases} \quad (30)$$

The boundary conditions for equation [27] are

$$\begin{cases} E_2(a,0) = E_\gamma(z) \\ E_2(\infty,z) = 0 \\ E_2(a,x) = E_\gamma(z).\gamma \\ E_2(a,\infty) = 0 \end{cases} \quad (31)$$

The boundary conditions for equation [28] are

$$\begin{cases} B_1(a,0) = B_\vartheta(z) \\ B_1(\infty,z) = 0 \\ B_1(a,x) = B_\vartheta(z).\vartheta \\ B_1(a,\infty) = 0 \end{cases} \quad (32)$$

The boundary conditions for equation [29] are

$$\begin{cases} B_2(a,0) = B_\sigma(z) \\ B_2(\infty,z) = 0 \\ B_2(a,x) = B_\sigma(z).\sigma \\ B_2(a,\infty) = 0 \end{cases} \quad (33)$$

where α and γ are the attenuation factors of the electrical fields; σ and ϑ are the attenuation factors of the magnetic fields; $B_\vartheta(z)$ and $B_\sigma(z)$ are the magnetic fields at the boundary of the MRI antenna; $E_\gamma(z)$ and $E_\alpha(z)$ are the electric fields at the boundary of the MRI antenna; x is the length of MRI antenna; β is the frequency of excited power; j is the radio frequency current; r represents the radius or horizontal component of the antenna; z represents the vertical component of the antenna; m represents the number of the protons; ξ represents the electrical permeability; μ_o represents the magnetic permeability; e_r is the spin factor which determines the protons spin along the horizontal component of the MRI; e_z is the spin factor which determines the protons spin along the vertical component of the MRI transmitting antenna; e_{r1} is the spin factor which determines the protons spin along the horizontal component within the electric field of the MRI receiving antenna; e_{z1} is the spin factor which determines the protons spin along the vertical component within the electric field of the MRI transmitting antenna; f_r is the spin factor which determines the protons spin along the horizontal component within the magnetic field of the MRI receiving antenna; f_{r1} is the spin factor which determines the protons spin along the horizontal component within the magnetic field of the MRI transmitting antenna; f_z is the spin factor which determines the protons spin along the vertical component within the magnetic field of the MRI receiving antenna; f_{z1} is the spin factor which determines the protons spin along the vertical component within the magnetic field of the MRI transmitting antenna.

Therefore, the total action of lagrangian density is given by

$$D = \iint \mathcal{L}_1 + \mathcal{L}_o \quad (34)$$

Then the Euler-Lagrange equation associated to the function $S = S(E_r, E_z, \mathrm{B_r}, B_z, r, \theta, z)$ gives rise to the following systems of equation

$$E_{rt} + \left[\frac{\hbar^2}{2m}|\mathrm{B_r} - eA|^2 + |B_z + V_o e|^2 - \left(\left|B_z - E_o e\left(\frac{a^2}{r} - r\right)\right|^2 - |B_z|\right) + 2E_o V_o e^2 + \beta e_r\right] E_r = \beta E_r e_r e^{-j\beta r}(sin\theta + cos\theta) \quad (35)$$

$$\frac{\partial}{\partial t}\left[(B_z + V_o e){E_r}^2\right] - \frac{\partial}{\partial t}\left[\left(B_z + E_o e\left(\frac{a^2}{x} - x\right)\right){E_r}^2\right] - \frac{1}{2}\frac{\partial B_z}{\partial t} = 0 \quad (36)$$

$$\frac{\hbar^2}{2m}{E_r}^2\frac{\partial}{\partial t}(\mathrm{B_r} - eA) = \beta B_r f_r e^{-j\beta r}(sin\theta + cos\theta) \quad (37)$$

$$\frac{\partial}{\partial t}E_z = \frac{\partial}{\partial t}E_z e_z e^{-j\beta r}(sin\theta + cos\theta) \quad (38)$$

$$2\left|B_z - E_o e\left(\frac{a^2}{r} - r\right)\right| E_r E_o e\left(\frac{a^2}{r^2} - 1\right) = \frac{j\beta}{8\pi}\left[\frac{E_r e_r}{r}(sin\theta + cos\theta) + \frac{2B_r f_r}{r}(sin\theta + cos\theta)\right]\beta e^{-j\beta r} \quad (39)$$

$$\frac{1}{8\pi}[\beta E_r(a,z)e_r + \beta B_r(a,z)f_r + E_z(a,z)e_z + B_z(a,z)f_z][cos\theta - sin\theta] = 0 \quad (40)$$

$$\frac{1}{8\pi}[-\frac{2}{z}e^{-j\beta r}sin\theta(B_z(a,z)f_z + E_z(a,z)e_z) - \frac{2}{z}e^{-j\beta r}cos\theta(B_z(a,z)f_z + E_z(a,z)e_z)] = 0 \quad (41)$$

Discussion

RF pulses are required to attain transverse magnetisation (equation 16b & 19). This can be initiated via the electromagnetic signatures expressed in equation [37]. We can easily obtain any solution of

the transverse magnetisation (when $\theta = \omega t; B_r = M_{yo}$) via this method

$$\omega\left(-\frac{\hbar^2}{2m\beta f_r}{E_r}^2\right)e^{j\beta r} = cos\omega t + cos^2\omega t \quad (42)$$

Equation [42] is related to the loss path equation stated by Basar et al., (19) where $j\beta r$ is the loss path, $cos\theta + cos^2\theta$ is the cumulative spin precession angle, $\frac{\hbar^2}{2m\beta f_r}{E_r}^2 = K$ is the RF pulse sequence, ω is the frequency. Taking the assumption that LPC was stated in equation (42) $\frac{j\beta r}{20}$, then the distance between the transmitting and receiving points are determined, as shown in Figure 7. Hence, the transformed equation (42) can be written as

$$LPC = 20\log_{\mathrm{K}}\left(-\frac{cos\omega t + cos^2\omega t}{\omega}\right) \quad (43)$$

The cumulative spin precession angle can be determined from the combination of deviated spins along with the same phase (as shown in Figure 3 below). A typical effect of the cumulative spin precession angle of individual protons in the human body Figure 3.

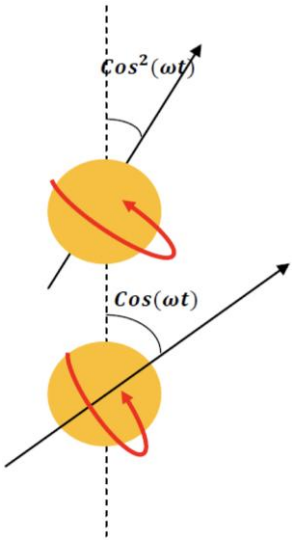

Figure 3: Physical model of cumulative spin precession angle

The MRI machine transmits a different pulse sequence (Figure 2B) when scanning a specific body mass. The pulse sequence through the free space and its losses are dependent on the frequency that was derived in equation [42]. The loss path has the same properties as the RF pulse sequence (i.e. frequency of exciting power) and differs from the usual loss path property that describes the transmitted power [19]. The linear dependence of the RF pulse, loss path and cumulative spin precession was investigated (Figure 4).

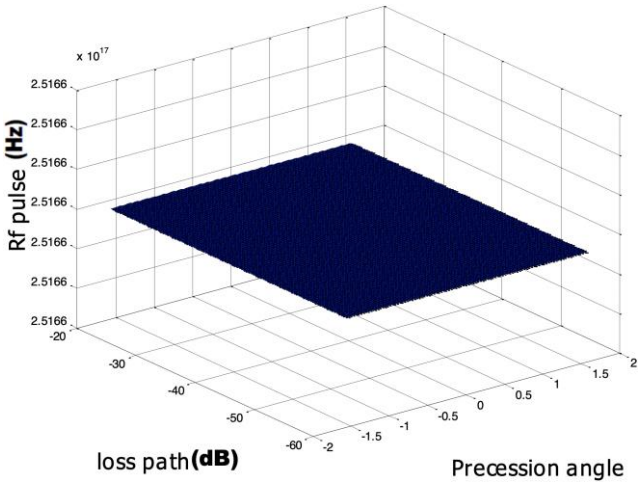

Figure 4: linearly dependence of the RF pulse, loss path and cumulative spin precession. A decrease in frequency translates in an increase in the RF pulse

The basis of linearity was initiated by the sudden decrease in the frequency of protons. These protons in the tissues increase the tendency of RF pulse to create imaging impact and vice-versa. The normal distribution of the RF pulse over tissues is represented in Figure 5.

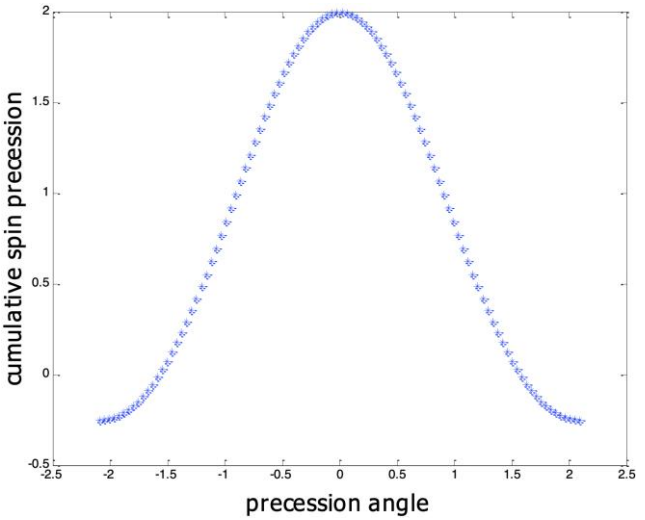

Figure 5: Distribution of RF for deep tissue analysis

Experimentally, the distribution of the RF pulse is not a smooth curve [20], [21]. One of the reasons attributed to this occurrence is the electromagnetic signatures of the MRI antenna. Recall the importance of the electromagnetic signatures had been mathematically illustrated in section three. The electromagnetic signatures are the specific combination of emitted, reflected or absorbed electromagnetic radiation (EM) at varying wavelengths and directions. This concept defines a unique set of combined frequencies, as seen in the loss path. Equation [42] is a typical electromagnetic signature of the MRI antenna. In a narrow view, i.e. at the low quantity of electromagnetic radiation, the system is

excited by differential amount of electrical energy. In a practical sense, the electromagnetic radiation in the MRI is moderate. The description of moderate electromagnetic radiation may be relative, i.e. depending on the operational specifics of the MRI machine. Practically, the energy in MRI operations is controlled by the integration of the function of equation [42] to account for the many-body effects shown in Figure 4 and 6 below. Since a safe MRI operation was assumed, the extremes of the equation [42] were not explained in this paper.

Practically, the determination of the RF value for deep tissue analysis is equal to the shadow fading for deep tissue implant [22] as shown in Figure (5). The effects of the RF pulse attenuation at varying frequencies were investigated (Figure 6A, B, C, and D). The RF pulse attenuation decreases at higher frequencies. At higher loss path, the RF pulse decreases drastically. The LPC for this mathematical experimentation of the MR process was found to be -56 dB and -20 dB. Further calculation of the LPC value using equation (43) could be extended to 6.7 dB.

It is easy to infer from Figure (6 A, B, C, and D), that the electromagnetic signature creates a non-uniform impact on the protons of the body. This phenomenon results in a process called quasi-resonance. In the quasi resonance state, all the protons do not come under the same influence of the radio frequency. This is partly because of the LPC. For easy identification, such protons are referred to as 'vagabond' protons. They reside mainly at the molecular boundaries and partially absorb the radio frequencies that in turn obstruct the transverse magnetisation signals. Hence, it was proposed that LPC could be initiated by the potential between molecular boundaries.

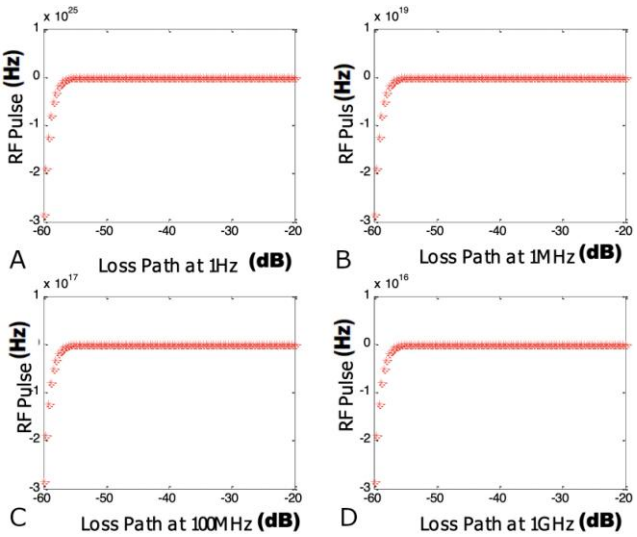

Figure 6: RF pulse attenuation at varying frequencies

The non-inclusion of the potential across molecular boundaries in the ab-initio Bloch NMR equations (23-29) conceals the error due to the existence of the 'vagabond' proton that resides in some tissues or tumours (Figure 7). The excess ejection of heat (due to the 'vagabond' protons at the molecular boundaries) is one of the challenges in MRI.

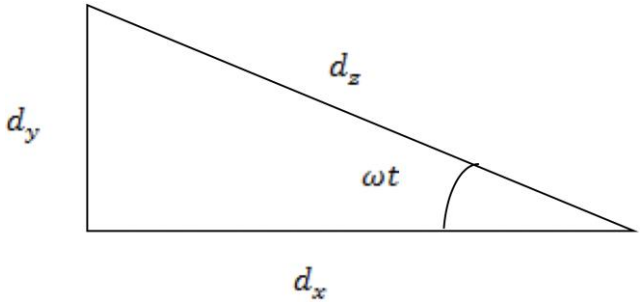

Figure 7: Angular resolution of the distance between the transmitting and receiving points

The 'vagabond' protons trap excesses energy as shown in equation [42]. Since the principle of conservation of energy must be obeyed, the 'vagabond' proton ejects the trapped energy in the form of heat. Some scientists and engineers had suggested that the challenges of MRI signal attenuation could be remedied by introducing the loopless MRI antenna (30) and MRI strip detectors [31], [32]. This idea is very effective to improve the signal-to-noise ratio (SNR) and field-of-view (FOV).

The LPC exists in the MR process. This can be seen in the reduction in RF pulse at higher frequencies. The LPC for MR processes was found to be -56 dB and 6.7 dB. The existence of the LPC led to another concept like the existence of the 'vagabond' proton. It was reported that the 'vagabond' proton supports poor imaging from the MR process. The LPC exposes the danger of improper design of the RF sequence. This fundamental error exposes the patient to a higher frequency that may be abnormal for certain regions of the body. Theoretically, incorporating the molecular boundaries potentials in the ab-initio MRI source code can solve the LPC. Practically, LPC can be resolved by introducing a detachable device like RF strip detector that would synchronise particles across different molecular boundaries and prevent patients from excessive exposure to high RF frequencies.

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Basic Science

Royal Jelly (Bee Product) Decreases Inflammatory Response in Wistar Rats Induced with Ultraviolet Radiation

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Keywords: Royal jelly; Ultraviolet radiation; Nrf 2; NF-κB; TNF alpha

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Abstract

BACKGROUND: Ultraviolet (UV) radiation damages human skin by triggering various types of cellular damage, several main factors involved are nuclear-related factor 2 (Nrf2), nuclear factor kappa-light-chain-enhancer of activated B cells (nF-kB) and pro-inflammatory cytokine, TNF alpha. Royal jelly (RJ) possesses the effect of protecting DNA and tissue against oxidative damage.

AIM: This study aimed to assess the efficacy of RJ as a protector of ultraviolet radiation, by assessing endogenous anti-oxidant expression (Nrf2), transcription factors (Nf-kB) and proinflammatory cytokines (TNF alpha).

METHODS: This study was an experimental study with post-test control group design. Thirty Wistar rats were induced by exposing 40 Watt UV-B lamps for 2 hours/day in 14 days. The rats were grouped into groups with RJ cream application with doses of 2.5%, 5%, and 10%, negative control with vaseline, and normal control. Examination of Nrf2 and NF-kB levels was carried out by ELISA. Quantitative analysis to obtain the percentage of TNF alpha expression on the tissue was entered into the ImageJ® program. Bivariate analysis was carried out by the T-test.

RESULTS: Nrf2 levels elevated following the increase of RJ dose, with the highest level was at RJ 10%. Nf-kB levels decreased following the increase of RJ dose, with the lowest level was at RJ 10%. TNF alpha expression was reduced in groups of RJ in various doses. Increased dose resulted in a more diminished level of TNF alpha.

CONCLUSION: Royal jelly cream application protected the skin from UV radiation by increasing cellular antioxidants and suppressing inflammatory cascade.

Introduction

Ultraviolet radiation (UVR) damages human skin by triggering various types of cellular damage, especially DNA damage and oxidative damage. This condition will increase the risk of skin cancer including skin melanoma [1], [2]. UVB radiation can cause loss of cellular integrity, direct damage to DNA and trigger various cellular responses including apoptosis [3] and inflammation [4] in skin cells including melanocytes (MC). However, the biological and physiological responses of normal MC to UVR are complex and are governed by various factors secreted by their neighbouring cells including keratinocytes (KC) for maintenance of MC homeostasis [5], [6], [7]. The micro-environmental conditions created by KC play a role in regulating MC responses including UVR-induced apoptosis and cell damage through paracrine factor secretions such as endothelin-1 peptides (ET-1), hypophysis and pituitary such as proopiomelanocortin (POMC), adrenocorticotropic hormones, β-endorphin and αmelanocyte-stimulating hormone (α-MSH) or corticotropin-releasing hormone (CRH) [8], [9], [10], [11], [12]. α-MSH has been recognized as an important paracrine factor that plays a protective role against UVB-induced radiation and DNA damage in MC humans. It also shows that the cytoprotective effect of α-MSH on UVR-mediated skin photodamage is associated with their ability to

suppress apoptosis, oxidative stress and inflammatory responses [13]. However, the mechanism involved in regulating the paracrine effect of KC affecting MC activity has not been investigated. Nuclear-related factor 2 (Nrf2) is the main transcription factor that regulates some phase II detoxification and antioxidant genes involved in cellular defence against oxidative stress. Nrf2 is believed to play a regulatory role in UVR-mediated oxidative stress associated with disorders in the physiology of skin cells including MC [14], [15]. Also, Nrf2 is involved in the regulation of paracrine factors such as epidermal growth factor family epigene in KC, which causes enlargement of the sebaceous gland in rats [16]. Modulation of Nrf2 can affect the function of KC associated with UVR response. Also, UVB irradiation can mediate apoptosis through oxidative stress activation from upstream mitogen-activated protein kinase (Mitogen-activated protein kinases (MAPKs), extracellular signal-regulated kinases (ERK), c-Jun N-terminal kinases (JNK), and p38) at MC and KC. This activates nuclear factor kappa-light-chain-enhancer of activated B cells (nF-kB) and activates pro-inflammatory cytokines such as: TNF alpha [17], [18].

Royal jelly (RJ) is a product of the cephalic gland secretion of worker bees and serves as the most important part of the honeybee larva's diet, playing a major role in caste differentiation [19]. For the first 2-3 days, RJ is the only food given to all young larvae in their ripening process, while for the queen, it is a special food for the entire period of her life. This is the reason the queen of bees lives longer than other bees. RJ, one of the most effective and beneficial drugs for humans, is widely used both in traditional medicine and modern medicine and it is a controversial food supplement. RJ has the effect of protecting DNA and tissue against oxidative damage [20], [21], [22], [23], [24].

This study was the first study to assess the efficacy of royal jelly as a protector of ultraviolet radiation, by assessing endogenous anti-oxidant expression (Nrf2), transcription factors (Nf-kB) and proinflammatory cytokines (TNF alpha) in the Wistar white Ratskin tissue.

Material and Methods

Subjects

This study was an experimental study with post-test control group design. Thirty Wistar rats, aged 20 weeks, were used in this study. White Wistar rats were obtained from the Eureka Research Laboratory in Palembang. This study had received ethical approval from the Research Ethics Committee, Faculty of Medicine, Universitas Sriwijaya (No. 198/kptfkunsrirsmh/2018). White rats were maintained in a room with a temperature between 20-24°C, and a dark-light cycle for 12 hours.

UV Induction and Royal Jelly Treatment

Before induction and treatment, rats were acclimatised for seven days. UV radiation induction was carried out by exposing 40 Watt UV-B lamp for 2 hours/day; the exposure was carried out for 14 days. The rats were grouped into 5 groups: (I) 10% RJ Group: five white rats were induced with UV-B and 10% royal jelly cream was applied for 14 days. (II) 5% RJ Group: five white rats were induced with UV-B and 5% royal jelly cream was applied for 14 days. (III) RJ Group 2.5%: five white rats were induced with UV-B and were applied with 2.5% royal jelly cream for 14 days. (IV) Negative control: five white rats were induced with UV-B and vaseline cream was applied for 14 days. (V) was control: five white rats were not induced with UV-B and no cream application.

ELISA of Nrf2 and NF-kB

Examination of Nrf2 and NF-kB levels was carried out from serum samples from Wistar rats obtained from the orbital vein as much as 1 mL. Then, the serum was centrifuged at 5000 rpm for 10 minutes. The supernatant was inserted into the tube and stored at -20°C. As much as 10 µL of supernatant from each sample was put into microplate well, then incubated and continued with the addition of HRP-conjugate, Chromogen A and B and Stop Solution. The optical density value was read with a microplate reader at 450nm wavelength. The ELISA was carried out according to the manufacturer's manual (Cloud-Clone Corp®, Texas, USA).

Examination of TNF Alpha Expression

Samples of skin tissue from each experimental subject were evaporated and put into a 10% NBF solution (Leica Biosystems, Wetzlar, Germany), followed by paraffin blocks and cutting samples with a thickness of 4 um. The sample was placed on a glass object and the dehydration process was carried out by entering the sample into multilevel alcohol starting from alcohol 96%, 80%, 70%, Xylane I, II and III. Furthermore, antigen retrieval was carried out using HIER (Heat Induced Epitop Retrieval) technique. Followed by administration of anti-TNF Alpha (Cloud-Clone Corp®, Texas, USA) antibodies (1: 700) in each sample. Then proceeded with the administration of biotynilated link antibodies and streptavidine peroxidase. Then followed by DAB chromogen and counterstain with hematoxylin (Cloud-Clone Corp®, Texas, USA). Furthermore, dehydration is carried out by adding samples to alcohol starting from alcohol 70%, 80%, 96%, and Xylan I, II, III (Sigma-Aldrich®, St. Louis, Missouri, USA). Then each sample was observed under a microscope with

400 times magnification. Photographs from each subsequent sample were entered into the ImageJ® program to be carried out on quantitative analysis to obtain the percentage of TNF alpha expression on the tissue.

Statistical Analysis

Statistical analysis was performed with SPSS 24.0 (SPSS Inc., Chicago, Illinois, USA). Data were presented with mean ± SD. Bivariate analysis was carried out by the T-test between groups. P-value was considered significant at < 0.05.

Results

As exhibited in Table 1, RJ groups with various doses showed multiplied the higher level of Nrf2 compared to the negative control. Nrf2 levels elevated following the increase of RJ dose, with the highest level was at RJ 10%. Nrf2 level of RJ 10% was almost 10-fold higher compared to negative control and almost 2-fold higher compared to normal control. Nrf2 level differences in all groups were statistically significant.

Table 1: Level of Nrf2 in Skin Tissue

Group	Nrf2 Level (pg/mL)	p-Value
RJ 10%	294.18 ± 16.21	0.021*. 0.027#
RJ 5%	184.83 ± 11.55	0.001*. 0.043#
RJ 2.5%	99.11 ± 7.17	0.001*. 0.001#
Negative control	31.23 ± 1.45	0.001#
Normal	156.23 ± 10.23	0.001*

*Independent T test VS negative control; #Independent T test VS normal control.

As shown in Table 2, RJ groups with various doses showed a lower level of Nf-kB compared to the negative control. Nf-kB levels decreased following the increase of RJ dose, with the lowest level was at RJ 10%. Nf-kB level of RJ 10% was almost similar to normal control. Nf-kB level differences in all groups were statistically significant.

Table 2: Level of Nf-kB in Skin Tissue

Group	Nf-kB Level (ng/mL)	p-Value
RJ 10%	2.98 ± 0.11	0.001*. 0.011#
RJ 5%	10.83 ± 1.02	0.001*. 0.008#
RJ 2.5%	21.11 ± 7.17	0.001*. 0.001#
Negative control	26.23 ± 1.98	0.001#
Normal	2.73 ± 0.12	0.001*

*Independent T test VS negative control; #Independent T test VS normal control.

As exhibited in Figure 1, TNF alpha expression increased about 30 times in UVB-induced rats (negative control) compared to the normal group that was not UVB-induced. TNF alpha expression was reduced in groups treated with RJ in various doses. Increased dose resulted in a more diminished level of TNF alpha.

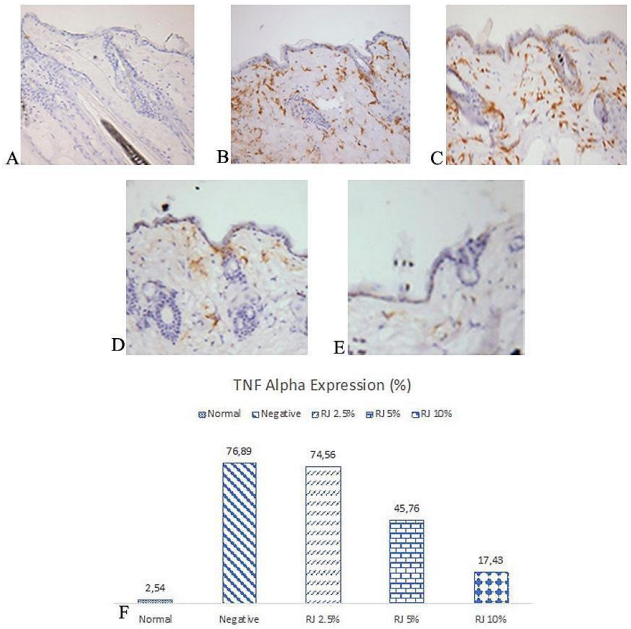

Figure 1: Expression of TNF Alpha in Skin Tissue (400x). A. Normal; B. Negative control; C. RJ 2.5%; D. RJ 5%; E. RJ 10%; F. TNF alpha expression (%)

Discussion

Ultraviolet is an electromagnetic wave with various roles in human life. Ultraviolet radiation of UV-B type causes inflammatory activation, in the form of TNF alpha expression, which increased about 30 times in UVB-induced White rats (negative control) compared to the normal group that was not UVB-induced. This showed that UVB radiation was able to trigger activation of the inflammatory cascade, in line with various studies that have previously explained [25].

Royal jelly (RJ) is a product of the cephalic gland secretion of worker bees and serves as the most important part of the honeybee larva's diet, playing a major role in caste differentiation [19]. For the first 2-3 days, RJ is the only food given to all young larvae in their ripening process, while for the queen, it is a special food for the entire period of her life. This is the reason the queen of bees lives longer than other bees. RJ, one of the most effective and beneficial drugs for humans, is widely used both in traditional medicine and in official medicine and it is a controversial food supplement. RJ has the effect of protecting DNA and tissue against oxidative damage [20], [21], [22], [23], [24]. These conditions indicate that RJ has the potential as a natural antioxidant that can suppress oxidative stress processes which are initiated by an inflammatory process induced by UVB radiation.

The group with RJ treatment showed that the higher the RJ dose, the more potential it was in

increasing the level of Nrf2. Nuclear-related factor 2 (Nrf2) is the main transcription factor that regulates some phase II detoxification and antioxidant genes involved in cellular defence against oxidative stress. The more cellular oxidants that occur will cause an increase in antioxidant production mediated by Nrf2. But, if there is a large amount of oxidant production, endogenous antioxidants are unable to compensate for the production of cellular oxidants, which will lead to a decrease in antioxidant production, which in turn will also reduce the expression of Nrf2 [26], [27], [28].

Application of RJ at a dose of 10%, 5% and 2.5% increase the level of NRF2. This showed the antioxidant potential of RJ, which suppressed cellular oxidants due to UVB radiation. In the presence of antioxidants from RJ, it would help endogenous antioxidants in overcoming cellular oxidants. The higher the level of Nrf2 shows the higher the endogenous antioxidants available in the body, so the lower the level of Nrf2 indicates the lower endogenous antioxidants available in the body. The more oxidants handled by endogenous antioxidants, the Nrf2 levels will decrease. RJ which is rich in antioxidants, will help endogenous antioxidants to suppress cellular oxidants. This caused in the groups with RJ, the level of Nrf2 were higher, which indicated the increasing number of antioxidants available in the body [29], [30], [31].

NF-kB (Nuclear factor-kappa Beta) is a transcription factor that will initiate the expression of pro-inflammatory cytokines, one of which is TNF-alpha. Application of RJ can reduce inflammation by suppressing oxidative stress [32], [33]. The mechanism of RJ in suppressing inflammation is by repressing oxidants in the body, thereby reducing the expression of NF-kB, which results in a decrease in the production of pro-inflammatory cytokines, TNF alpha.

In conclusion, royal jelly cream application protected the skin from UV radiation by increasing cellular antioxidants and suppressing inflammatory cascade.

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Association of Gene Polymorphism of *Bactericidal Permeability Increasing Protein* Rs4358188, *Cluster of Differentiation 14* Rs2569190, *Interleukin 1β* Rs1143643 and *Matrix Metalloproteinase-16* Rs2664349 with Neonatal Sepsis

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Keywords: Neonatal sepsis; Single nucleotide polymorphism; BPI; CD14; IL1β; MMP16; Polymorphism

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Abstract

BACKGROUND: Neonatal sepsis is a health problem because it causes serious morbidity and mortality in neonate intensive care units. The susceptibility of neonates occurs due to the immaturity of immune system development as well as due to maternal and environmental risk factors that can cause infection. Identification of genetic variation in genes involved in the inflammatory process can help clarify the pathophysiology of sepsis in high-risk patients, useful for the development of new diagnostic tools, and specific management plans for more accurate predictions of patient's prognosis.

AIM: This study aims to determine the association between gene polymorphism of *BPI* rs4358188, *CD14* rs2569190, *IL1β* rs1143643 or *MMP16* rs2664349 and the incidence of neonatal sepsis.

METHODS: Cross-sectional observational studies with genomic DNA samples from infants with sepsis and non-sepsis which were stored according to the standard storage of genetic materials in the Biomedical Laboratory of Faculty of Medicine Universitas Andalas Padang City, Indonesia. This study is part of a previous study by Rukmono P. Continued with PCR examination, sequencing and bioinformatics analysis.

RESULTS: Only *IL1β* rs1143643 G > A gene polymorphism was associated with the incidence of neonatal sepsis and was statistically significant (p = 0.017). No significant association was found between gene polymorphisms of *BPI* rs4358188 G > T, *CD14* rs2569190 A>G or *MMP16* rs2664349 G > A and neonatal sepsis (p > 0.05).

CONCLUSION: Gene polymorphism of *IL1β* rs1143643 G > A is associated with the incidence of neonatal sepsis.

Introduction

Neonatal sepsis is a health problem because it causes severe morbidity and mortality in neonate intensive care units. The susceptibility of neonates is due to immaturity in the development of an immune response or due to maternal and environmental risk factors that can cause infection [1].

The *World Health Organization* (WHO) estimates 1 million deaths each year from neonatal sepsis and 42% of these deaths occur within the first week of life [2]. The incidence of neonatal sepsis in the United States ranges from 1 to 4 per 1000 live births and from 2.4 to 16 per 1000 live births in Southeast Asia. In Indonesia, the neonatal mortality rate caused by infection and sepsis reached 1 to 10 per 1000 live births and reached 13 to 27 per 1000 live births in babies born under 1500 g [3].

The cause of neonatal sepsis is multifactorial and could have a maternal, neonatal or environmental basis. In recent years, many studies have investigated the association of genetic variation to the incidence of neonatal sepsis. Study into the association of genetic variation to neonatal sepsis is very important because identifying genetic variations in genes that are

involved in bacterial-induced cell responses and those associated with the pathogenesis of sepsis can help clarify the pathophysiology of sepsis in a group of high-risk patients. It is useful for the development of new diagnostic tools and specific management plans for more accurate prediction of patient prognosis [1].

To find treatment solutions for neonatal sepsis incidence, further studies on aspects of the immune system need to be carried out, primarily associated with the inflammatory process. Several genes that are considered to be associated with it and have not been explicitly studied in Indonesia are *Bactericidal Permeability Increasedasing Protein* (BPI), *Cluster of Differentiation 14* (CD14), *Interleukin 1 Beta* (IL1β) and *Matrix Metalloproteinase-16* (MMP16).

This study aims to reveal the association of gene polymorphism of *BPI* rs4358188, *CD14* rs2569190, *IL1β* rs114364003 and *MMP16* rs2664349 with the incidence of neonatal sepsis.

Material and Methods

Study design and research sample

The study is an observational study with a cross-sectional design using genomic DNA extraction samples from sepsis and non-sepsis infants which were stored according to standards for genetic material in the Biomedical Laboratory of FK UNAND. The materials used in the study are part of a previous study by Rukmono P. The Team of Research Ethics Committee approved the study, Faculty of Medicine, Universitas Andalas, Padang No: 532/KEP/FK/2017.

Operational definitions

The variables of this study included several independent variables: Gene Polymorphism of *Bactericidal Permeability Increasing Protein* rs4358188, *Cluster of Differentiation* 14 rs2569190, Interleukin 1β rs1143643 and *Matrix Metalloproteinase*-16 rs2664349; and a dependent variable is neonatal sepsis.

Research procedure

The criteria for neonatal sepsis which is used, is the presence of clinical symptoms of infection confirmed by positive blood culture results indicating bacteremia. The samples are then carried out by PCR examination, electrophoresis, DNA restriction, sequencing, and bioinformatic analysis.

Data analysis

The analysis was performed using the chi-square test to determine the association between gene polymorphism and neonatal sepsis, with a level of $p < 0.05$ considered as statistically significant. Data analysis was carried out by using *STATA 14.2 (Stata Corporation).*

Results

The samples obtained were from 30 neonates with neonatal sepsis and 30 neonates with unproven neonatal sepsis (controls) (Table 1).

Table 1: Demographic characteristics of study subjects

Variable	Neonates		p
	Proven sepsis (n = 30)	Unproven sepsis (n = 30)	
Gender			
Male	15 (50.00%)	23 (76.67%)	0.061
Female	15 (50.00%)	7 (23.33%)	
Age			
< 3 days	26 (86.67%)	29 (96.67%)	0.353
≥ 3 days	4 (13.33%)	1 (3.33%)	
BW			
≤ 2500 g	9 (30.00%)	12 (40.00%)	0.588
> 2500 g	21 (70.00%)	18 (60.00%)	
APGAR score at 1 minute			
≤ 3	1 (3.33%)	0 (0.00%)	1.000
> 3	29 (96.67%)	30 (100%)	
Gestational age			
28–31 weeks	2 (6.67%)	3 (10.00%)	
32–35 weeks	4 (13.33%)	8 (26.67%)	0.483
36–38 weeks	8 (26.67%)	8 (26.67%)	
≥ 38 weeks	16 (53.33%)	11 (36.67%)	
Maternal fever (≥ 38°C)			
Yes	14 (46.67%)	13 (43.33%)	1.000
No	16 (53.33%)	17 (56.67%)	
Thick-smelling amniotic fluid			
Yes	16 (53.33%)	8 (26.67%)	0.065
No	14 (46.67%)	22 (73.33%)	

Table 1 showed the demographic characteristics, consisting of gender, birth weight, APGAR score at first minute, gestational age, and occurrence of maternal fever and presence of thick-smelling amniotic fluid.

Table 2: Clinical symptoms in study subjects

Clinical symptoms in study subjects	Neonates		p
	Proven sepsis (n = 30)	Unproven sepsis (n = 30)	
Crying			
Strong	23 (76.67%)	26 (86.67%)	0.453
Moaning	6 (20.00%)	4 (13.33%)	
Unreacted	1 (3.33%)	0 (0%)	
Suction reflex			
Strong	14 (46.67%)	19 (63.33%)	0.489
Weak	14 (46.67%)	10 (33.33%)	
Vomiting	1 (3.33%)	1 (3.33%)	
Nothing	1 (3.33%)	0 (0%)	
Seizure			
Yes	1 (3.33%)	1 (3.33%)	1.000
No	29 (96.67%)	29 (96.67%)	
Lethargy			
Yes	13 (43.33%)	6 (20.00%)	0.096
No	17 (56.67%)	24 (80.00%)	
Chest retraction			
Yes	8 (26.67%)	4 (13.33%)	0.333
No	22 (73.33%)	26 (86.67%)	

Obtained more neonates aged < 3 days (86.67%) who suffered sepsis compared to neonates aged ≥ 3 days (13.33%) and more neonates born with

thick-smelling amniotic fluid (53.33%) had proven sepsis although not statistically significant (p > 0.05). Clinical symptoms in study subjects (Table 2).

Table 2 showed the clinical symptoms of the study subjects which included crying, suction reflexes, seizures, lethargy, and chest retraction, and there was no statistically significant association between these characteristics (p > 0.05), (Table 3).

Table 3: Gene polymorphism of *BPI, CD14, IL1β*, and *MMP16*

Type of mutation	Allele	f	%
	AA (*homozygous*)	7	11.67
BPI rs4358188 G > A	GA (*heterozygous*)	23	38.33
	GG (*wild type*)	30	50.00
	GG (*homozygous*)	17	28.33
CD14 rs2569190 A > G	AG (*heterozygous*)	34	56.67
	AA (*wild type*)	9	15.00
	AA (*homozygous*)	16	26.67
IL-1β rs1143643 G > A	GA (*heterozygous*)	29	48.33
	GG (*wild type*)	15	25.00
	AA (*homozygous*)	35	58.33
MMP16 rs2664349 G > A	GA (*heterozygous*)	24	40.00
	GG (*wild type*)	1	1.67

Table 3 known, only three SNPs were found in this study population, namely *CD14, IL1β*, and *MMP16* gene polymorphism, and no *BPI* gene polymorphism was found. Association of genetic polymorphism with neonatal sepsis (Table 4).

Table 4: Association of genetic polymorphism with neonatal sepsis

Gene and allele of the polymorphism	Group				p
	No sepsis	%	Sepsis	%	
BPI rs4358188 G > T					
No mutations	15	25.0	15	25.0	1.00
Mutation	15	25.0	15	25.0	
CD14 rs2569190 A > G					
No mutations	3	5.00	6	10.0	0.472
Mutation	27	45.0	24	40.0	
IL1β rs1143643 G > A					
No mutations	12	20.0	3	5.0	0.017
Mutation	18	30.0	27	45.0	
MMP16 rs2664349 G > A					
No mutations	1	1.67	0	0	1.00
Mutation	29	48.33	30	50.0	

Table 4 showed the four SNPs in the study samples that were proven or unproven to have sepsis. Only gene polymorphism of *IL1β* rs1143643 G > A was associated with the incidence of neonatal sepsis and was statistically significant (p < 0.05). However, no significant association was found between gene polymorphism of *BPI* rs4358188 G > T, *CD14* rs2569190 A > G or *MMP16* rs2664349 G > A and neonatal sepsis (p > 0.05).

Discussion

Identification of genetic variation in neonatal sepsis is essential because infants, especially those with very low birth weights (VLBW) or born prematurely, have immature immune systems and innate immunity to bacterial infections is disrupted. This causes infants to be at risk of sepsis or severe sepsis. Therefore, identification of these genetic variations can help to clarify sepsis pathophysiology in a group of high-risk patients.

About demographic characteristics of the study subjects, no significant association was found between these characteristics and neonatal sepsis. There was no significant association between gender and neonatal sepsis (p > 0.05). This result contrasts with the results of Shivaprasad B's study in which male infants had sepsis risk factors that were 3.1-fold higher than female infants. Shane L. Andi also found a higher incidence of sepsis in term male infants than term female infants, although this association has not been found in premature infants [4]. Sepsis is multifactorial, and inflammatory cytokine effects on sepsis development can be modified by age, gender, and several environmental factors.

In the age group of the study subjects, no significant association was found (p > 0.05). Neonatal sepsis is divided into two categories based on time of onset, namely neonatal early-onset sepsis (EOS) if symptoms occur at < 72 hours, and neonatal late-onset sepsis (LOS) if symptoms occur at ≥ 72 hours. The incidence of neonatal early-onset sepsis is reported to be 0.98 infections per 1000 live births [4]. While the estimated number of LOS cases nationally is unknown.

About the birth weight group, there were no significant differences in the sepsis and non-sepsis groups regarding birth weight (p > 0.05). The incidence of sepsis was inversely proportional to birth weight, namely 10.96 per 1000 live births with a birth weight of 401 – 1500 g, 1.38 for 1501 – 2500 g, and 0.57 for > 2500 g [4].

With regard to the APGAR score at 1 minute, one neonate (3.33%) had an APGAR score of ≤ 3 and 29 neonates (96.67%) a score of > 3 of APGAR score in the sepsis sample group, and 0 (0.00%) neonate with ≤ 3 of APGAR score and 30 (100.00%) neonates with > 3 of APGAR score, with p-value of 1,000, showed no significant differences between sepsis and non-sepsis groups based on APGAR score at 1 minute. This is not by the study conducted by Gebremedhin D, where the APGAR score at 5 minutes and shortly after birth showed a significant association with the risk of neonatal sepsis. Neonates with an APGAR score of < 7 at 5 minutes have a higher risk of developing neonatal sepsis than neonates with an APGAR score of ≥ 7 (OR = 68.9; 95% CI 3.63, 1307.90). Similarly, neonates who cry immediately at birth are 99% less likely to experience sepsis than neonates who do not cry immediately at birth (OR = 124.0; 95% CI 6.5, 2379) [5]. This may be due to the nature of crying, which is a physiological event, and changes associated with this event.

About gestational age, no significant differences were found between sepsis and non-sepsis groups. Based on the literature, the overall case fatality rate of neonatal sepsis is 16%, with an incidence that is inversely proportional to gestational

age: 54% at 22–24 weeks of gestational age, 30% at 25 – 28 weeks, 12% at 29 – 33 weeks, and 3% at greater than 37 weeks [4]. While for the risk of LOS incidence based on gestational age is 36.3% in neonates with a gestational age of < 28 weeks at least having one episode of LOS compared to 29.6%, 17.5% and 16.5% in moderate preterm neonates (29–32 weeks of gestational age), late preterm (33–36 weeks of gestational age) and a term neonates, respectively.

There was no significant association between the maternal fever group during labour (≥ 38°C) and the incidence of sepsis ($p > 0.05$). This was not by the results of previous studies, which found that neonates born to mothers who had a fever during labour had a 6-times greater risk of experiencing sepsis than neonates born to mothers who did not experience intrapartum fever [5], [6]. Intrapartum fever is considered to be an indicator of maternal infection which is often transmitted to the baby *in utero* or as it passes through the birth canal and often causes EOS. Maternal fever without accompanying chorioamnionitis signs can also increase the risk of sepsis but is often accompanied by a cause of noninfectious maternal fever such as dehydration or epidural anaesthesia [7]. According to Verma P et al., some maternal factors that predispose to sepsis include vaginal examinations that are too frequent (23.25%), maternal fever (33.33%) and history of foul-smelling amniotic fluid (24.72%) [8].

In the sepsis group, 16 neonates (53.33%) were from mothers with thick-smelling amniotic fluid and 14 neonates (46.67%) from mothers without a history of thick-smelling amniotic fluid. Whereas in the unproven sepsis group, there were eight neonates (26.67%) from mothers with thick-smelling amniotic fluid and 22 (73.33%) from mothers without a history of thick-smelling amniotic fluid ($p > 0.05$). The results of this study are consistent with two previous studies which reported that foul-smelling amniotic fluid, prematurity, low birth weight, residency, parity, and ANC facility services were not statistically associated with the risk of neonatal sepsis. However, regarding absolute numbers of sepsis cases, there was a higher incidence of thick-smelling amniotic fluid and vice versa [5]. Rawat S. also reported that the discovery of foul-smelling amniotic fluid is evidence of anaerobic bacteria and is one of the predisposing factors for sepsis, but there is insufficient evidence confirm this as an independent risk factor for neonatal sepsis [7].

Clinical symptoms were examined in this study, including crying, suction reflexes, seizures, lethargy and chest retraction. After statistical analysis, no significant differences were found in the group that was proven to have sepsis or not proven to have neonatal sepsis against crying ($p = 0.453$), suction reflexes ($p = 0.489$), seizures ($p = 1.000$), lethargy ($p = 0.096$) and chest retraction ($p = 0.333$). This suggests that both groups had almost the same clinical symptoms, so it does not affect the results of the study.

About *BPI* rs4358188 gene polymorphism in the sepsis group (30 subjects), 12 subjects experienced heterozygous polymorphism (GA) of the rs4358188 *BPI* gene, three subjects with homozygous polymorphisms (AA) and 15 wild-types (GG) subjects. Whereas in the no sepsis group (30 subjects), 11 subjects experienced heterozygous polymorphisms (GA) of the rs4358188 *BPI* gene, four subjects with homozygous polymorphisms (AA) and 15 wild-types (GG) subjects.

BPI is a gene that encodes factors that play an essential antibacterial and anti-inflammatory role and are commonly found in neutrophil azurophilic granules, playing an essential role in defence against Gram-negative infections. Esposito et al. reported that the AG genotype of BPI rs4358188 was associated with a reduced risk of sepsis [4]. However, Abu Maziad's study did not show an association between *BPI* polymorphisms and susceptibility to sepsis [9]. Michalek et al. also reported a negative association between *BPI* SNP and sepsis in children aged 0 – 18 years where the GG genotype of *BPI* rs435188 was associated with increased susceptibility to severe sepsis and adverse perinatal outcomes [10].

In the case of polymorphism in the *CD14* rs2569190 gene, 16 subjects experienced heterozygous polymorphism (AG) of the rs2569190 *CD14* gene, eight subjects experienced homozygous polymorphism (GG), and there were six wild-types (AA) subjects in the proven neonatal sepsis group. In the unproven neonatal sepsis group (30 subjects), 18 subjects were found with heterozygous polymorphism (AG) of the rs2569190 *CD14* gene, nine subjects experienced homozygous polymorphism (GG), and the remaining three subjects were wild-type (AA).

CD14 is a component of lipopolysaccharide receptor molecules and an important recognition receptor that plays a key role in the immune response and inflammation. Previous studies have shown that *CD14* gene polymorphisms are involved in several inflammatory diseases, such as ulcerative colitis and Crohn's disease. Previous reports have shown that the biological association of *CD14* rs2569190 gene polymorphism with the survival of patients with A allele can be caused by a strong pro-inflammatory response in patients, along with higher *CD14* expression [11].

Studies have shown that GG genotype of *CD14* rs2569190 gene is associated with an increased risk of severe sepsis and death in sepsis shock patients undergoing major surgery [1], [11]. Patients with GG genotype of rs2569190 had a shorter probability of survival compared to AA/AG genotype of rs2569190 in 60 days (62.3% vs 50%), and 90 days (62.3% vs 52.6%) ($p = 0.046$) compared to AA/AG genotypes. A to G polymorphism at -159 positions in the region of *CD14* (rs2569190) gene promotion can cause different activities in promoter

arrangement. In this context, the G allele of rs2569190 seems to have implications for decreasing *CD14* transcription regulation and also decreasing mCD14 expression and circulating sCD14 levels [11].

The study conducted by Wang reported that there was no evidence that rs2569190 was associated with susceptibility to sepsis [12]. In 2013, a meta-analysis by Zhang et al. concluded that *CD14* rs2569190 polymorphism was not a relevant risk factor for sepsis and mortality. The authors found that patients with AA/AG genotype had a higher risk of death than patients with the GG genotype (Asian population). This finding is likely to be a result of the small number of studies associated with sepsis mortality that were included in the meta-analysis [13].

In the sample group with *IL1β* gene polymorphism, 20 subjects experienced heterozygous polymorphism (GA) of rs1143643 *IL1β* gene, seven subjects experienced homozygous polymorphism (AA), and the remaining three subjects were wild-type (GG). Whereas in the unproven group of neonatal sepsis totalling 30 subjects, nine subjects were found who experienced heterozygous polymorphism (GA) of rs1143643 *IL1β* gene, nine subjects experienced homozygous polymorphism (AA), and the remaining 12 subjects were wild type (GG).

Interleukin 1β is a key pro-inflammatory cytokine that is produced early in response to microbial invasion and plays an important role in the pathogenesis of sepsis and sepsis shock. These molecules stimulate the production of prostaglandins and nitric oxide, the two vasodilatory mediators found in sepsis.

Esposito et al. reported that the CT and TT genotypes of *IL1β* rs1143643 gene were associated with a significant increase in the overall risk of sepsis [4]. The three polymorphisms of *IL1β* rs1143634, rs1143633 and rs1143643 were associated with the risk of allergic asthma ($p = 0.034$, OR = 1.523; $p = 0.024$, OR = 1.471; $p = 0.044$, OR = 1.420) [14]. The study by Abu Maziad et al. did not find an association between *IL1β* gene polymorphism and sepsis. This discrepancy may be due to differences in the definition of sepsis and its severity, and differences in some general characteristics of the subjects, included ethnicity, compared to those used in Esposito's study [9].

Concerning *MMP16* gene polymorphism, 12 subjects experienced heterozygous polymorphism (GA) of the rs2664349 *MMP16* gene, 18 subjects experienced homozygous polymorphism (AA), and there were no subjects with wild type (GG). In the unproven neonatal sepsis group, totalling 30 subjects, 12 subjects experienced heterozygous polymorphism (GA) of the rs2664349 *MMP16* gene, 17 subjects who experienced homozygous polymorphism (AA) and one wild type (GG) subject.

One of the advantages of the PCR sequencing method the detection of other nucleotides besides the nucleotide targets, for example, SNP *MMP16* rs2616505 A > G, rs11785236 A > G, and rs10504853 T > G; these were also found in this study and SNP has not been studied previously in Indonesia.

The results of this study on increasing MMP expression after exposure to LPS indicate that protease can affect the pathogenesis of endotoxemia so that MMP levels are said to be associated with sepsis severity. In this study, MMP may play a role in the incidence of sepsis because almost all samples had mutations, although it was not statistically significant. MMP is regarded as a time bomb because most of the samples had mutations in both sepsis and non-sepsis patients. Therefore, currently, MMP-based therapy (MMP-inhibitor) is being developed in patients with sepsis and sepsis shock, for example, glucocorticoids, retinoids and progesterone which can suppress MMP expression [4].

MMP16 is a zinc-dependent enzyme, and this element is very important for the normal functioning of the innate or adaptive immune systems [15]. The possibility that MMP genetic variation significantly affects susceptibility to infectious disease in humans is still very small [16]. A consistent discovery to many gene expression studies that patients with pediatric septic shock are characterised by extensive repression of genes that directly participate in zinc balance or directly depend on them to perform normal functions [17], [18], [19], [20].

Esposito et al. reported that the GG genotype of rs2664349 was associated with an increased risk of significant sepsis. This is the first report of the potential effects of *MMP16* genetic variation on sepsis and seems to be in agreement with recent evidence that MMP is, not only a matrix degradation enzyme as previously thought, but also has multiple immunomodulation mechanisms. SNP rs2664349 not only affects pulmonary expression, *MMP16* function and risk of bronchopulmonary dysplasia in preterm infants but also affects to MM2 activation, MMP which plays a core role in monocyte chemoattraction and subsequently affects the response to infectious agents [4], [21].

In conclusion, this analysis confirmed gene polymorphism of *IL1β* rs1143643 G > A is associated with the incidence of neonatal sepsis.

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Comparison of Glomerular Filtration Rate Measurement Methods between Radionuclide *in vivo* Scintigraphic Gates' and Plasma Sampling

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Abstract

AIM: To correlate between the radionuclide *in vitro* plasma sampling method (using single and dual blood samples) and Gates' GFR measurement using Tc-99m diethylene triamine penta-acetic acid (Tc-99m DTPA) renal scintigraphy (*in vivo* method).

METHODS: This study included 40 renal donors (group 1) and 40 patients with obstructive uropathy (group 2). Group 1 included 22 males and 18 females with an age range from 22 to 65 years, while group 2 included 24 males and 16 females with age range 27 to 64 years. Both groups subjected to renal Scintigraphy after administration of 5 mCi 99m-Tc DTPA, GFR was calculated using Gates' method (*in vivo* method), then plasma sampling was acquired at 60 mins and 180 mins post-injection of the tracer, samples were counted in well counter and GFR was calculated using *in vitro* technique either using single plasma sample (SPSM 60 mins) or dual sample (DPSM 60 & 180 min). Additionally, GFR was measured by estimated equations based on serum creatinine.

RESULTS: In group 1, the mean GFR using *in vivo* Gates' method was 115.7 ± 29 ml/min, while using the SPSM was 100.1 ± 16.1 ml/min, and the DPSM was 100.3 ± 20.1 ml/min. In group 2, mean GFR using *in vivo* method was 74.1 ± 14.5 ml/min, while using *in vitro* SPSM it was 77.5 ± 24.9 ml/min and DPSM was 76.8 ± 24.8 ml/min. There was no significant difference between mean GFR values using *in vivo* and *in vitro* methods (single or dual samples) in group 1 and 2 ($p > 0.05$). There is high significant correlation between SPSM and DPSM in groups 1 and 2 ($r = 0.90$, $r = 0.91$ respectively), moderate significant correlation was found between *in vivo* Gates' method and *in vitro* SPSM in group 1 and 2 ($r = 0.46$ and 0.57 respectively) and moderate correlation was evident between *in vivo* and *in vitro* DPSM in both groups ($r = 0.42$ and 0.68 respectively). By using the DPSM as the reference standard significant high correlation was found with SPSM and significant-high moderate correlation with *in vivo* Gates' scintigraphic method. Conclusion: *In vitro* plasma sampling considered as a reliable, accurate |method for GFR calculation yet it considered relatively complex, both single and dual sample *in vitro* techniques showed a very high correlation, and hence SPSM can replace DPSM.

CONCLUSION: Renal scintigraphy and GFR estimation using Gates' *in vivo* method is considered inaccurate, yet given its simplicity in performance it can still be used if corrected GFR is standardised for Egyptian population-based on studies with large numbers of patients from multiple centres.

Introduction

Glomerular filtration rate (GFR) is the volume of fluid filtered from the renal glomerular capillaries into the Bowman's capsule per unit time [1]. The Kidney Disease Outcome Quality Initiative (K/DOQI) of the National Kidney Foundation clinical practice guidelines identified GFR as the keystone for the definition and staging of chronic kidney disease including obstructive uropathies [2].

Inulin clearance has long been regarded as the 'gold standard' method for GFR calculation [3]. Yet it was restricted in clinical practice as it considered a complex technique that requires constant intravenous infusion and bladder catheterisation [4]. Evaluation of GFR using camera-based Tc-99m DTPA renal scintigraphy is a noninvasive method, less time consuming and does not require urine or blood samples collection. It also can identify the individual renal function, whereas other methods evaluate the global renal function. The major disadvantages of scintigraphy include the use of radioactive isotopes, specialised Gama camera needs, and expertise in evaluating the procedure [5].

GFR can be accurately calculated from

the rate of clearance of a tracer activity (commonly used Tc-99m DTPA) from the plasma, which considered a precise method simulating inulin clearance. Initially, multisampling technique was used yet it was exhaustive and difficult to perform in routine clinical practice. Simpler methods have been proposed in clinical practice in which the GFR is estimated from only one or two plasma samples (based on empiric relationships relating an apparent tracer volume with various GFR regression equations) rather than from a multi-sample time-activity curve [6].

Another less accurate method for GFR assessment includes estimated equations based upon serum creatinine such as the Cockcroft-Gault equation (CG), the Modification of Diet in Renal Disease (MDRD) Study equations, and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. That provides a quick and simple estimate for GFR [7] however they are limited by the use of serum creatinine which depends on variations in creatinine production also they are less accurate in certain populations including diabetic patients with high GFR [8]. In light of the above factors; it was decided to compare the single and double plasma sampling method with scintigraphic Gates' *in vivo* method and estimated equations to observe the reliability of these measures in routine clinical practice.

Material and Methods

This comparative study included 80 subjects, 40 renal donors considered as control (group 1) and 40 patients diagnosed as obstructive uropathy (group 2), they were selected from the patients who were sent for routine renal study in Nuclear Medicine Unit, Cairo University during the period from July 2013 till April 2014. The study was approved by the ethical committee. The Inclusion criteria included patients above 18 years old with serum creatinine level within the normal range for both groups, while exclusion criteria included patients under 18 years old, patient with a history of marked renal impairment with GFR < 30 ml/min and high serum creatinine level (> 1.5). Both groups were subjected to full clinical history taking, and serum creatinine level is measured and recorded.

In vivo Gate's method: patients are well hydration and voiding was done just before the beginning of the study**.** Pre – injection syringe containing 185 MBq, Tc-99m DTPA (5 mCi) was counted using dual-head gamma camera (Philips-Axis) before injection. Then an intravenous bolus injection of the tracer was done followed by dynamic imaging acquisition in the posterior position. The post-injection syringe was counted at the end of study similar to pre-injection. The difference between the pre and the post-injection counts provided the total injected dose. Region of interest (ROI) for each kidney was drawn manually and semi-lunar background ROIs were placed around the lower outer renal margins. The background-corrected time-activity curve was generated, and the renal uptake of each kidney from 2 to 3 min after the injection was calculated. Afterimage acquisition, patient's weight and height were entered into the computer software system, on which all imaging data were recorded, and the GFR was automatically calculated according to the Gate's algorithm [9].

In vitro plasma sampling method: Tc-99m-DTPA plasma clearance measured by SPSM and DPSM. After scintigraphy, the site of injection on the arm was scanned under the Gamma camera. The residual radioactivity at the injection site should be less than 0.1% in all subjects, venous blood samples (10 ml) were collected in a syringe from the contralateral arm at 60 and 180 min through. The blood samples were centrifuged at 1000 g for 15 min to separate the red blood cells from the plasma, then 1 ml of plasma from the sample as well as the standards was counted in well counter of (Atom lab 960 thyroid uptake system) for 1 min after 24 hours. The decay of radioactivity was corrected. Time at which the blood sample was taken was recorded on the worksheet. The blood samples taken at 60 min and 180 min were used for the DPSM and a sample taken at 180 min was used for SPSM. Russell's method was used for *in vitro* GFR estimation [10]. Estimation equations for each patient based upon serum creatinine were calculated including the Cockcroft-Gault equation and 2009 CKD-EPI equations.

Statistical methods: All statistical calculations were done using computer programs SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 17 for Microsoft Windows. Data were statistically described in terms of mean ± standard deviation (± SD). Comparison of numerical variables between the study groups was made using Student t-test, Paired t-test and Chi-square test. Linear Correlation Coefficient was used for detection of correlation between two quantitative variables in one group. Also, standard linear least-squares regression analysis was used, p-values of 0.05 or less in the linear regression analysis were considered significant. Bland and Altman's analysis were referred to an agreement between the two methods for independent samples.

Results

No significant difference concerning age and gender between both groups was detected. By using

the *in vivo* scintigraphic method, the mean GFR is in group 1, and group 2 was 115.7 ± 29.0 ml/min and 74.1 ± 14.5 ml/min respectively. The difference in mean values between both groups where statistically significant (p ≤ 0.001) (Table1).

Table 1: Mean and range of GFR as measured by a radionuclide *in vivo* method in both groups

Groups	GFR in vivo						T-Test	
	Range			Mean	±	SD	t	P-value
Group 1	70.5	-	169.0	115.7	±	29.0	-8.106	< 0.001*
Group 2	42.3	-	98.1	74.1	±	14.5		

No significant difference was found between the mean GFR values using *in vitro* SPSM and DPSM in both groups; mean GFR in group 1 for the SPSM & DPSM was 100.1 ± 16.1 ml/min and 100.3 ± 20.1 ml/min respectively, while it was 77.5 ± 24.9 ml/min, and 76.8 ± 24.8 ml/min in group 2 respectively (p-value 0.6 and 0.8). However, there is a significant difference between both groups by applying each *in vitro* method (SPSM and DPSM) separately (p-value < 0.001) (Table 2).

Table 2: Mean and range of GFR as measured using radionuclide *in vitro* method (single and dual plasma samples) in group1 and 2

Groups		GFR In Vitro						
		Group 1			Group 2			P-value
Single	Range	69.3	-	122.6	33.5	-	135.8	< 0.001*
	Mean ± SD	100.1	±	16.1	77.5	±	24.9	
Dual	Range	70.9	-	138.2	39.2	-	139.6	< 0.001*
	Mean ± SD	100.3	±	20.1	76.8	±	24.8	
	T	0.446			-0.187			
	P-value	0.658			0.852			

Creatinine based equations: using CG equation in both group 1 and 2, mean value of GFR was 143.1 ± 6.4 ml/min, 104.35 ± 27.41 ml/min respectively, whereas the CKD-EPI method means GFR values were 109.41 ± 18.7 ml/min, 85.21 ± 22.39 ml/min respectively. The difference between the two equations in both groups is statistically significant (p < 0.001) as shown in (Table3).

Table 3: Mean and range of GFR as measured by creatinine-based estimated equations

Groups		GFR		T-Test
		Group 1	Group 2	P-value
CG-EQU	Range	71.0 - 198.0	53.0 - 155.0	
	Mean ± SD	143.1 ± 36.4	104.4 ± 27.4	< 0.001*
CKD-EPI EQU	Range	64.0-129.0	45.0 - 124.0	
	Mean ± SD	109.4 ± 18.767	85.2 ± 22.4	< 0.001*
Paired t-test	T	5.83	6.24	
	P-value	< 0.001*	< 0.001*	

Correlations between different methods of GFR measurement

There is a highly significant correlation between *in vitro* SPSM and DPSM in both groups, (r = 0.90) for group 1 and (r = 0.91) for patients group as demonstrated in (Figure 1).

A moderate significant correlation was found between *in vivo* and *in vitro* SPSM in both groups (r = 0.46 and 0.57). Also, a moderate correlation was evident between *in vivo* and *in vitro* DPSM in both groups (r = 0.42 and 0.68).

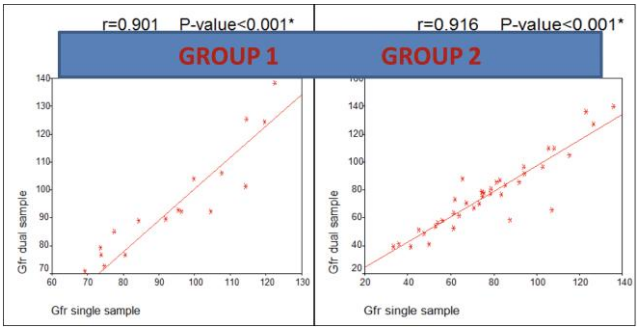


Figure 1: Scattered plot showing a linear correlation between single and dual plasma sampling using radionuclide in vitro methods for measuring GFR in both groups (r, 0.901 and 0.916) respectively and (P-value < 0.001)

Group 1 showed a low moderate significant correlation between radionuclide SPSM and DPSM *in vitro* method and CG creatinine-based equation (r = 0.43 and 0.33) respectively, while there is no significant correlation in GFR estimation between them in group 2. CKD-EPI 2009 equation demonstrates moderate significant correlation in GFR estimation compared to *in vitro* (SPSM & DPSM) in both group 1 and 2 (r = 0.46 and 0.37) and (r = 0.38 and 0.46) respectively.

Table 4: Linear regression between the dual sample *in vitro* technique and other methods in group1

Group 1	Standardised Coefficients		R^2
	r	Sig.	
(Dual Sample)		0.00	44.20%
GFR invivo	0.68	0.00	
(Dual Sample)		0.17	
GFR SPSM	0.90	0.00	80.74%
(Dual Sample)		0.00	8.66%
GFR CG-EQU	0.33	0.04	
(Dual Sample)		0.00	12.19%
GFR CKD-EPI EQU	0.38	0.02	

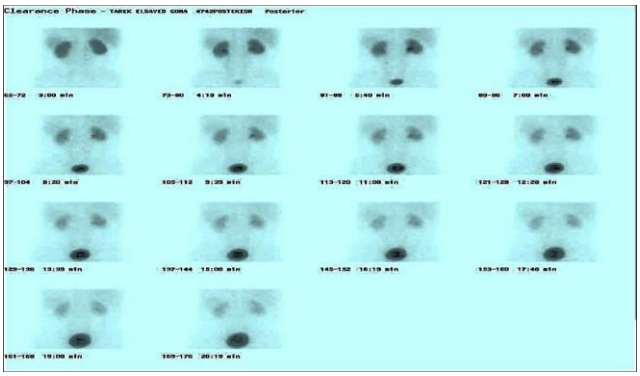
Taking the double sample radionuclide *in vitro* technique as a reference standard; linear regression analysis is considered to be significant (p < 0.05) against *in vivo* Gates' method, SPSM *in vitro* and estimated creatinine equations (CG and CKD-EPI 2009) methods respectively in control group**.** The accuracy of regression equations of dual sample radionuclide *in vitro* is highest against single sample technique (80.7%) while is moderate with *in vivo* Gates' method (44.2%) and very low against CG, and CKD-EPI creatinine-based method was (8.6% and 12.19%) respectively (Table 4 and 5).

Table 5: Correlations between DPSM and other different methods for GFR estimation in group1 (renal donors)

Methods	r -value	Correlation
DPSM & SPSM	0.91	High
DPSM & in vivo	0.68	High moderate
DPSM & CKD-EPI	0.38	Low moderate
DPSM & CG	0.33	Weak

Discussion

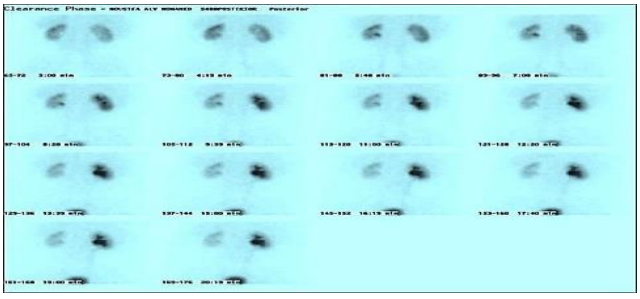
Glomerular filtration rate (GFR), the best overall index of renal function, many methods are developed to estimate GFR to obtain more accurate value and simpler procedure including the equations based on serum creatinine and serum cystatin C, and renal dynamic imaging method [11], [12]. Cr-51-EDTA and Tc-99m-DTPA are among the most commonly used radionuclide tracers for measuring GFR. Studies have shown that their renal clearance correlates well with inulin clearance which was considered the standard gold method.



GFR In vivo	GFR SPSM	GFR DPSM	GFR-CG equ	GFR-CKD EPI equ
121.2	114.6	125.27	183	125

Figure 2: Male donor, 41 years old with normal GFR value by different methods

Plasma clearance of Tc-99m-DTPA using *in vitro* plasma sampling method correlates well with inulin clearance (standardised estimation error is 3.5 ml/min) [13], [14]**.** Based on study results, the DPSM in a mono-compartment model is more accurate in GFR determination than the SPSM [15], this method is taken as a reference in our study as inulin clearance was not available for our setup.



GFR In vivo	GFR SPSM	GFR DPSM	GFR-CG equ	GFR-CKD EPI equ
65.5	87.59	78.59	88	52

Figure 3: Male patient 54 years old, complaining of right loin pain 2 months ago, diagnosed radiologically as right renal stone with grade II to III backpressure. There is a normal value of GFR using in vitro method as compared to in vivo method

Similarly, because of the satisfactory accuracy and relative simplicity of 99mTc-DTPA dual plasma sample clearance, this method was taken as the reference approach in determining GFR by the Nephrology Committee of Society of Nuclear Medicine [16].

The results of the present study demonstrate that the DPSM correlate well with the SPSM in both groups (r = 0.91). Similar results were reported in a study by Mulligan et al., [17]. The DPSM using Russell's formula considered as a reliable method for the valid estimate of true GFR. Also, in a study by Itoh et al., [18] Russell's SPSM was compared with 10 sample method, and the coefficient was 0.971. Furthermore, Zuo et al., [19] reported that the DPSM should be used when GFR is less than 45 ml/min.

In our study, GFR ranged 33.5-135.8 ml/min with a mean value of 77.5 ± 24.9 ml/min using SPSM, while using DPSM ranges 39.2-139.6 ml/min with mean GFR value of 36.8 ± 24.8 in obstructive uropathy group. The Gates *in vivo* [20] method was considered feasible and very simple when compared to the plasma sampling method, which was a bit complex yet more accurate.

Jackson et al., [21] reported that the Gates method tended to overestimate GFR in comparison to the dual sample *in vitro* method. Itoh [22] also reported overestimated GFR values with the Gates method and indicated that the overestimation might be attributable to insufficient correction for background activity in the kidney**.** In the present study *in vivo* GFR measurement using the Gates method also tends to overestimate GFR, the value ranges 42.3-98.1 ml/min with a mean value of 74.1 ± 14.5 ml/min in group2. GFR estimation was performed in 133 patients using: A) gamma camera uptake method (modified Gates, Gates); B) predicted creatinine clearance method (Cockcroft-Gault, CG); and C) single- or two-plasma clearance method (PSC). The PSC was chosen as a reference (Same as in the current study). In comparison with the GFR by PSC, the Gates tended to overestimate the GFR, as found in our study. This study concluded that The Gates correlates well with the PSC, while in our study, it showed a moderate correlation.

Itoh et al., [22] showed that GFR estimation using by *in vitro* method is better than the CG method, which tended to underestimate the GFR. In our study GFR values using CG method ranges from 71-198 with a mean value of 143.1 ± 36.4 in group1 with low, moderate correlation (r = 0.33) in both SPSM &DPSM. The estimated creatinine equations show a weaker correlation than Gates as compared to the *in vitro* techniques. However, in group1 the DPSM and *in vivo* camera-based method showed a mean difference of -15.43 ± -8.92(95% confidence interval CI). Whereas for CKD-EPI method, the mean difference was -9.09 ± 1.37, 95% CI. Accordingly, we concluded that both the Gates *in*

vivo and the CKD-EPI equation tended to overestimate GFR, especially in the range of high GFR (group 1).

In conclusion, dual sample *in vitro* method (DPSM) was considered as the reference with good correlation with the SPSM. Whereas neither Gates method nor CKP-EPI predicted creatinine equation could calculate GFR accurately as they tend to overestimate GFR measurement, especially in the range of high GFR. Our study was limited by the small number of patients. Gold standard "inulin" *in vitro* GFR measurement was not available for comparison. Also, normal GFR in the Egyptian population has not been standardised specially in children were *in vitro* SPSM and DPSM will be a proper method for GFR.

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Basic Science

Combination of Human Amniotic Fluid Derived-Mesenchymal Stem Cells and Nano-hydroxyapatite Scaffold Enhances Bone Regeneration

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Abstract

BACKGROUND: Human amniotic fluid-derived stem cells (hAF-MSCs) have a high proliferative capacity and osteogenic differentiation potential *in vitro*. The combination of hAF-MSCs with three-dimensional (3D) scaffold has a promising therapeutic potential in bone tissue engineering and regenerative medicine. Selection of an appropriate scaffold material has a crucial role in a cell supporting and osteoinductivity to induce new bone formation *in vivo*.

AIM: This study aimed to investigate and evaluate the osteogenic potential of the 2nd-trimester hAF-MSCs in combination with the 3D scaffold, 30% Nano-hydroxyapatite chitosan, as a therapeutic application for bone healing in the induced tibia defect in the rabbit.

SUBJECT AND METHODS: hAF-MSCs proliferation and culture expansion was done *in vitro,* and osteogenic differentiation characterisation was performed by Alizarin Red staining after 14 & 28 days. Expression of the surface markers of hAF-MSCs was assessed using Flow Cytometer with the following fluorescein-labelled antibodies: CD34-PE, CD73-APC, CD90-FITC, and HLA-DR-FITC. Ten rabbits were used as an animal model with an induced defect in the tibia to evaluate the therapeutic potential of osteogenic differentiation of hAF-MSCs seeded on 3D scaffold, 30% Nano-hydroxyapatite chitosan. The osteogenic differentiated hAF-MSCs/scaffold composite system applied and fitted in the defect region and non-seeded scaffold was used as control. The histopathological investigation was performed at 2, 3, & 4 weeks post-transplantation and scanning electron microscope (SEM) was assessed at 2 & 4 weeks post-transplantation to evaluate the bone healing potential in the rabbit tibia defect.

RESULTS: Culture and expansion of 2nd-trimester hAF-MSCs presented high proliferative and osteogenic potential *in vitro*. Histopathological examination for the transplanted hAF-MSCs seeded on the 3D scaffold, 30% Nano-hydroxyapatite chitosan, demonstrated new bone formation in the defect site at 2 & 3 weeks post-transplantation as compared to the control (non-seeded scaffold). Interestingly, the scaffold accelerated the osteogenic differentiation of AF-MSCs and showed complete bone healing of the defect site as compared to the control (non-seeded scaffold) at 4 weeks post-transplantation. Furthermore, the SEM analysis confirmed these findings.

CONCLUSION: The combination of the 2nd-trimester hAF-MSCs and 3D scaffold, 30% Nano-hydroxyapatite chitosan, have a therapeutic perspective for large bone defect and could be used effectively in bone tissue engineering and regenerative medicine.

Introduction

Human amniotic fluid stem cells (hAF-MSCs) derived from the second trimester are of particular importance as a source of a multipotent fetal stem cell and characteristically as a rich source of fetal progenitor cells including osteoblasts. Successful differentiation into osteogenic stem cells *in vitro* proposes their crucial role in regenerative medicine and bone regeneration [1].

Amniotic mesenchymal stem cells (AF-MSCs) have been used in animal models as a pre-clinical application. AF-MSCs have been applied on critically sized femoral defects of nude rat in combination with biomaterial scaffold and showed bone formation in rat femoral defect [2].

In contrast to embryonic stem cells, undifferentiated AF-MSCs propagate widely without inducing tumour [3]. Unlike adult-derived stem cells, the AF-MSCs cell lines propagated up to 250 population doublings retained long telomeres and a normal chromosomal karyotype [3]. Human amniotic fluid-derived stem cells (hAF-MSCs) are extensively multipotent and have been induced to differentiate into cell types representing each embryonic germ layer, including cells of osteogenic, adipogenic, chondrogenic, myogenic, endothelial, neuronal, and hepatic lineages [3-6].

To preserve and retain the physiological function and microstructure of the defected bones, regenerative medicine was explored very extensively during the last decades. Novel treatment techniques include utilising of nanocomposite scaffolds and sophisticated carriers for the targeting and delivery of bioactive molecules, mesenchymal stem cells and/or growth factors, providing both the structural exactness and the biochemical information to cells when they are differentiated into a particular type of tissue [7].

Nano-hydroxyapatite (HA) is considered as the most inorganic mineral in human bone tissue, therefore, HA artificial conformable materials are largely employed in the curing of bone defects originated by traffic accidents, trauma and bone disease [8-10]. In the bone defects remediation, some problems are expected to take place, such as low rate bone healing. The slower healing rate is especially recognised for patients with metabolic bone disorders and local osteoporosis [11].

Despite the magnificent role done by HA, stile it poses some drawbacks when it is used alone such as mechanical failure in load-bearing sites. In addition to that, the well-known composition of natural bone is considered as a composite material. This composite is made of inorganic (HA) and organic (collagen of type (I)) materials. Therefore, many of studies discussed the concept of the incorporation of the inorganic materials within the biopolymer matrix to form a composite material mimicking the microstructure of the bone [9, 12, 13].

Accordingly, nanocomposite scaffolds consisting of biodegradable polymers and/or ceramics consolidate the beneficial peculiarities of both materials. The effective carrier scaffolds can maintain their microstructure, physicochemical and physicomechanical properties at the implantation site [14]**.** Chitosan (C) is considered as a suitable polysaccharide for the fabrication of 3D scaffolds due to its impressive properties, such as biodegradability, biocompatibility, non-toxicity and bio-functionality [15].

The pre-clinical application of combined Stem cell technology with 3D biodegradable and nanostructure scaffold is a new trend of therapy in tissue engineering and bone repair, which is going to be investigated, evaluated and applied in this study.

Human AF-MSCs have the advantage of being a versatile precursor cell with great expansion capability. However, few studies have reported research results related to Nano-hydroxyapatite/chitosan (HA/C) scaffolds combined with hAF-MSCs in both *in vitro* and *in vivo*. Therefore, the aim of this study was directed to investigate the effect of the combination of 2nd-trimester hAF-MSCs with chitosan polymer scaffold contain 30% of HA ($^{W}/_{W}$ %) in the treatment of bone defect assessment.

Subjects and Methods

Second-trimester amniotic fluid samples (collected between the 14th and 18th weeks of gestation) were obtained by amniocentesis from six women, three samples only (n = 3) were successfully isolated. The age range was between 24 to 36 years old. The Ethics Committee of the National Research Centre, Cairo, Egypt, approved the study protocol and all participants gave informed consent.

Isolation of Mesenchymal stem cells

Mesenchymal stem cells were isolated from the collected amniotic fluid was done by centrifugation and pelleting of cells [3]. Subsequently, the cells were cultured in alpha minimal essential medium (α-MEM; Gibco BRL, Life Technologies B.V., Breda, Netherlands) containing 20% fetal bovine serum (FBS; Gibco ERL), 1% glutamax (Invitrogen), 100 U/ml penicillin (Gibco ERL), and 100 U/ml streptomycin (Gibco ERL) and 4 ng/ml basic fibroblast growth factor (bFGF; Sigma) and incubated at 37°C under standard conditions with 5% CO_2.

Culture, expansion and proliferation of hAF-MSCs

These steps were maintained in regular proliferation media to reach about 80% confluence, and then cells were passage and reseed. Manual scraping technique using cell scraper (Corning incorporated, Costar, Mexico) and collection of the cells, followed by centrifugation and re-suspension in regular proliferation media then re-seeding of cells in culture plates. Human AF-MSCs proliferation was done at 70% confluence of the 3rd passage and were used for osteogenic differentiation by exchanging the culture media into Dulbecco's Modified Eagle's Medium (DMEM) containing 20% FBS, 50 mmol/l L-

ascorbic acid 2-phosphate,10 mmol/l β-glycerol phosphate and 0.1 mmol/l dexamethasone as well as 100 µg/ml penicillin/ streptomycin and 1% glutamax [16]. A comparable control culture (MSCs in proliferation media) was made for each sample. Two similar sets that differ in incubation time were simultaneously generated; one set (plates in differentiation media and control plates) was maintained in corresponding culture media for 14 days before passed into characterisation protocols. The second set; same kind of plates retained in its culture media for 29 days before characterisation.

Osteogenic differentiation characterisation

This step was done using Alizarin Red staining for the detection of mineralised nodules developed in the differentiated cultures. In briefly, plates were washed three times with PBS and fixed in 70% ethanol at room temperature for an hour, washed with dH_2O before adding 2% Alizarin Red (pH 4.2). The plates were incubated at 37ºC for an hour with gentle shaking. Plates were washed with dH_2O until the dye's colour disappears. Carefully aspirate dH_2O, wash the plates with PBS and add enough dH_2O to cover the cellular monolayer to be ready for image capture by inverted microscope [17].

Expression of the surface markers in AF-MSCs

At the third passage (P3), AF-MSCs were assessed using Flow Cytometer, fluorescein-activated cell sorting (FACS) Calibur (BD Biosciences, USA). Cells were trypsinised (0.25% trypsin and 0.01% EDTA; w/v), washed twice with PBS supplemented with 0.5% Bovine Serum Albumin (BSA; Sigma-Aldrich, Saint Louis, MO, USA), and resuspended in PBS at a concentration of 2×10^5cells/20µl. Subsequently, the labelled cells were incubated with 10µl of the following fluorescein-labelled antibodies, CD34-PE, CD73-APC, CD90-FITC, and HLA-DR-FITC (BD Biosciences, USA) in dark place for 30 min at room temperature. Isotype-matched controls were performed for every analysis for the evaluation of possible nonspecific staining and autofluorescence [18].

Flow cytometry analysis operation started, and then a single colour immunofluorescence protocol was defined. A gate was set to include the population of interest, followed by running samples and 10.000 events were collected. Data analysis was executed to determine percentage positivity for the antibody [18]. Detailed immunophenotype analysis was carried out by flow cytometry using relevant markers. The cultured AF-MSCs express on their surface HLA-DR, CD 34, CD73, and CD90 and the percentages of positivity were calculated.

Osteogenic differentiation of AF-MSCs

Osteogenic differentiation of AF-MSCs performed on the 3D scaffold of 10 mm diameters and 3 mm heights *in vitro*. 3D Scaffold was used as a carrier for AF-MSCs, placed in 6-well culture plates, washed 3 times with 70 % ethanol, exposure to U.V for an hour and then washed twice with osteogenic medium (an hour for each rinse). AF-MSCs were seeded on the 3D scaffold at a density of $10x10^6$ and incubated at 37°C in osteogenic differentiation medium, DMEM containing 20% FBS, 100 µg/ml penicillin & streptomycin and 1% glutamax, L-ascorbic acid 2-phosphate, β-glycerol phosphate and dexamethasone for two and three weeks [16]. The scaffold carrying osteogenic differentiated AF-MSCs cells was fixed at 21st day using Glutaraldehyde and analysis by scanning electron microscope (SEM) [19].

In vivo study

The *in vivo* study, transplantation of human amniotic fluid stem cells (hAF-MSC) at the third passage (P3) was seeded on the 3D scaffold, 30% Nano-hydroxyapatite chitosan, and osteogenically differentiated for 3 weeks in osteogenic media and then applied to the induced tibia defect in the rabbit.

Ethics Approval

Ethical approval for this study was obtained from the National Research Centre Ethics Committee (approval number/16/263).

Animals and experiment design

A total of ten, six-month-old New Zealand White rabbits (1.5 – 2 Kg) were used for this study. Rabbits were classified into 3 groups as follows; G1: included 4 rabbits and were sacrificed at 2 weeks post-transplantation, G2: included 2 rabbits and were sacrificed at 3 weeks post-transplantation, and G3: included 4 rabbits and were sacrificed at 4 weeks of post-transplantation (Table 1). The defect has been transplanted with osteogenic differentiated hAF-MSCs progenitors cells seeded on the 3D scaffold *in vitro* (considered as an experimental target), and the tibia defect received 3D scaffold without osteogenic differentiated hAF-MSCs cells (considered as the control).

Table 1: Animal Groups Classification

Groups	G1	G2	G3
Rabbits no.	4	2	4
Sacrificed/Post-operation	2 weeks	3 weeks	4 weeks

Preoperatively, each rabbit has fasted for 12 hours. General anaesthesia was induced by intramuscular injection of ketamine hydrochloride (35 mg/kg BW) and xylazine (5 mg/kg BW) and

maintained throughout the surgical procedure.

The external incision in the tibia, the skin incisions (approximately 1.5 cm) were carried out (initiated) and extending to the muscles layers deep to the bone level, producing a defect cavity in the tibia 0.5 mm in diameter and 0.5 mm in depth. The cavities were prepared using standard round bur in a contra-angle handpiece running at approximately 10,000 rpm and abundantly irrigated with saline solution. Finally, the flaps were repositioned carefully and sutured in layers. Postoperative pain was managed by injection of buprenorphine (50 µg/kg BW) at every 2 hours for the first day. This experiment was conducted following the national and European guidelines for animal experiments.

The hAF-MSCs (approximately 2 million cells) at the third passage (P3) were seeded on the 3D scaffold, 30% Nano-hydroxyapatite chitosan, for two days in regular proliferation media and then replaced by osteogenic differentiation media after PBS washing, and incubated for 3 weeks at 37 ºC. Scanning electron microscope (SEM) was assessed to evaluate the osteogenic differentiation potential of hAF-MSCs osteogenic differentiated cells seeded on the 3D scaffold, 30% Nano-hydroxyapatite chitosan, *in vitro.* Meanwhile, osteogenic differentiated hAF-MSCs cells seeded on the 3D scaffold, 30% Nano-hydroxyapatite chitosan, was applied and fitted in the defect area of rabbit tibia to evaluate the new bone formation and bone healing impact.

Histological examination

Bone samples were fixed in 10% buffered formalin. Samples were decalcified in 0.5 M EDTA, pH 8.3 (Sigma-Aldrich, St. Louis, MO, USA). Parietal bones were then rinsed in PBS, dehydrated with graded ethanol, and embedded in paraffin. Transversal serial sections 5-6 µm thick were deparaffinized in xylene, hydrated with a series of graded ethanol, stained with hematoxylin/eosin (H&E), and examined under the optical microscope (CX41, Olympus, Tokyo, Japan) [20].

Preparation of Nano-hydroxyapatite

Artificial HA was prepared in the laboratory using the sol-gel method with an atomic ratio of Ca/P = (1.67) and pH = (10). Firstly, dissolving of 37.8 gm of calcium nitrate tetrahydrate in 1000 ml bi-distilled water. Meanwhile, 12.66 gm of diammonium hydrogen orthophosphate were also dissolved in another 1000 ml bi-distilled water containing polyethylene glycol as a dispersant agent. The required amounts of both stock solutions to give the desired Ca/P atomic ratio of 1.67 were mixed in room temperature, and the pH of the resulted mixture was adjusted to about 10 by the addition of a dilute NH_4OH solution with proper strength. The above stapes were followed by ageing and vigorous stirring of the obtained solution at its boiling point for about 2 h in a sealed container. The HA was collected from the solution by filtration and was washed three times with bi-distilled water, followed by drying at 100^{o}C overnight. A calcination temperature above 800^{o}C was applied to the dried powder to eliminate the other impurities such as nitrates and ammonia. The particle size and the morphology of the prepared HA were determined using TEM.

Preparation of scaffold Nano-composites

Acetic acid was used as the solvent to prepare the polymer solutions. By using 2 v % acetic acid solution, chitosan was dissolved using magnetic stirrer for 3 h, and the polymer solution was left overnight at room temperature to remove the air bubbles trapped in the viscous solution. Afterwards, 30% of hydroxyapatite was dispersed in bi-distilled water by 30 min ultrasonic treatment. The dispersed HA powders were mixed with the chitosan solution under agitation. The homogeneously mixed solution was poured in a syringe which was used as cylindrical moulds and immediately taken to deep freeze at –20^{o}C. After 24 h freezing the samples were quickly lyophilised for 36 hours. Finally, the scaffolds were cut into discs and analysed by XRD, and SEM-EDX measurements before *in vitro* and *in vivo* studies.

Transmission Electron Microscope

The morphology and particle size of the prepared hydroxyapatite was analysed using transmission electron microscope-TEM (JEM2010, Japan) working at 200 kv. Practically, 10 mg of HA powder was dispersed ethanol using the ultrasonic bath, and few drops of prepared suspension were dropped on the Cu grid. Afterwards, the grid was left to dry at room temperature and was further investigated by the TEM.

X-ray diffraction analysis (XRD)

X-ray diffraction analysis (XRD) was used to identify the phase of the prepared samples. The data was collected from a (Diano corporation made in the USA) diffractometer using monochromatic CuK_{α} radiation (λ=1.5406**Å**) with scanning rate 0.1^{o} in the 2θ ranging from 10 to 80^{o} step radiation. Voltage and current were set at 40 kV and 40 mA, respectively.

Scanning Electron Microscope

The scaffold microstructure was studied using JSM 6360 LV SEM (JEOL, Japan). In detail, the cross-sectional view for the prepared scaffold was studied through mounting the internal scaffold structure on metal stubs and was coated with gold

before being examined. Additionally, X-ray elemental analysis (EDAX) was carried out for the samples pre-*in vitro* and *in vivo* studies to assess the presence of the inorganic elements within the examined scaffold.

Moreover, SEM images were recorded for the scaffold with and without hAF-MSCs osteogenic differentiated cells *in vitro* and *in vivo* to evaluate the effect of the combination of hAF-MSCs osteogenic differentiated cells and scaffold on bone healing rate. Practically, *in vitro* SEM images were obtained upon seeding of hAF-MSCs on the scaffold surface in 12 well plates for 21 days in the osteogenic media. In details, the scaffolds were washed 3 times with 70 % ethanol, exposed to U.V light for 2 h, and then washed twice with the culture medium.

Results

Proliferation and culture expansion of hAF-MSCs

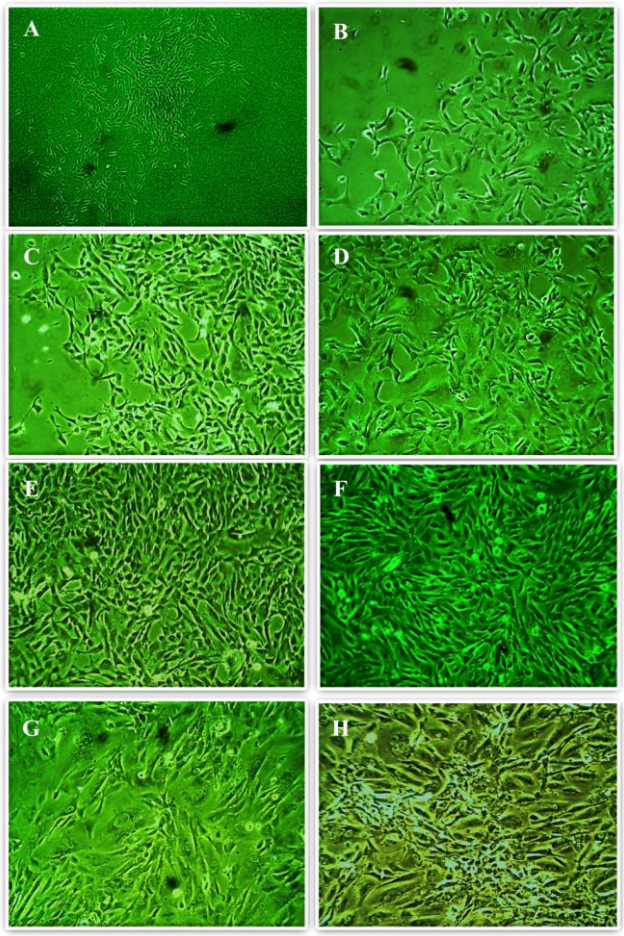

Figure 1: Proliferation and culture expansion of hAF- MSCs at different passage. (A & B) P0 at 7 & 21 days respectively; (C&D) P1 at 7 &17 days respectively; (E&F) P2 at 7 &19 days respectively; (G&H) P3 at 7 & 21 days respectively, magnification X10

Human AF-MSCs was cultured and expanded in regular proliferation media. Colonies of hAF-MSCs appeared in the primary culture (P0) after 7-10 days from the initial cell plating day. Cells aggregated from central to peripheral side of the plate till reaching 75% confluence by the end of this passage (Figs. 1A, B respectively). At the beginning of the first passage (P1), hAF-MSCs displayed typical fibroblast-like cells morphology and presented a higher proliferation rate by the end of this passage (Figs. 1C, D respectively). Human AF-MSCs presented typical mesenchymal cell morphology in the subsequent passages and experienced further proliferation and expansion in the second passage (P2) (Figs. 1E, F) and third passage (P3) (Figs. 1G, H).

Osteogenic differentiated AF-MSCs on 14th and 28th days

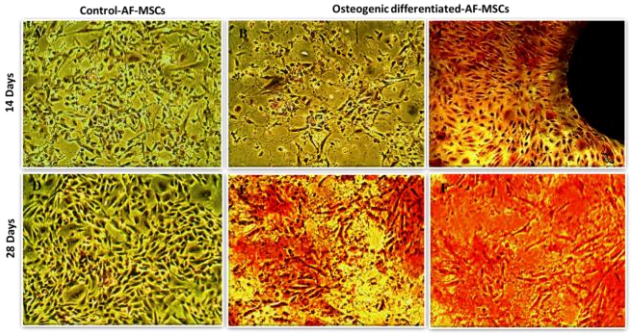

Figure 2: Alizarin red staining of osteogenic differentiated AF-MSCs on 14 and 28 days, A) Control at 14 days of culture showed no red staining; B) Osteogenic differentiated AF-MSCs cells at 14 days showed few stain spots in centre of the plate; C) moderate red staining patches were detected in peripheral side of the plate; D) Control at 28 days of culture showed very weak red staining spots; E) Strong red staining patches of osteogenic differentiated AF-MSCs at 28 days in the central of the plate; F) Stronger red staining intensity of osteogenic differentiated AF-MSCs cells in peripheral side of the plate

Osteogenic differentiation of hAF-MSCs seeded on Scaffold after three weeks in vitro

The hAF-MSCs cells were seeded and osteogenically differentiated on the 3D scaffold, Nano-hydroxyapatite chitosan, in the osteogenic media for 3 weeks *in vitro,* small to large black areas represented scaffold filled with AF-MSCs osteogenic differentiated and calcium deposits all over the plate were observed using an inverted microscope (Figs. 3A & B). Moreover, this finding was confirmed by using the electron microscope scanned (EMS) indicating the osteogenic differentiated hAF-MSCs was penetrated the pore and filled with calcium nodule deposits which will be ready for animal transplantation, bone repair, and regeneration (Fig. 3D) compared to the non-seeded scaffold (Fig. 3C).

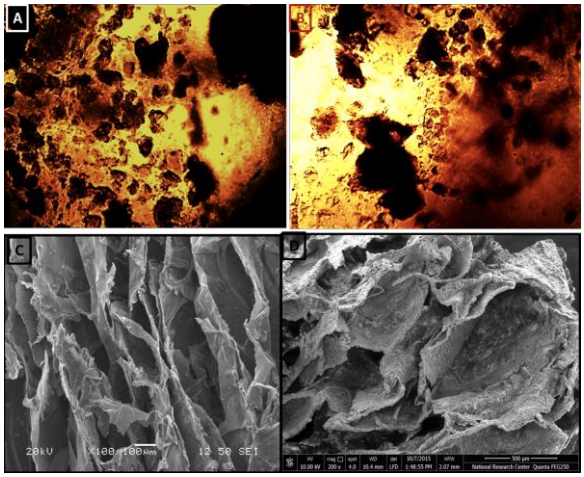

Figure 3: Osteogenic differentiation of hAF-MSCs seeded on Scaffold after 3 weeks in vitro. (A) & (B) Osteogenic differentiated of hAF-MSCs seeded on scaffold represented by inverted microscope images. (C) Non-seeded Scaffold (SEM, Scale Bar: 100 µm); (D) hAF-MSCs osteogenic differentiated seeded on the scaffold (SEM, Scale Bar: 500 µm)

Flow cytometric analysis

Flow cytometric analysis was performed to detect the AF-MSCs immunophenotypes.

Table 2: Flow-cytometry parameters in hAF-MSCs

hAF-MSCs Surface marker	Positivity rate
CD73	42.1%
CD90	2.4%
CD34	0.2%
HLA-DR	0.1%

The percentage were positive for both CD73 and CD90 cell surface markers showed 42.1 % and 2.4% respectively, while were negative for both CD34 and HLA-DR cell surface markers showed 0.2%, and 0.1% respectively (Table 2) (Fig. 4).

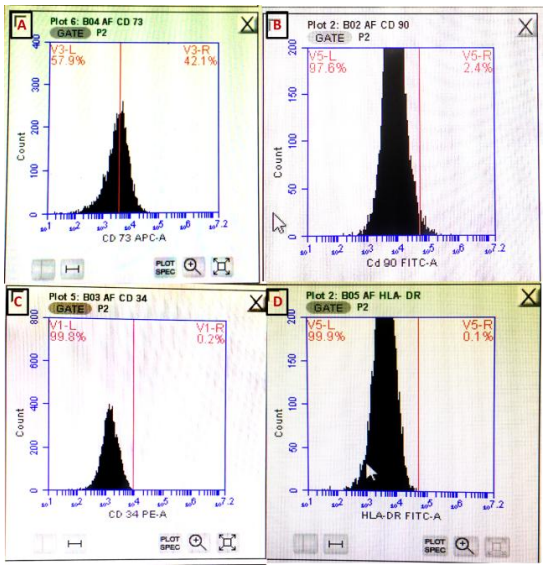

Figure 4: Flow cytometric analysis for hAF-MSCs. (A) & (B) hAF-MSCs were positive for both CD73 and CD90 cell surface markers 42.1% & 2.4% respectively. (C) & (D) hAF-MSCs were negative for both CD34 and HLA-DR cell surface markers 0.2% & 0.1% respectively

Histological results

At two weeks post-transplantation, histological examination of sections transplanted with osteogenic differentiated hAF-MSCs cells seeded on the 3D scaffold, 30% Nano-hydroxyapatite chitosan, showed large areas of newly formed bone that were observed directly nearby to the host bone as compared to the control (Fig. 5A). In contrast, the control (non-seeded scaffold) showed areas filled with structurally and morphologically rearranged fibrous tissue consistent with the failure to repair the bone defect (Fig. 5B).

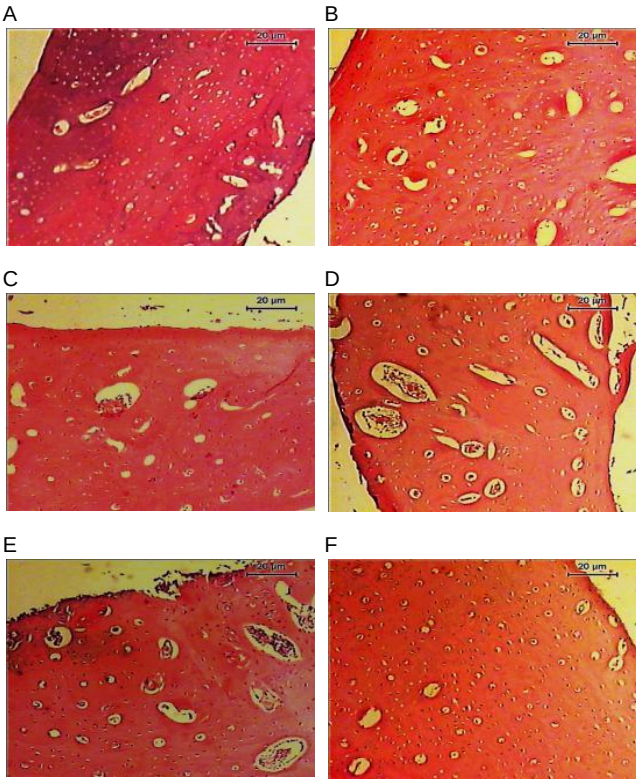

Figure 5: Histopathological Sections of rabbits transplanted by hAF-MSCs and Scaffold. A): Osteogenic differentiated hAF-MSCs seeded on the 3D scaffold, 30% Nano-hydroxyapatite chitosan after 2 weeks showing large areas of new bone formation visible immediately nearby to the host bone compared with the control (non-seeded scaffold). Notice the occurrence of an infinite network of vessels is obviously in the not yet mineralized construct area, B) Control, after 2 weeks showing areas filled with structurally and morphologically prearranged fibrous tissue consistent with failure to repair the bone defect; C) hAF-MSCs/scaffold, after three weeks shows large areas of mature formed in the defect. Few vacuoles filled with collagen were noticed D) Control after 3 weeks shows limited bone regeneration and large bone defect remaining. Notice that the defect is filled primarily with connective tissues; E) hAF-MSCs/scaffold after 4 weeks show large mature bone mostly filed the whole defect. Few vacuoles filled with collagen were noticed; F) Control after 4 weeks shows limited bone regeneration and large bone defect remaining. Notice that the defect is filled primarily with connective tissues (H&E stain; Scale bar: 20 µm)

At three weeks post-transplantation, sections of bone defect transplanted with osteogenic differentiated hAF-MSCs/scaffold showed large areas of mature formed bone in the defect site. Few vacuoles filled with collagen were noticed as compared to control one (Fig. 5C). However, the

control (non-seeded scaffold) showed limited bone regeneration with a large bone defect area remaining. The defect was filled primarily with connective tissues (Fig. 5D).

At four weeks post-transplantation, histological examination of bone **s**ections defect transplanted with osteogenic differentiated hAF-MSCs/scaffold showed mature bone formation mostly filled the whole defect area. Few vacuoles filled with collagen were noticed (Fig. 5E). However, the control (non-seeded scaffold) showed limited bone regeneration and large bone defect area remaining. Notice that the defects were filled mainly with connective tissues (Fig. 5F).

Morphology of the prepared Nano-hydroxyapatite

The morphology and the size of the prepared Nano-hydroxyapatite were evaluated by TEM as represented in Fig. 6. The Nano-hydroxyapatite particles are presented in the nanoscale with a range of 20-30 nm. Semisphere morphology was observed for aggregated particles of hydroxyapatite.

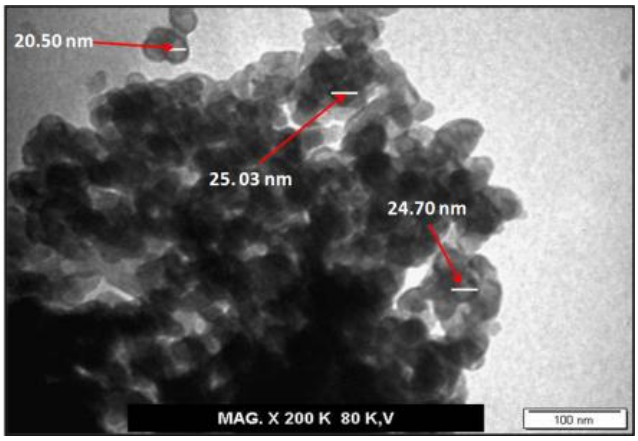

Figure 6: TEM image of the prepared Nano-hydroxyapatite (TEM, Scale Bar: 100 µm)

Scanning Electron Microscope results

The XRD technique is employed to assess the phase purity, the level of crystallinity and changes of the prepared nanocomposites. XRD method is based on the coherent scattering of X-rays by an ordered lattice, in this case, a crystal in which the atoms are periodically ordered. Interference of the radiation with the crystal lattice leads to a distinct distribution of diffracted intensity as a function of the measuring angle 2θ.Fig.7a shows the X-ray diffraction pattern of the prepared hydroxyapatite (HA). Several peaks were observed at approximately 25.8°, 29.1, 30.6° and 31.8°C, these peaks are corresponding to the diffraction planes (211), (002), (210) and (300) of the hydroxyapatite (HA) crystallites, respectively, that confirms the formation hydroxyapatite as early reported [21, 22]. It is worthy noted that upon the formation of chitosan/hydroxyapatite composite scaffold, no remarkable changes were observed for the crystallisation of the HA particles. However, minor crystallisation decrease was noted (Fig.7b). In addition to that, the characteristic crystalline peaks of HA were significantly shifted, due to the expected interface binding that takes place between hydroxyapatite particles and chitosan matrix.

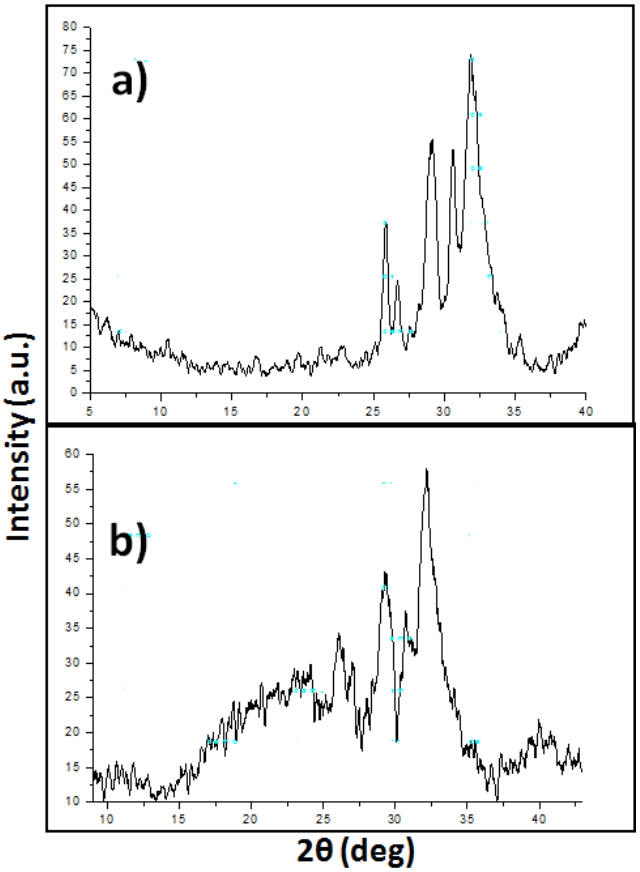

Figure 7: XRD analysis of a) the prepared Nano-hydroxyapatite and b) chitosan /hydroxyapatite composite scaffold

The scanning electron microscope (SEM-EDX) micrographs of the prepared scaffold composite (HC30) is shown in Fig. 8. The SEM images provide direct information about the mullet-porous structure of the as-prepared hydroxyapatite/chitosan scaffold nano-composite. SEM images demonstrated a large number of pores around the size of 200 µm–700 µm. These pores are connected to form an interconnected porous network. The rough surface that was observed for prepared composites was attributed to the incorporation of hydroxyapatite in the composite. The HA particles were embedded well in the chitosan matrix. From the EDX spectra of the prepared hydroxyapatite /chitosan scaffold nanocomposite (Fig. 8), illustrated the recorded elements within the investigated scaffold as following; Ca, P, C and O.

Furthermore, the cells attachment on the scaffold surface cultured in the osteogenic medium at culture time 21 days (Fig. 9a), was also investigated using SEM. “C” corresponds to cells, “J” is the cell-cell

junctions, “M” mineralisation is also detected. Extracellular matrix (EX), attached to scaffold surface, of chitosan /hydroxyapatite composite scaffold.

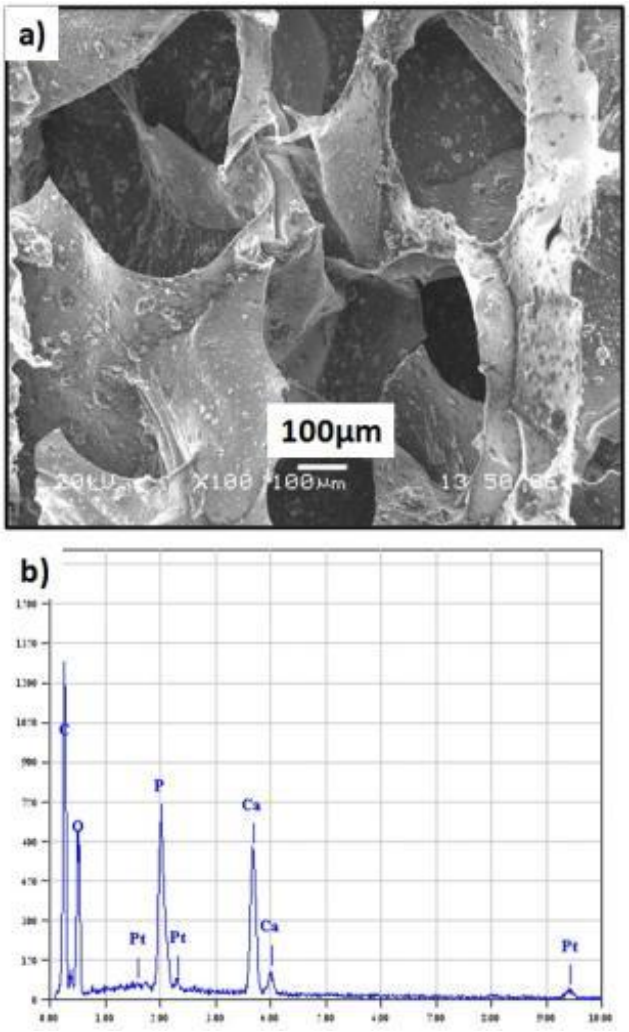

Figure 8: Illustration of a) SEM image for the microstructure (Scale Bar: 100 µm) and b) EDX elemental analysis for the chitosan /hydroxyapatite composite scaffold

In details, it is very clear that human amniotic fluid-derived stem cells (hAF-MSCs)are grown on scaffold surface and have displayed an elongated shape and spread out discretely within the pores and on the surface of the scaffold.

Also, mineral aggregates formation was noted after 21 days of culture, thus denoting the osteogenic activity of prepared scaffold. The higher magnification image (Fig.9b) that show the surface of the cells on prepared scaffolds appeared to be smoother than that on the scaffolds, indicating greater extracellular matrix (EX) depositions and fibrous networks on scaffolds surface as well as attached cells.

In addition to that, the *in vivo* bone healing was investigated by using SEM (Fig.10). Post-transplantation of the prepared scaffold was transplanted into the rabbit's tibia defect, revealed normal healing of the bone tissues with no abnormalities over the site of the operation.

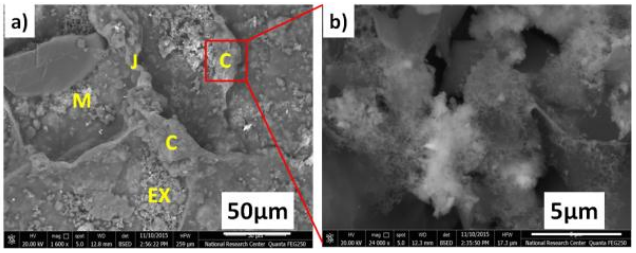

Figure 9: SEM images of a) chitosan/hydroxyapatite composite scaffold and b) Zoom in mineralisation on the scaffold surface (SEM, Scale Bar: 50 and 5 µm, respectively)

All rabbits were healthy and did not present signs of oedema during the post-transplantation period. The scaffolds were in direct contact with new bone without any fibrous encapsulation and almost completely covered with new bone (NB) indicating that scaffolds were highly bioactive as well as biocompatible.

Few remains of scaffolds were still found in the defect, as well as unfilled margins (Figs.10a &10c). They were in direct contact with new bone without any fibrous encapsulation and almost completely covered with bone indicating that scaffolds were highly bioactive as well as biocompatible. The induced bone defect in the rabbit tibia transplanted with the non-seeded scaffold at two and four weeks post-transplantation was semi-healed with highly mineralised bone matrix, and the defect holes remain semi-opened (Figs. 10a & 10c).

For the defect transplanted with osteogenic differentiated hAF-MSCs/scaffold at 2 weeks post-transplantation (Fig. 10b), new bone formation progressed from the deep end walls of the defect regions in-wards. Complete bone integration was detected for the transplanted osteogenic differentiated hAF-MSCs/scaffold at 4 weeks post-transplantation (Fig.10d).

The new bone was formed in continuity with the transplant surface from the cortical bone at the defect site into the marrow space. Also, the results of SEM made for the non-seeded scaffolds transplanted *in vivo* revealed that the bioactive degradable scaffolds have a great response with the host bone and the remodelling proceeded at the scaffolds-bone interface. The induced bone defects in the rabbit tibia at 4 weeks post-transplantation were completely closed by mineralised (M) bone matrix, collagen fibres (CF) and differentiated cells (DC) (Fig. 10 d). There was evidence of new bone formation on the surface of all implants with no evidence of fibrous encapsulation.

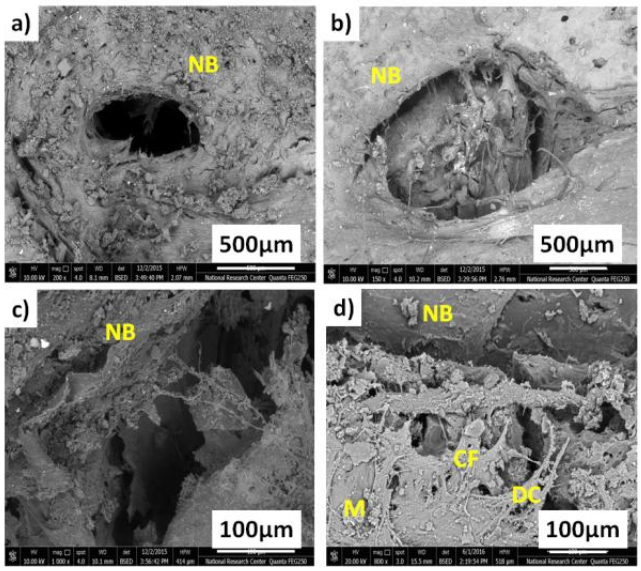

Figure 10: SEM images of chitosan/hydroxyapatite composite scaffold after in vivo implantation. a) control, non-seeded Scaffold after 2 weeks, b) osteogenic differentiated hAF-MSCs cells seeded on Scaffold after 2 weeks, c) control, non-seeded Scaffold after 4 weeks and d) osteogenic differentiated hAF-MSCs cells seeded on Scaffold after 4 weeks (SEM, Scale Bar: 100 µm)

Discussion

Human amniotic stem cell (hAF-MSCs) has a high proliferative capacity and osteogenic differentiation potential *in vitro* provides a very promising source for bone repair applications. Moreover, the combination of hAF-MSCs and three-dimensional (3D) scaffold is considered to be a promising approach for therapeutic purpose applications in bone tissue engineering and regenerative medicine.

In our previous study, results revealed the 2nd-trimester hAF-MSCs used in repairing the induced spinal cord defect in rat model had the priority of bone healing efficiency compared to both the 3rd-trimester hAF-MSCs and hBM-MSCs transplanted into the same animal model [23]. Accordingly, we suggested that the 2nd-trimester hAF-MSCs cells may represent a valuable healing prospect in combination with 3D scaffolds for bone regeneration in the rabbit tibia defect.

The assessment of the combination of the 2nd-trimester hAF-MSCs with the 3D scaffold, 30% Nano-hydroxyapatite chitosan, in our study demonstrated the osteogenic induction for three weeks *in vitro;* cells penetrated the scaffold porous, filled with calcium deposited and were ready for repairing the bone defect in a rabbit model. This finding is consistence with Maraldi et al., who conducted his study on a rat model. They support the idea of the efficiency of scaffold material, fibroin, combined with AF-MSCs in healing critical-size bone defects [24]. Our hAF-MSCs subjected an osteogenic differentiation *in vitro* for 21 days on the 3D scaffold, 30% Nano-hydroxyapatite chitosan, showed great mineralisation and repairing potential for the induced bone defect in the rabbit. Interestingly, Peister et al. demonstrated that AF-MSCs have the effect to differentiate for 28 days on 3D medical-grade poly-e-caprolactone (mPCL) scaffolds *in vitro* producing seven times more mineralised matrix when transplanted subcutaneously in the rat model. Findings of this study suggest that AF-MSCs could be an effective cell source for efficient repair of large bone defects [25].

However, our *in vivo* study revealed the combination of osteogenic differentiated hAF-MSCs cells and scaffold enhanced the bone healing efficiency in the bone defect of rabbit at 4 weeks post-transplantation comparing to the control (non-seeded scaffolds). Roccio et al., in 2012, carried out a comparative assessment of fibroin scaffolds combined with human dental pulp stem cells (hDP-MSCs) and amniotic fluid stem cells (hAF-MSCs) for repairing the cranial bone defects in immunocompromised rats [26]. They reported a significant efficient healing property of the combination of stem cells/fibroin bioengineered scaffold in repairing large animal cranial defects. This finding could be a starting point for human large bone defects regeneration in craniofacial surgery [26]. Moreover, Kim et al., have already noted an inconsistency with Roccio`s results that hAF-MSCs seeded on another type of 3D scaffold, collagen matrix extracted from porcine bladder submucosa matrix (BSM) and poly (lactide-co-glycolide) (PLGA), offered an appropriate microenvironment that enhanced osteogenic differentiation of hAF-MSCs *in vitro* and could be used in bone tissue engineering [27].

In the present study, AF-MSCs were examined for surface and differentiation markers percentages on cells using flow cytometer as an indication of Mesenchymal stem cells (MSC). Flow cytometric assays were performed within the tested cell population for CD34, CD90, and CD73 in addition to HLA-DR. Isolated AF-MSCs cells in this study revealed moderate expression of CD73 (42.1%) in addition to the negativity of CD34 and HLA-DR (0.2% and 0.1 %respectively) and weak expression of CD90 (2.4%). Regarding CD34, our results came by De Rosa et al., and Fei et al., studied the phenotypic characterisation of AF-MSCs [28, 29]. By flow cytometric assays; found them negative for CD34. Moreover, our results also agreed with Zhou et al., stated that AF-MSC must express CD105, CD73 and CD90 and lack the expression of CD45, CD34, CD14, CD11b, CD79a, CD19 and HLA-DR surface molecules. In the same context, in this study, CD73 was positively expressed (42.1%); while CD34 and HLA-DR were negatively expressed (0.2% and 0.1% respectively); results of which had the same opinion

as Markmee et al., found CD73 (49.85%), CD34 (0.3%) and HLA DR (0%) [30].

Nevertheless, Spitzhorn et al. found that AF-MSCs obtained during C-sections showed the typical MSC cell surface marker expression of CD73, CD90, and CD105 by the parallel absence of the hematopoietic markers CD14, CD20 and CD34 [31]. The analysis of the isolated cells in Fei et al., study, revealed negative expression of the hematopoietic stem cell marker, CD34 and some cells were weakly positive for MHC Class II antigens (HLA-DR and HLA-DQ) [29], which agree with the results found in the present study.

Additionally, regarding, HLA-DR testing, cells were found negative as the previous results of Zhou et al., [32]. However, our study didn't reveal strong positivity for CD90, unlike Zhou et al., Savickiene et al., and Markmee et al. [30], [32], [33]. On the contrary, Fei et al. found isolated cells in his study not expressing markers of hemopoietic lineage such as CD105 (SH2), CD70 (SH3/4), CD29, CD16, CD44, and CD90 [29].

The analysis of our histopathological results revealed a distinctive increase in bone regeneration in the tibia defect of a rabbit when received the osteogenic differentiated hAF-MSCs cells/scaffold composite system which mostly filled with mature bone and few vacuoles filled with collagen at 4 weeks post-transplantation. However, the control (non-seeded scaffold) presented fewer, smaller mineralised tissue, limited new bone formation, and there was a large bone defect area still not filled with bone. These findings identified the highest bone healing efficiency with complete healing of the bone defect at 4 weeks post-transplantation. However, the least bone healing potential was observed at 2 weeks post-transplantation; these findings were consistency with the Scanning electron microscope (SEM) results. There is a satisfactory agreement between our results and those of Rodrigues et al., despite the difference in scaffold type, transplantation time, an animal model [34].

It is well known that hydroxyapatite mineral forms about 70 wt. % of natural bone. Collagen and water represent the remaining portion (20-30) wt. %. Nano-structured hydroxyapatite crystals are aligned along the collagen fibres within the water phase [35]. Herein, the prepared hydroxyapatite possessed similar morphology and particle size for those of the natural bone. The XRD results for the prepared scaffolds revealed that incorporation of the Nano-hydroxyapatite didn't alert the physical stability and crystalline nature of obtained scaffold. The low crystallinity of the obtained HA that was much similar to the apatite in natural bone could improve the biodegradability (Fig. 7). It is well known that bone biological characterises of scaffolds are affected by their chemical composition, crystallinity, crystal orientation and porous structures [36].

SEM images revealed the scaffolds would allow the attachment and spreading of the cells while keeping a normal cellular morphology as confirmed with recorded pore sizes (200 µm – 700 µm). These pores are associated with each other to form an interconnected porous network. This network directly facilitated the normal activity of the cells such as nutrition transport, ions exchange and elimination of the cells biological leftover. Porous structure enables the cells infiltration within the pore cavities and provides enough nutrition and blood supply through penetration and circulation [37], [38].

It is worth to highlight that the choice of scaffold depends on the specific tissue to be engineered. It is generally recognised that the chemical properties of materials can influence the cellular behaviour of osteoblasts [12], [39]. SEM images for the current investigated scaffold suggested that the 30 CH scaffold of higher support osteogenic activity and these results were in the same line with early reported studies [12], [39].

Chitosan-nanocomposite scaffolds, the biomaterials that were utilised in the current research, are deemed osteoconductive materials to both cell adhering and tissue ingrowth, and it couples biomaterial resorption with bone formation [40], [41]. However, The SEM images of hAF-MSCs seeded on the 3D scaffold, 30% Nano-hydroxyapatite chitosan, have demonstrated a higher healing potential compared to the control, non-seeded scaffold, 30% Nano-hydroxyapatite chitosan at 2 and 4 weeks post-transplantation.

Our investigations into this area are still ongoing, and further investigations on studying different stem cell sources as well as various scaffold materials will advance our knowledge about the contribution of stem cells progenitor and scaffold composite system to the field of bone tissue engineering.

In conclusion, based on our *in vitro* and *in vivo* findings, we suggest that the 2nd-trimester hAF-MSCs in combination with the 3D scaffold, 30% Nano-hydroxyapatite chitosan, could significantly promote and enhance the bone healing efficiency in the rabbit model. Furthermore, this approach can be used as an outstanding system for functional bone treatment for the repair of large bone defect in human, bone tissue engineering and regenerative medicine.

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Basic Science

Preparation and Evaluation of Sunscreen Nanoemulsions with Synergistic Efficacy on SPF by Combination of Soybean Oil, Avobenzone, and Octyl Methoxycinnamate

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Keywords: Soybean oil; Avobenzone; Octyl methoxycinnamate; Nanoemulsion; Sunscreen

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Abstract

BACKGROUND: Soybean oil contains vitamin E and acts as a natural sunscreen which can absorb Ultra Violet (UV) B light and has antioxidant properties to reduce the photooxidative damage that results from UV-induced Reactive Oxygen Species production. The UV blocking from most natural oils is insufficient to obtain a high UV protection. The strategies for preparations of sunscreen products with high SPF can be done by nanoemulsion formulation and Ultra Violet filter combinations of Soybean Oil, Avobenzone and Octyl methoxycinnamate.

AIM: The purpose of this study was to prepare and in vitro efficacy evaluation of sunscreen nanoemulsion containing Soybean oil, Avobenzone and Octyl methoxycinnamate.

METHODS: The sunscreen nanoemulsions were prepared by the high energy emulsification method. The formulation uses a combination of 3% Avobenzone, 7.5% Octyl methoxycinnamate, with different ratio of Soybean oil and Liquid Paraffin. The nanoemulsion was evaluated for droplet sizes by using particle size analyzer, physical stability in room temperature (25 ± 2°C during experiment for 12 weeks of storage, physical stability (cycling test), phase separation by centrifugation at 3750 rpm for 5 hours, pH, viscosity, and Sun Protection Factor (SPF) value by UV spectrophotometric. The SPF value of sunscreen nanoemulsion was compared to sunscreen nanoemulsion without Soybean Oil and sunscreen emulsion. Particle morphology observation of nanoemulsion by using Transmission Electron Microscope.

RESULTS: The sunscreen nanoemulsion formulation containing a combination of 3% Avobenzone, 7.5% Octyl methoxycinnamate with a ratio of 2.73% Soybean Oil and 0.27% Paraffin Oil resulted in the smallest average droplet size of 68.47 nm. The sunscreen nanoemulsion without Soybean Oil had an average droplet size of 384.07 nm. The globules size was increased during the experiment for 12 weeks of storage at room temperature, but there was no phase separation after centrifugation. The formulation of sunscreen emulsion, phase separation was formed after centrifugation. The nanoemulsion had a pH value of 7.23 ± 0.06 and a viscosity value of 133.33 ± 7.22 cP. The sunscreen nanoemulsion containing a combination of 3% Avobenzone, 7.5% Octyl methoxycinnamate 2.73%, Soybean Oil, 2.73% and 0.27% Liquid Paraffin had SPF value (21.57 ± 1.21) higher than sunscreen nanoemulsion without Soybean Oil (16.52 ± 0.98) and sunscreen emulsion (15.10 ± 0.22). The TEM analysis of globules morphology showed that the sunscreen nanoemulsion formed a spherical globule.

CONCLUSION: The sunscreen nanoemulsion containing a combination of 3% Avobenzone, 7.5% Octyl Methoxycinnamate, 2.73% Soybean Oil and 0.27% Liquid Paraffin showed synergistic sunscreen efficacy on SPF. This sunscreen nanoemulsion is more stable than sunscreen emulsion formulation during the experiment for 12 weeks at room temperature.

Introduction

The excessive exposure of human skin to Ultra Violet Radiation (UVR) may cause sunburn, erythema, photoaging, and increase the risk of skin cancer. UVR causes DNA damage and genetic mutations, which subsequently lead to skin cancer. The regular use of sunscreens protects the skin from the harmful effects of UV radiation, particularly the UVB (290-320 nm) and UVA (320-400 nm). UVC (200-290 nm) radiation is filtered by the atmosphere before it reaches the earth. UVB induces photoaging and mutagenic damage to nucleic acids. UVA promotes ROS (Reactive Oxygen Species) accumulation. ROS also induce direct cell damage, carcinogenesis and contribute to photoaging [1].

UV filters (“sunscreens”) are designed to

protect the skin from the harmful effects of solar radiation, particularly the UVB (290-320 nm) and UVA (320-400 nm). UVB induces photoaging and mutagenic damage to nucleic acids. UVA promotes ROS (Reactive Oxygen Species) accumulation. ROS also induce direct cell damage, carcinogenesis and contribute to photoaging [2].

Photoprotection involves both primary protective factors (sunscreens) and secondary factors (e.f., Antioxidants, osmolytes, and DNA repair enzymes) that can disrupt the photochemical cascade triggered by UV-penetration, thereby limiting skin damage. The sunscreens should provide broad-spectrum UV protection for the presence of active ingredients, which attenuate the transmission of UV radiation onto the skin by absorbing, reflecting or scattering the incident radiation [3], [4].

Nowadays, there is an increasing interest in reducing the use of synthetic UV-filters by incorporating in natural sunscreen compounds that exhibit a similar filtering activity and possess radical scavenger properties, providing broad-spectrum sunscreen product with antioxidant properties [5], [6].

The efficacy of sunscreen products usually measured in the form of sun protection factor (SPF), which can be evaluated by *in vitro* or *in vivo* techniques. UVB protection is measured by a product's SPF, which theoretically indicates that products with high SPFs provide more protection against the hazardous effects of sunlight than those with low SPFs.

The UV blocking from most natural oils is insufficient to obtain significant UV protection [7]. The strategies for preparations of sunscreen products with high SPF are nanotechnology formulations [8] and UV filter combinations [9], [10]. In this study, soybean oil was used as a natural UV filter, avobenzone and OMC as synthetic UV filters.

Soybean oil is a vegetable oil extracted from the seeds of the soybean. It is a natural sunscreens oil with UV B filter effect by absorbing them and antioxidant effect to reduce the photooxidative damage that results from UV-induced ROS production. Soybean oil contains (71.3 ± 6.4) mg/kg alpha-tocopherol and (273.3 ± 11.1 mg/kg gamma-tocopherol [11]. The results of the analysis of Indonesian Oil Palm Research Institute showed that soybean oil used in this study contains polyunsaturated fatty acids (46.4% linoleic acid) and 554 ppm Vitamine E. Vitamin E absorbs strongly in the UV-B region (280-320 NM) [12].

Experiments in vivo showed that soybean-germ oil (SGO) possesses a remarkable protective activity against UVB-induced skin inflammation, probably due to its radical-scavenging components, mainly tocopherols and polyunsaturated fatty acids [13].

Avobenzone is among the most common UV filters present on the market, due to the broad absorption spectrum in the UVA region. It is insoluble in water but freely soluble in organic solvent and oil. However, it suffers photo-degradation, giving rise to new compounds responsible for photoallergic and phototoxic reactions. This UVA filter is commonly used in concentrations between 3.0 to 10.0%. Therefore, the concentration of Avobenzone used in this study was 3%, and paraffin liquid was used for ensuring the Photostability of Avobenzone [14], [15]. Octyl methoxycinnamate (OMC) is one of the most commonly used UVB filters in sunscreen products, due to its high absorption capacity in the short wavelength region (290–320 NM). The approved concentration of OMC is 7.5-8.5% The concentration of OMC used in this study was 7.5% [16].

Nanoemulsion is very attractive to be applied in cosmetics (sunscreen products) because nanoemulsion has droplet size (20-500 NM) smaller than conventional emulsion (0.1-100 µm), so it is more stable, can prevent creaming, sedimentation or coalescence, besides also increase the solubility of an insoluble active ingredient in water [17]. Nanoemulsion has low viscosity, and transparent visual aspect, and a high surface area allows effective delivery of the active ingredient for the skin, thereby increasing the efficacy (SPF value) of the sunscreen product [18], [19].

Thus, the aims of this study were to investigate effects of nanoemulsion formulations on SPF values of sunscreen nanoemulsions containing the blends of herbal oil (soybean oil) and organic UV filters (Avobenzone and OMC) by *in vitro* (spectrophotometric) method and to verify the synergistic efficacy by a combination of the UV filters.

Material and Methods

The sunscreen nanoemulsion and emulsion was formulated using Soybean Oil (CV. Surya Agung, Jakarta), Avobenzone, Octyl methoxycinnamate (India), Tween 80, Ethanol and water demineralised were purchased from PT. Bratachem, Butylated hydroxytoluene, Liquid paraffin, Methylparaben, Propylparaben, Propylene glycol, Sodium CMC, Span 80 and Glycerin were purchased from CV. Rudang Jaya Medan Indonesia.

Nanoemulsion was prepared by using variations of Soybean oil and paraffin Liquid. Tween 80 as a surfactant and ethanol as a co-surfactant were used in the preparation of nanoemulsion. Oil phase consists of Avobenzone, Soybean oil, Paraffin Liquid, Butylated hydroxytoluene, Octyl methoxycinnamate (OMC) and Propylparaben, while the water phase was prepared by dissolving Methylparaben in hot water demineralised. This

solution was then cooled down and added with Tween 80. This water phase then stirred with a magnetic stirrer for 30 minutes. Nanoemulsion was obtained by adding oil phase into the water phase, then homogenised with magnetic stirrer HI 190 M (Hanna Instruments) at 3500 rpm for 6 hours and sonicated using sonicator (Branson) for 1 hour to obtain a transparent, yellowish colour nanoemulsion.

Emulsion system consists of an oil phase and water phase. The mixture of Avobenzone, OMC, Soybean oil, Liquid paraffin, Propylparaben, Butylated hydroxytoluene and Span 80 were heated in a water bath at 70°C (Oil phase). The mixture of Methylparaben, Tween 80, propylene glycol and Glycerin were heated in the water bath at 70°C (water phase). The water phase was then added to the sodium Carboxy Methylcellulose (CMC) and was stirred quickly to avoid the formation of air bubble. The oil phase was then added to the mixture, then stirred until an emulsion was produced.

The nanoemulsion and emulsion were stored in a room temperature (25 ± 2°C) for 12 weeks and evaluated the physical stability, including consistency, odour, colour and phase separation.

Nanoemulsions globule size was determined by using particle size *analyser* (Analysette 22 Nanotec Fritsch) at room temperature for 0, 6, 12 weeks. Observation of phase separation of nanoemulsions and the emulsion was done by using a centrifuge (Hitachi CF 16 R X II) at 3750 rpm for 5 hours.

The pH of the nanoemulsions was determined by using a pH meter (Hanna) and viscosity by using viscometer Brookfield DV-E with specific spindle (spindle 62) after the nanoemulsions were storage for 0, 4, 8, and 12 weeks at room temperature.

The Evaluation of nanoemulsions stability at low and high temperatures (cycling test) was done by storing it's at low temperature (4 ± 2°C) in refrigerator for 24 hours, then directly stored in high temperature (40 ± 2°C) in climatic chamber for another 24 hours (1 cycle). This test was done with 6 cycle repetition.

Evaluation of sunscreen activity was performed using one gram of sunscreen nanoemulsion or emulsion diluted in ethanol 96% at a final concentration of 200 ppm analysed by UV Spectrophotometry (Shimadzu) from 290-320 nm with the interval of 5 nm and 10 nm with the interval from 320-400 nm. Calculate the average of three determinations and calculate SPF by Mansur equation [20].

The SPF determination which is the correlation between the erythemogenic effect (EE) and the radiation intensity at each wavelength (I) and is adjusted according to Eq:

$$SPF = CF \times \sum_{320}^{290} Abs \times EE \times I$$

Where: Correction factor (CF) is 10, EE is the erythemogenic effect of radiation on wavelength, I is the intensity of solar light at each wavelength, and Abs is an absorption value from the sample [21].

Results

The nanoemulsions were prepared in 6 formulas, as shown in Table 1. All the nanoemulsions were yellowish, clear and transparent (Figure1).

Table 1: Composition of sunscreen nanoemulsion containing Soybean Oil, Avobenzone and OMC

Material	Quantity of 100 mL (%)					
	F1	F2	F3	F4	F5	F6
Soybean oil	0	0.5	1.5	2.5	2.73	3
Liquid paraffin	3	2.5	1.5	0.5	0.27	0
Avobenzone	3	3	3	3	3	3
OMC	7.5	7.5	7.5	7.5	7.5	7.5
Ethanol 96%	26	26	26	26	26	26
Tween 80	34	34	34	34	34	34
Propylene glycol	5	5	5	5	5	5
Butylated hydroxytoluene	0.1	0.1	0.1	0.1	0.1	0.1
Methylparaben	0.1	0.1	0.1	0.1	0.1	0.1
Prophylparaben	0.02	0.02	0.02	0.02	0.02	0.02
Water demineralized	Up to 100 mL	Up to 100 mL	Up to 100 mL	Up to 100 mL	Up to 100 mL	Up to 100 mL

The formula for emulsion preparation was shown in Table 2. The emulsion formed was milky-white in colour and was not transparent.

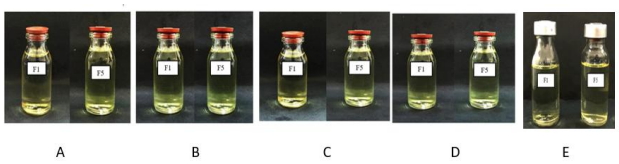

Figure 1: Appearance of the prepared sunscreen nanoemulsions F1 (without Soybean Oil) and F5 containing Soybean Oil, Avobenzone and OMC; A) Before storage; B) After storage for 4 weeks; C) After storage for 8 weeks; D) After storage for 12 weeks at room temperature; E) After cycling test

The results of physical stability evaluation of the nanoemulsions were shown in Figure 1. Nanoemulsions were stored at room temperature (25 ± 2°C) for 12 weeks.

Table 2: Formula of sunscreen emulsion containing Soybean oil, Avobenzone and OMC

Material	Quantity of 100 mL (%)
Soybean oil	2.73
Liquid paraffin	0.27
Avobenzone	3
OMC	7.5
Tween 80	3.6
Span 80	1.4
Glycerin	13
Propylene glycol	10
Butylated hydroxytoluene	0.1
Methylparaben	0.1
Prophylparaben	0.02
Sodium CMC	2
Water demineralised	Up to 100 mL

The cycling test was done by storing it's at low temperature (4 ± 2°C) in the refrigerator for 24 hours,

then at high temperature (40 ± 2°C) in the climatic chamber for another 24 hours (1 cycle). This test was done with 6 cycle repetition. The result of physical stability evaluation of emulsion was shown in Figure 2.

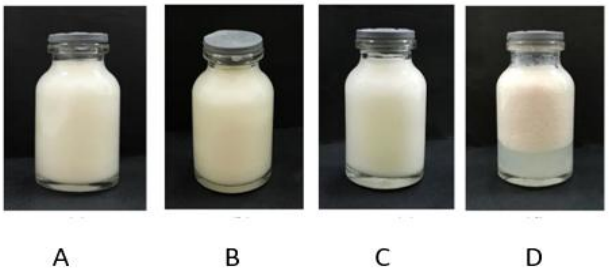

Figure 2: Appearance of the prepared sunscreen emulsion containing Soybean oil, Avobenzone and OMC; A) Before storage; B) After storage for 4 weeks; C) After storage for 8 weeks; E) After storage for 12 weeks) at room temperature

The results of centrifugation test showed that all the nanoemulsion were stable, there is no discolouration and phase separation or creaming after centrifugation, but the emulsion was not stable, colour changes occur and the formation of phase separation (Figure 3).

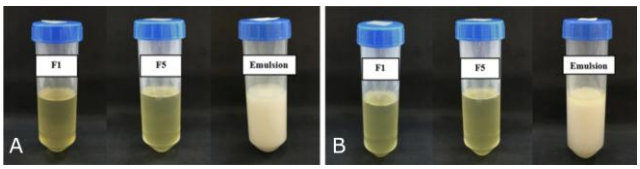

Figure 3: Appearance of the prepared sunscreen nanoemulsion without Soybean oil (F1) and containing Soybean oil (F5); and emulsion containing Soybean oil; A) Before; B) After centrifugation

Nanoemulsion (F5) has the smallest average globule size (Table 3).

Table 3: Average globule size of sunscreen nanoemulsions and emulsion

Formula	The ratio of Soybean Oil and Liquid Paraffin		Average Globule Size (nm)
	Soybean Oil	Liquid Paraffin	
F1	0	3	384.07
F2	0.5	2.5	17100
F3	1.5	1.5	14310
F4	2.5	0.5	538.90
F5	2.73	0.37	68.47
F6	3	0	560.18

Globule size of nanoemulsion and emulsion were increased after storage at room temperature (Table 4).

Table 4: Average globule size of sunscreen nanoemulsion and emulsion containing Soybean oil (F5) during storage for 12 weeks at room temperature

Formula	Time (week)	Average Globule Size (nm)
Nanoemulsion (F5)	0	68.47
	4	404.09
	8	619.82
	12	863.36
Emulsion	0	1294.96
	4	2869.46
	8	4727.27
	12	7417.51

There was a decrease in pH and increase in viscosity from sunscreen nanoemulsion formulation after storage at room temperature for 12 weeks, but viscosity was decreased after storage at high temperature for 12 weeks (Table 5).

Table 5: pH of sunscreen nanoemulsion containing Soybean oil (F5) during storage for 12 weeks at room and high temperature

Formula	Time (week)	pH ± SD		Viscosity ± SD	
		25 ± 2°C	40 ± 2°C	25 ± 2°C	40 ± 2°C
Nanoemulsion (F5)	0	7.23 ± 0.06	7.23 ± 0.06	133.33 ± 7.22	133.33 ± 7.22
	4	6.90 ± 0.20	6.63 ± 0.06	162.50 ± 12.50	120.83 ± 7.22
	8	6.63 ± 0.06	6.30 ± 0.00	245.83 ± 7.22	104.17 ± 7.22
	12	6.20 ± 0.00	5.93 ± 0.06	383.33 ± 14.43	75.00 ± 0.00

N = 3.

The pH and viscosity of sunscreen emulsion formulation were decreased during storage for 12 weeks at room temperature (Table 6).

Table 6: pH and viscosity of sunscreen emulsion containing Soybean oil during storage for 12 weeks at room temperature

Formula	Time (week)	pH ± SD	Viscosity± SD
Sunscreen Emulsion	0	6.63 ± 0.06	8100.00 ± 0.00
	4	6.43 ± 0.10	6933.33 ± 28.87
	8	6.30 ± 0.00	5800.00 ± 0.00
	12	5.93 ± 0.06	4400.00 ± 0.00

N = 3.

Shape and size of nanoemulsion were investigated using TEM (JEOL JEM 1400). This evaluation was performed on nanoemulsion (F5) with the smallest particle size among all nanoemulsion formulations. Figure 4 shows that sunscreen nanoemulsion containing Soybean oil, Avobenzone and OMC has a spherical morphology.

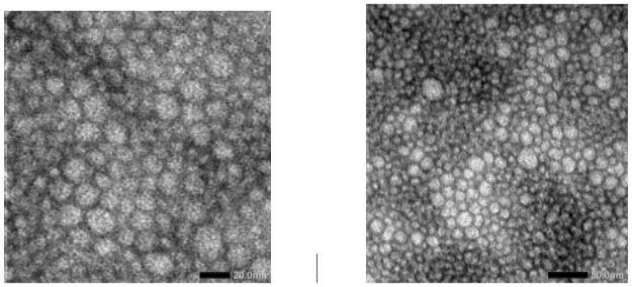

Figure 4: TEM Images of sunscreen nanoemulsion containing Soybean oil, Avobenzone and Octyl methoxycinnamate

The results of determination of the SPF value are shown in Table 7. The results showed that the SPF value of sunscreen nanoemulsion containing Soybean oil, Avobenzone and OMC is higher than the emulsion.

Table 7: SPF value of sunscreen nanoemulsions (F5) and emulsion

Formula	Sun Protection Factor (SPF) Value						Average SPF Value
	I	II	III	IV	V	VI	
Nanoemulsion F5	21.15	20.56	20.64	23.82	21.98	21.32	21.57 ± 1.21
Emulsion	14.81	14.96	14.98	15.33	15.15	15.37	15.10 ± 0.22

N = 6.

Discussion

The sunscreen nanoemulsion formulation (F5) containing a combination of 3% Avobenzone, 7.5% Octyl Methoxycinnamate with a ratio of 2.73% Soybean Oil and 0.27% Liquid Paraffin showed the smallest average droplet size of 68.47 nm. This formulation was selected for stability and sunscreen activity evaluation. The sunscreen nanoemulsion without Soybean Oil had an average droplet size of 384.07 nm. This is because avobenzone is more soluble in soybean oil. However, the optimum formulation is obtained from a combination of 2.73% soybean oil and liquid paraffin 0.275 as the oil phase compared to formulations using only soybean oil or liquid paraffin. Selection of an appropriate oil phase is very important, mainly in case of O / W nanoemulsions. Usually, the oil which has the maximum solubilising potential for active substance is selected as an oily phase for the formulation of nanoemulsions. This helps to achieve maximum active substance loading in the nanoemulsions [22]. The droplet size of nanoemulsions and emulsion were increased during 12 weeks of storage at room temperature, but the nanoemulsion formulation (F5) still in the nano-size range.

The sunscreen emulsion containing a combination of 3% Avobenzone, 7.5% Octyl Methoxycinnamate had an average droplet size of 1294.96 nm greater than sunscreen nanoemulsion formulation. The formulation of nanoemulsion was prepared based on high energy emulsification method, in which mechanical energy input is applied by High-Shear Stirring (magnetic stirrer 3500 rpm) and sonication. Thus, droplet sizes of the internal phase can be significantly decreased [23].

The sunscreen nanoemulsion was stable during the experiment for 12 weeks of storage at room temperature (25 ± 2°C), and high temperature (40 ± 2°C). There was no discolouration, changes in consistency, odour and phase separation during the experiment for 12 weeks storage at a variation temperature in the nanoemulsion, but the emulsion showed discolouration and phase separation (not stable) for 12 weeks storage at room temperature.

Centrifugation test was performed to determine the stability of nanoemulsion. The centrifugation test describes the stability of one year of storage. All of the nanoemulsions were stable with no phase separation or creaming after centrifugation at 3750 rpm for 5 hours. However, the emulsion was not stable with the formation of phase separation.

The results of pH evaluation all nanoemulsions show that there were decreases in pH value and the pH value of formula F5 is close to the neutral pH of human skin normally ranges from 4.5 to 6.0 [24]. The viscosity of all nanoemulsions was increased during storage for 12 weeks at room temperature, but the decrease in high temperature. The viscosity of emulsion was decreased during storage for 12 weeks at room temperature; this is caused by the occurrence of phase separation caused by damage to the interface layer.

The SPF value of sunscreen nanoemulsion formulation (F5) containing a combination of 3% Avobenzone, 7.5% Octyl Methoxycinnamate, 2.73% Soybean Oil and 0.27% Liquid Paraffin was higher than sunscreen nanoemulsion formulation without Soybean Oil (F1) and emulsion preparation. This is because Soybean Oil has properties that can absorb UVB rays. Nanoemulsion technologies, which is being applied to enhance the solubility of lipophilic substance (Avobenzone) and had a smaller globule size and also is more stable throughout the stability experiment. So, they absorb more ultraviolet light which results in higher SPF values.

The sunscreen nanoemulsion containing a combination of 3% Avobenzone, 7.5% Octyl Methoxycinnamate, 2.73% Soybean Oil and 0.27% Liquid Paraffin showed synergistic efficacy sunscreen on SPF and more stable compare with sunscreen emulsion formulation.

The in vitro photoprotective efficacy assessment demonstrated that there was a synergism between the formulation with the combination of 2,73% Soybean Oil, 3% Avobenzone, and 7.5% Octyl methoxycinnamate. The sunscreen nanoemulsion more stable than sunscreen emulsion during the experiment for 12 weeks at room temperature. The SPF value of this nanoemulsion more higher than nanoemulsion without Soybean oil and emulsion preparation.

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Basic Science

Optimisation of Cobalt Oxide Nanoparticles Synthesis as Bactericidal Agents

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Abstract

AIM: With the increased bacterial resistance and the resulting problems in recent years, it seems necessary to find new biocompatible compounds to confront this problem. This research was conducted to optimise the synthesis of cobalt oxide nanoparticles with the highest antibacterial activity.

METHODS: In the present study, 9 experiments were designed using the Taguchi method. The effect of three factors of cobalt nitrate, KOH and the stirring time in the synthesis of cobalt oxide nanoparticles with the highest antibacterial activity was investigated. The bactericidal effect of synthesised nanoparticles was evaluated using the colony-forming unit (CFU) and disk diffusion methods. The characteristics of nanoparticles were studied using the Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and the scanning electron microscopy (SEM).

RESULTS: The results indicated that all three evaluated factors were effective on the antibacterial properties of the synthesised nanoparticles. The best antibacterial activity of cobalt oxide nanoparticles was observed in experiment 9 (cobalt nitrate 0.6 M, KOH 2M and stirring time 60 min). The study of nanoparticles synthesised by FTIR, XRD, and SEM confirmed the formation of cobalt oxide nanoparticles with size (24 nm) and a proper structure (spinel structure).

CONCLUSION: Due to the optimal antibacterial properties of the synthesised cobalt oxide nanoparticles, they can be used in the fabrication of dental and medical equipment with antibacterial properties.

Introduction

Despite significant advances in the treatment of diseases in recent decades, no proper cure has been still found for some diseases such as cancer [1], [2], cardiovascular diseases [3], chronic pains [4], diabetes [5], autoimmune diseases [6], [7], AIDS [8], [9], and microbial infections [10], [11]. Some common microbial infections with bacterial origin are strongly annoying. Since the discovery of bacteria, the researchers have always been looking for effective ingredients against them. The widespread introduction of antibiotics was made early in the last century. Since then, the bacterial resistance has grown significantly to the available antibiotics [12]. Over the years, bacteria have achieved effective mechanisms to cope with antibiotics through chromosomal mutations and genetic exchanges [13]. *Escherichia coli* and *Staphylococcus aureus* cause some of the most common infections in a variety of situations in society and hospitals. According to a report by the World Health Organization, the bacteria of *Escherichia coli* and *Staphylococcus aureus* have shown resistance to some common antibiotics in more than 50% of cases [14]. The development of resistance to antibiotics has limited their service life. Finding an antimicrobial agent is not an easy task due to poor penetration of the

compounds into the bacterial cells [15]. The traditional organic compounds used for the disinfection of toxic have side effects such as toxicity for humans and sensitivity to high temperatures and high pressures.

In contrast, inorganic compounds are nontoxic, exhibit high antibacterial properties at low concentrations and are resistant to unfavourable and unbalanced conditions [16]. Due to the increased resistance of bacteria to antibiotics and their adaptability characteristics, finding new solutions to eliminate bacteria has become a priority nowadays. Over the past few decades, the use of nanotechnology and the synthesis and production of nanoparticles have brought new hopes for overcoming the antibacterial resistance.

Cobalt oxide is one of the transition metal oxides, which is in the form of black powder with antimicrobial and magnetic properties. The magnetic nanoparticles are particles with an independent nature and a maximum dimension of 100 nm, which have magnetic properties. Due to its semistable three phases with various crystalline structures, cobalt is one of the most important magnetic metals. The crystalline cobalt oxide structures include the Hexagonal Closed Packed (HCP) phase, Face-Centered Cubic (FCC) phase, and the Epsilon phase [17]. Due to the high capacity for use in various areas, magnetic nanoparticles of iron, cobalt, and nickel have drawn a lot of attention. The cobalt oxide nanoparticles, according to their properties, are used in various fields such as the synthesis of sensors, magnetic materials, electrochemical systems, smart absorbers, catalysts and in the medical area. Considering the use of cobalt oxide nanoparticles in different fields, optimising their synthesis seems to be important in terms of their application. The structures, sizes, morphologies and surface properties of nanoparticles can be improved by controlling the effective factors in the synthesis process [18], [19]. Therefore, changing and targeted determining the properties of nanoparticles can be considered according to their function and activity. Accordingly, this research was designed to optimise the synthesis of cobalt oxide nanoparticles as an antibacterial agent by using the co-precipitation method and evaluate its properties by employing Infrared Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and scanning electron microscopy (SEM) devices.

Material and Methods

Synthesis of cobalt oxide nanoparticles

The cobalt oxide nanoparticles were synthesised using the coprecipitation method. The Qualitek-4 software and Taguchi method were used to optimise the synthesis of cobalt oxide nanoparticles with the highest antibacterial activity. To do so, 9 experiments were designed and the effect of 0.2, 0.4 and 0.6 M levels of $Co(NO_3)_2.6H_2O$ and the levels of 1, 2 and 3 M of KOH at the stirring times of 30, 60, and 90 min were evaluated on the antibacterial activity of the synthesized nanoparticles (Table 1).

Table 1: Taguchi design of experiments and antibacterial effect of manufactured cobalt oxide nanoparticles on gram-positive and gram-negative bacteria

Experiment	Cobalt nitrate (M)			KOH (M)			Stirring time (min)			Bacterial survival (Log_{10} CFU/ml)	
	0.2	0.4	0.6	1	2	3	30	60	90	Gram-positive	Gram-negative
1	0.2			1			30			6.42	6.76
2	0.2			2			60			5.84	6.13
3	0.2			3			90			5.10	5.32
4	0.4			1			60			5.32	5.51
5	0.4			2			90			5.60	5.71
6	0.4			3			30			4.94	5.06
7	0.6			1			90			4.40	4.75
8	0.6			2			30			4.76	4.89
9	0.6			3			60			3.72	3.97

A volume of 100 ml of KOH solution at concentrations of 1, 2 and 3 M and cobalt nitrates with the concentrations of 0.2, 0.4, and 0.6 M were prepared in separate containers. Then, according to the nine tests suggested by the Taguchi method, the solutions of KOH at different concentrations were added drop-by-drop to the containers containing cobalt nitrate solution while continuously stirring, which were mixed and combined at 30, 60 and 90 min, respectively. Initially, pink sediment was formed, which was easily oxidised in contact with the air, and dark sediment was obtained. The dark sediment was separated by centrifugation and washed out with deionised water several times. It was then dried in an oven at 100°C for 24 h. The cobalt hydroxide powder was calcined in air at 400°C furnace for four hours to obtain the cobalt oxide nanoparticles.

Antibacterial activity

The antibacterial activity level of synthesised cobalt oxide nanoparticles according to 9 experiments suggested by Taguchi method against *Staphylococcus aureus* and *Escherichia coli* bacteria was studied using the colony-forming unit method. Bacterial suspensions were made from both bacteria with an approximate concentration of 10^8 CFU/ml, which were shaken along with the synthesised nanoparticles for 6 h. The solutions containing bacterial suspensions and nanoparticles were incubated for 24 h at 37°C on a nutrient agar culture medium. After incubation, the colonies growth rate was calculated. Then, cobalt oxide nanoparticles were produced using the suggested conditions by Taguchi method, and their bactericidal effect was surveyed using CFU and disk diffusion methods. CFU method was done similar before and for disk diffusion test homogenous suspensions of *Staphylococcus aureus* and *Escherichia coli* bacteria were cultured by a swab on a nutrient agar medium. The discs containing cobalt oxide nanoparticles and Gentamycin (positive

control) were then placed on the media and incubated for 24 h at 37°C. Afterwards, the diameter of the zone of inhibition was measured for each disk [10].

Characteristics

After determining the optimal conditions based on the Taguchi method, the nanoparticles of cobalt oxide were synthesised under the proposed optimum conditions (cobalt nitrate 0.6 M, KOH 3M and stirring time 60 min) and their characteristics were evaluated. The FTIR spectrum of cobalt oxide nanoparticles was provided by the alpha spectrometer (Bruker, Germany) after the preparation of KBr tablets from the specimens. The X-ray diffraction test was performed by Philips X 'Pert (40 kV, 30 mA) to analyse and evaluate the crystalline structure formed in the synthesised nanoparticles. The microscopic images were taken of the cobalt oxide nanoparticles by a high-resolution scanning electron microscopy (TESCAN, Czech Republic) to examine the morphology and determine the size of the synthesised nanoparticles.

Statistical analysis

The data were analysed using Qualitek-4 software (Nutek Inc., MI, USA). All tests were carried out three times with three replicated, and their averaging results were reported.

Results

One of the most important features of metal nanoparticles received considerable attention in recent years is their use as antibacterial compounds. Aimed at this, we used the Taguchi method to assess the effect of three factors of cobalt nitrate, KOH, and stirring time on the antibacterial activity of cobalt oxide nanoparticles (Table 1). The results indicated that the synthesised nanoparticles in experiment 9 (cobalt nitrate 0.6 M, KOH 2M, and stirring time of 60 min) had the highest antibacterial activity against gram-positive bacteria (3.72) and gram-negative bacteria (3.97).

The effect of each factor on the antibacterial properties of the synthesised cobalt oxide nanoparticles is displayed in Table 2.

Table 2: The main effects of factors of cobalt nitrate, KOH and stirring times at different levels on the bacterial survival

Factors	Gram positive bacteria (*S. aureus*)			Gram-negative bacteria (*E. coli*)		
	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3
Cobalt nitrate	5.79	5.29	4.29	6.07	5.43	4.54
KOH	5.38	5.40	4.59	5.67	5.58	4.78
Stirring time	5.37	4.96	5.03	5.57	5.20	5.26

Cobalt nitrate, KOH, and the stirring time showed the highest performance in reducing the growth of *Staphylococcus aureus* in the third level (4.29), in the third level (4.59), and the second level (4.96), respectively. The studied factors had similar effects on the *Escherichia coli* as well. The optimal performance of cobalt nitrate (4.54) and KOH (4.78) was seen at the third level, while the stirring time (5.20) showed its bests at the second level.

Table 3 shows the interaction effect between the concentrations of the cobalt salt, KOH and the stirring time. The interaction effect rate of the studied factors varied from 15.92 to 40.37 in the gram-positive bacteria and showed changes from 14.87 to 44.62 on the gram-negative bacteria. The highest interaction effect rate was seen in the case of KOH × Stirring time, while the lowest interaction rate was related to cobalt nitrate × KOH on both gram-positive and gram-negative bacteria.

Table 3: The interactions among studied factors on the survival rate of bacteria

	Interacting factor pairs	Column	Severity Index (%)	Optimum conditions
Gram-positive bacteria (*S. aureus*)	KOH × Stirring time	2×3	40.37	[3,2]
	Cobalt nitrate × Stirring time	1×3	17.77	[3,2]
	Cobalt nitrate × KOH	1×2	15.92	[3,3]
Gram-negative bacteria (*E. coli*)	KOH × Stirring time	2×3	44.62	[3,2]
	Cobalt nitrate × Stirring time	1×3	19.35	[3,2]
	Cobalt nitrate × KOH	1×2	14.87	[3,3]

The analysis of variance of the examined factors (cobalt nitrate, KOH, and stirring time) is presented in Table 4. According to the results, the factors of cobalt nitrate, KOH and the stirring time respectively indicated the optimal effect on the synthesis of nanoparticles with the highest antibacterial activity against gram-positive and gram-negative bacteria.

Table 4: The ANOVA test for studied factors on reducing the growth of bacteria

Type of bacteria	Factors	DOF	Sum of Squares	Variance	F-Ratio (F)	Pure Sum	Percent (%)
Gram positive bacteria (*S. aureus*)	Cobalt nitrate	2	3.47	1.73	49.74	3.40	66.35
	KOH	2	1.29	0.64	18.53	1.22	23.86
	Stirring time	2	0.29	0.14	4.19	0.22	4.34
Gram negative bacteria (*E. coli*)	Cobalt nitrate	2	3.56	1.78	39.18	3.47	65.25
	KOH	2	1.43	0.71	15.76	1.34	25.22
	Stirring time	2	0.23	0.12	2.57	0.14	2.69

DOF, degree of freedom.

The optimum conditions for the synthesis of cobalt oxide nanoparticles with the most favourable antibacterial activity are reported in Table 5. The results suggested that the use of cobalt nitrate and KOH at the third level and the stirring time at the second level can provide the optimal antibacterial performance for the synthesis of cobalt oxide nanoparticles. The antibacterial performance rate of synthesised nanoparticles in the optimal conditions improved compared to the average performance of the synthesised nanoparticles in the 9 experiments.

Table 5: Predicted the optimal conditions for producing cobalt oxide nanoparticles with the highest antibacterial activity

Factors	Gram-positive bacteria (*S. aureus*)		Gram-negative bacteria (*E. coli*)	
	Level	Contribution	Level	Contribution
Cobalt nitrate	3	0.83	3	0.81
KOH	3	0.54	3	0.56
Stirring time	2	0.16	2	0.14
Total contribution from all factors		1.53		1.51
Current grand average of the performance		5.12		5.34
Bacterial survival at optimum condition		3.59		3.83

Table 6 shows the level of antibacterial activity of the cobalt oxide nanoparticles synthesised under the proposed optimum conditions against *Staphylococcus aureus and Escherichia coli* bacteria. Evaluating the bactericidal effect of synthesised cobalt oxide nanoparticles by CFU method demonstrated that the growth rate of gram-positive and gram-negative bacteria reduced to 3.60 and 3.78, respectively. In the disc diffusion method, inhibition zone diameters for gram-positive and gram-negative bacteria were 15 and 14.66, respectively. The slight difference was observed in the bactericidal activity of cobalt oxide nanoparticles and antibiotic (gentamicin) against *Staphylococcus aureus* and *Escherichia coli* bacteria that denoting the favourable bactericidal effect of synthesised cobalt oxide nanoparticles.

Table 6: The bactericidal activity of synthesised cobalt oxide nanoparticles in optimal conditions

Type of essay	Type of bacteria	Bactericidal activity	
		Cobalt Oxide	Gentamycin
Bacterial survival (Log_{10} CFU/ml)	Gram positive bacteria (*S. aureus*)	3.60	2.56
	Gram negative bacteria (*E. coli*)	3.78	2.84
Zone of inhibition (mm)	Gram-positive bacteria (*S. aureus*)	15	17
	Gram negative bacteria (*E. coli*)	14.66	15.66

Examining the properties of nanoparticles showed that the synthesised nanoparticles have a suitable structure and size for biological applications. Figure 1 shows the FTIR spectrum of cobalt oxide nanoparticles in the range of 400-4000 cm^{-1} wavelengths. Some peaks were found in the range of 667 cm^{-1} and 574 cm^{-1} in the FTIR spectrum of cobalt oxide nanoparticles, which represented the synthesis of cobalt oxide nanoparticles. The peaks observed in the FTIR spectrum of the synthesised nanoparticles confirm the spinel structure of the cobalt oxide nanoparticles.

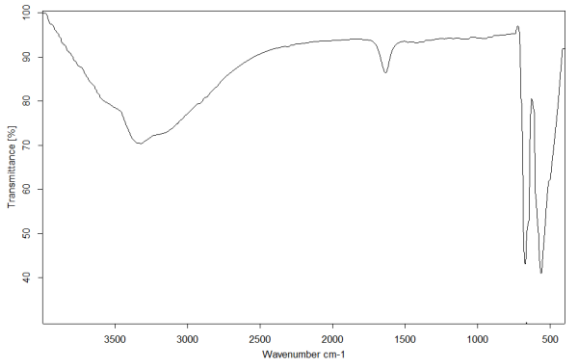

Figure 1: FTIR spectra synthesised cobalt oxide nanoparticles

The phase formation and crystallography of cobalt oxide nanoparticles were investigated using the X-ray diffraction (Figure 2). The nature of peaks in the XRD pattern of cobalt oxide nanoparticles was by the JCPDS 74-2120, suggesting the cubic structure of the synthesised nanoparticles. Considering the magnetic properties of these nanoparticles, they are suitable for use in various fields, including biological and medical applications.

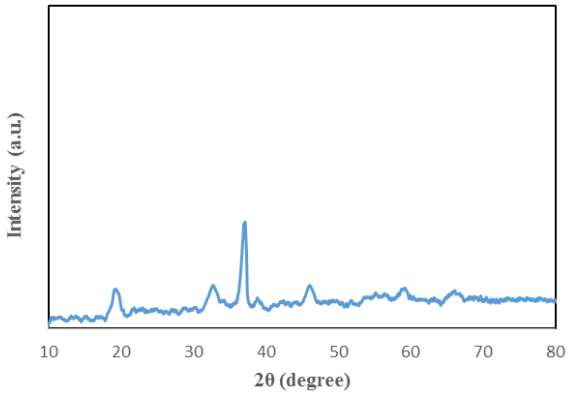

Figure 2: XRD pattern of synthesised cobalt oxide nanoparticles

The scanning electron microscopy images were used to study the structure and morphology of the synthesised cobalt oxide nanoparticles (Figure 3). The analysis of the scanning electron micrographs revealed the agglomeration of some cobalt oxide nanoparticles. According to the SEM image, the approximate average size of the synthesised nanoparticles was about 24 nm. The nanoparticles had a relatively rugged surface and a spherical shape, resulting in higher adsorption sites and their better interactions.

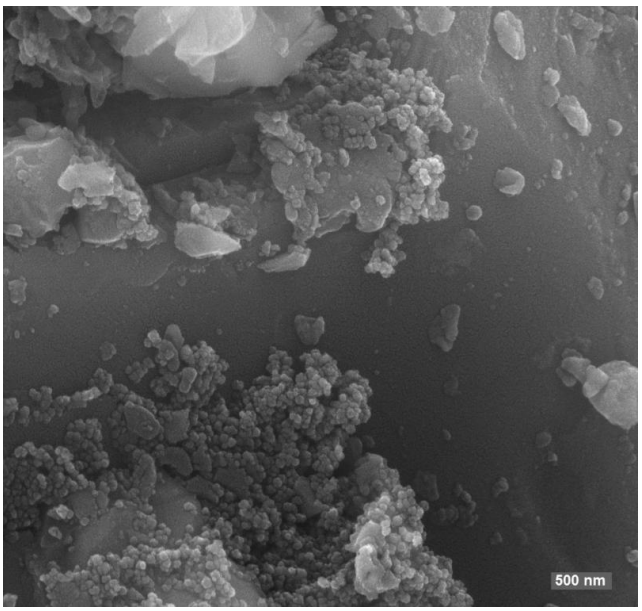

Figure 3: SEM image of synthesised cobalt oxide nanoparticles

Discussion

Regarding the use of metal oxide nanoparticles in various fields, optimising their synthesis can be important in terms of their application. Previous studies have shown that the factors effective in the synthesis of nanoparticles can affect their shape, size, and properties [10], [20].

The evaluation of the antibacterial properties of the synthesised cobalt oxide nanoparticles indicated a significant reduction in the colony formation of studied gram-positive and gram-negative bacteria. Consistent with the results of this study, previous studies have reported the antibacterial activity of cobalt oxide nanoparticles alone or in combination with other materials [21], [22], [23]. In research, Alsharaeh et al. [24] evaluated the synthesis of graphene-cobalt oxide nanocomposite and its antibacterial properties. According to them, a compound contacting cobalt oxide nanoparticles shows a desirable antibacterial effect against gram-negative bacteria. The precise mechanism of action of the cobalt oxide nanoparticles, which kills the bacteria, is still unclear and no specific mechanism has been suggested in this regard. However, due to mechanisms of action of other nanoparticles, probably processes such as the induction of oxidative stress, the release of toxic metal ions and damage to the cell membrane and interfering its activities can generate the antimicrobial properties of cobalt oxide nanoparticles [25], [26].

Due to the rapidly growing use of metal nanoparticles in the therapeutic applications, the targeted optimisation of their synthesis seems to be important regarding their application. Applying the Taguchi method, we determined the optimum conditions for the synthesis of cobalt oxide nanoparticles with the highest antibacterial activity (experiment 9). The results on antibacterial activity of factors effective in the synthesis of cobalt oxide nanoparticles showed that the cobalt nitrate and KOH at the third level and the stirring time at the second level had a greater effect on the antibacterial properties of the synthesized nanoparticles against the gram-positive bacteria of *Staphylococcus aureus* and the gram-negative bacteria of *Escherichia coli*. The results of FTIR, XRD, and SEM analyses revealed that the cobalt oxide particles were synthesised in nanoscale with a spherical form, a relatively uniform size and a high crystallisation degree. Due to the optimal antibacterial activity and proper stability of the cobalt oxide nanoparticles, they can be used as an alternative to conventional antibacterial compounds to combat the pathogenic bacteria.

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Basic Science

Protective Effects of Propolis Extract in a Rat Model of Traumatic Brain Injury via Hsp70 Induction

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Keywords: Propolis; Hsp70; Caspase 3; Apoptosis-inducing factor (AIF); Traumatic brain injury; Apoptosis

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Abstract

BACKGROUND: Traumatic brain injury (TBI) is one of the major global health problems. Secondary brain injury is a complex inflammation cascades process that causes brain cell apoptosis. Propolis is a natural product that has neuroprotective property.

AIM: This study aimed to investigate the effect of propolis toward Hsp70 expression with apoptosis marker in brain tissue after TBI.

METHODS: Thirty-three Sprague Dawley rats were randomised into three treatments group, i.e. sham-operated controls, closed head injury (CHI), and CHI with propolis extract (treatment group). In the treatment group, propolis was given 200 mg/kg per oral for 7 days then harvested brain tissues after sacrificed by cervical dislocation at day 8. We investigated Hsp70, Caspase 3, apoptosis-inducing factor (AIF), and TUNEL assay expression using immunohistochemistry staining. Statistical test using one-way ANOVA test and Tukey HSD as post hoc test.

RESULTS: Mean of positive Hsp70 stained cells in group 1 was 6.82 ± 2.14, group 2 was 3.91 ± 2.26, and group 3 was 9.64 ± 3.53 with a significant difference of Hsp70 expression distribution within groups ($p = 0.0001$). Mean of positive caspase 3 stained cells in group 1 was 5.45 ± 2.30, group 2 was 13.82 ± 2.44, and group 3 was 7.03 ± 1.54 with a significant difference of caspase3 expression distribution within groups ($p=0.0001$). Mean of positive AIF stained cells in group 1 was 5.36 ± 2.11, group 2 was 12.82 ± 1.40, and group 3 was 8.09 ± 1.81 with a significant difference of AIF expression distribution within groups ($p = 0.0001$). Mean of positive TUNEL assay stained cells in group 1 was 4.82 ± 2.04, group 2 was 11.55 ± 1.51, and group 3 was 7.64 ± 1.96 with a significant difference of TUNEL test expression distribution within groups ($p = 0.0001$).

CONCLUSION: Propolis may protect brain cell from apoptosis after injury by maintaining Hsp70 expression in addition to antioxidant and anti-inflammatory.

Introduction

Traumatic brain injury (TBI) is one of the major global health problems. Based on the WHO report, mortality and morbidity caused by TBI have increased significantly and also make an economical problem for families and societies [1]. Incidence of TBI was varied between regions, and the commonest is road traffic injuries. Estimation of worldwide TBI is 69 million (95% CI 64-74 million) lives each year from all causes with the severest burden in Western Pacific and Southeast Asian countries [2].

Brain contusion, haemorrhage, and axonal shearing are primary brain injury that occurred directly after injury and preventable. Secondary brain injury occurred later and activated complex inflammation cascades, excitotoxicity, and reactive oxygen species (ROS) that will cause apoptosis of brain cells. Apoptosis can be activated by caspase-dependent and caspase-independent pathway. Activation of the intrinsic and extrinsic pathway from caspase-dependent pathways will activate caspase 3 and trigger apoptosis process [3], [4].

Propolis was used as traditional medicine and had been investigated for its efficacy through researches. The main components of propolis are caffeic acid phenethyl ester (CAPE) that proved had antimicrobial, antioxidant, anti-inflammatory, cytotoxicity, and neuroprotective effects. Researches proved that the antioxidant effect of propolis by increased malondialdehyde (MDA) level and reduce superoxide dismutase (SOD) and glutathione peroxidase (GPx) level. CAPE from propolis also inhibits apoptosis by preventing caspase 3, nitric oxide synthase, and cytochrome productions [5], [6]. This study aimed to investigate the effect of propolis toward Hsp70 expression with apoptosis marker in brain tissue after TBI.

Material and Method

Propolis extract

Propolis collected at East Java, Indonesia was washed to clean all residual dirt. Propolis was mixed with 70% ethanol (100 gr in 500 cc water) with 50 rpm for 24 hours. Macerate filtrate was evaporated using rotary evaporator within 2 hours. Every 100 gr propolis produced 50 ccs of propolis extract.

Application of TBI

This research used 33 male Sprague Dawley rats with weight 250-400 gr and separated into three groups viz. group 1 as the negative control, group 2 as the positive control, and group 3 as the treatment group. TBI was made by using modified Feeney's weight-drop model. After general anaesthetic with ketamine HCl intramuscular (100 mg/kg body weight), mid frontal of rat' skull was exposed and dropped with 40 mg metal mass from 1.5 m height. Group 3 were given with 200 mg propolis extract/kg from oral for 7 days. At day 8, all rats were sacrificed by cervical dislocation after general anaesthesia. Brain samples were fixed with 10% formalin then contusion area of brains was processed to paraffin-embedded for immunohistochemistry (IHC) staining purpose. This research has been approved by the Health Research Ethical Committee Medical Faculty of Universitas Sumatera Utara / H. Adam Malik General Hospital.

Immunohistochemistry staining

IHC staining of proBDNF, Caspase3, Hsp70, and apoptosis-inducing factor (AIF) from paraffin-embedded of lesion cortical brain used the avidin-biotin-peroxidase complex method. 5 mm thickness paraffin-embedded sections were dewaxed, rehydrated, and microwave for 10 minutes. 3% H2O2 was used to block the activity of endogenous peroxidase then rinsed with phosphate-buffered saline (PBS). The tissue section was incubated with normal rabbit serum and incubated with a monoclonal antibody (Santa Cruz) at room temperature. These samples were rinsed with PBS and incubated with secondary antibody for 30 minutes. Tissue sections were rinsed twice with PBS and developed with 0.05% 3, 3 diamino-benzinetetrahydrochloride and slightly counterstained. Terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) method was used to analysis apoptotic DNA fragmentations from all samples. All samples were evaluated by one pathologist (blinded) and the first author (not blinded). Positive signal of IHC was found in the cytoplasm and counted with a binocular microscope with 100x magnify in 10 high power fields.

Statistical analysis

All counts were showed in mean and standard deviation. One-way ANOVA test was used to compare between groups and Tukey HSD for post hoc test. Significant Differences when $P < 0.05$.

Results

HSP70 expression

Mean of positive Hsp70 stained cells in group 1 was 6.82 ± 2.14, group 2 was 3.91 ± 2.26, and group 3 was 9.64 ± 3.53. Distribution of Hsp70 expression within groups has a significant difference ($p = 0.0001$).

Caspase 3 expression

Mean of positive caspase 3 stained cells in group 1 was 5.45 ± 2.30, group 2 was 13.82 ± 2.44, and group 3 was 7.03 ± 1.54. Distribution of caspase3 expression within groups has a significant difference ($p = 0.0001$).

Apoptosis-inducing factor (AIF) expression

Mean of positive AIF stained cells in group 1 was 5.36 ± 2.11, group 2 was 12.82 ± 1.40, and group 3 was 8.09 ± 1.81. Distribution of AIF expression within groups has a significant difference ($p = 0.0001$).

TUNEL assay expression

Mean of positive TUNEL assay stained cells in group 1 was 4.82 ± 2.04, group 2 was 11.55 ± 1.51, and group 3 was 7.64 ± 1.96. Distribution of TUNEL test expression within groups has a significant

difference (p = 0.0001).

Table 1: All markers (Hsp70, caspase 3, AIF, and TUNEL assay) expression in A) Negative control group; B) Positive control group; C) Propolis treatment group

Marker	Groups	N	Mean	Standard deviation	P
Hsp70	Negative control	11	6.82	2.136	0.0001
	Positive control	11	3.91	2.26	
	Propolis treatment	11	9.64	3.53	
Caspase 3	Negative control	11	5.45	2.30	0.0001
	Positive control	11	13.82	2.44	
	Propolis treatment	11	7.82	1.54	
Apoptosis inducing factor (AIF)	Negative control	11	5.45	2.30	0.0001
	Positive control	11	13.82	2.44	
	Propolis treatment	11	7.03	1.54	
TUNEL assay	Negative control	11	4.82	2.04	0.0001
	Positive control	11	11.55	1.51	
	Propolis treatment	11	7.64	1.96	

Discussion

Propolis composes of flavonoids, phenolic acid, steroid, essential oils. However, CAPE is the major component for antioxidant and anti-inflammatory effect. Secondary brain injury can be prevented based on these properties [6]. Spinal cord injury experiment using rat model with the administration of 200 mg/kg intraperitoneally reduce caspase-3 and cathepsin B expression compared to trauma group significantly [7]. Other animal experiment using rats that kept at 40°C for 12 hours to give heat stress then administrated propolis extract orally as a supplement showed low caspase-3 and malondialdehyde (MDA) expression with high glutathione (GSH) expression close to control group [8].

Our study showed that group 3 with propolis administration was significantly lower caspase-3 expressions than group 2 although not as low as group 1 and for AIF expression, group 3 was significantly lower than group 2 and as low as group 1. So propolis extracts had been proven to inhibit caspase-dependent and independent pathways. This study showed propolis could reduce AIF level in the treatment group similar to the negative control group compare to caspase level, so propolis is more prominently in inhibiting independent pathway. All these processes will decrease apoptotic events downstream and finally reduce the apoptosis in the brain cells. Apoptosis is programmed cell death that caused by several conditions, including brain injury. Apoptosis is the final result of caspase-dependent and independent pathways process including nuclear condensation, cell body shrinkage and fragmentation of DNA. The typical morphological phenotype of apoptotic cells is the reduction of cell volume. TUNEL assay can detect apoptotic cells that undergo vast DNA degradation during the late phase of apoptosis.[9] Traumatic brain injury experiment using rat model with 200 mg/kg propolis extract every day were significantly increased Bcl-2 level but decrease TNF-α, apoptosis, and necrosis expression [10], [11]. Our study also showed that propolis could reduce positive TUNEL assay level significantly in group 3 compared with group 2 and has a positive TUNEL assay level as low as group 1. This TUNEL assay proved that propolis could reduce apoptosis after traumatic brain injury.

Hsp70 has been proved to have protection properties in brain injury and cerebral schema. Hsp70 can prevent the process of caspase-dependent and independent pathways by inhibiting the release of cytochrome c, Apaf-1, and caspase3. Neuroprotective properties of Hsp70 by decreasing apoptotic events downstream [12], [13]. Animal experiment with turkey pout that administrated herbicide intravenously then fed with propolis showed normalised Hsp70 expression to a similar level of the control group [14]. Our experiment showed that group 3 had Hsp70 level significantly higher than group 2 but no significant different than group 1. This means propolis can normalise the Hsp70 level similar to normal condition or negative control group. Propolis treatment group in our study showed lower caspase-3, AIF, and TUNEL assay expression with higher Hsp70 expression than group 2 that significant different ($p < 0.05$). Hsp70 expression between group 1 and group 3 was not different significantly which mean propolis has the ability to increase Hsp70 level in the treatment group similar to the control group without trauma. We suggested that propolis can preserve Hsp70 expression in the injury area. Based on this, we proposed that propolis's neuroprotective properties also from Hsp70 induction, besides from anti-inflammatory and antioxidant properties.

In conclusion, apoptotic markers (caspase 3, AIF, and TUNEL assay) reduced significantly by propolis treatment compare to injury groups ($p < 0.05$). Propolis also can preserve Hsp70 expression similar to the control group. So it was shown that propolis has a potential effect in protecting brain cells from injury by preserving Hsp70 expression in addition to anti-inflammation and antioxidant properties. However, further clinical researches are needed for clinical use of propolis.

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Clinical Science

The Interaction of Social, Physical and Nutritive Factors in Triggering Early Developmental Language Delay in a Sample of Egyptian Children

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Abstract

BACKGROUND: Language acquisition and child development during the early years of life depend on multiple interacting factors.

AIM: To explore potential factors that can impact language development in 2 groups of Egyptian children, one with normal language development and the second with delayed development. Also, to explore to what extent can the involvement of impaired motor development potentiate the risk of developmental language delay.

METHODS: This cross-sectional case-control study involved Egyptian children belonging to the middle socioeconomic class between 18 and 36 months of age. Children were classified according to their performance on language domain of Bayley Scales of Infant and Toddler Development (Bayley-III) into two groups, infants with the average or above score (control group) and those having below-average scores (cases). Motor development was assessed on the same scale. Factors affecting language development were tested, including socio-demographic, obstetric, and maternal medical factors in addition to Infant Feeding Practices.

RESULTS: The independent factors lowering the language scores were early introduction of complementary food, low family income, history of delivery problems, pregnancy-related diseases of the mother, and maternal education. Impaired motor development appears as a further highly significant risk factor to the previously mentioned factors

CONCLUSION: In Egyptian children, delayed language development is severely affected by the interaction of medical, social and nutritional factors. Providing adequate maternal health care during pregnancy and childbirth, regular developmental monitoring at each child visit, and screening for such risk factors, can reduce size of the problem and promote child's social and psychological development.

Introduction

Language development is an essential part of a child's overall development. It builds the child's ability to communicate, express and perceive feelings. It also promotes thinking, problem-solving, and enhancing and maintaining relationships [1]. A child's early years of language development are crucial for the basis of school readiness, such as literacy skills, social and psychological growth [2].

Children develop receptive and expressive language skills at varying rates. The term Developmental language delay (DLD) or late talker is used to identify children aged 18-36 months who fail to attain the least expressive vocabulary milestones expected for their age and sex [3].

The prevalence of communication disorders in the international literature varies according to the age of affected children and methods of assessment. It was found that 61% of 24-months old children display expressive speech disorders [4], [5]. Developmental language disorder (DLD) comprising the largest

disability group in pre-school-aged children. Approximately 7% of the population is expected to have DLD [6]. Egyptian studies concerned with the prevalence of communication disorders are infrequent [7], [8]. Gharib et al. recorded that prevalence of confirmed delayed language development (DLD) in the Egyptian sample was 6.4% [8]. Unnoticed and unmanaged impaired language development can have a significant serious effect on a child's development, affecting educational, social, and psychological progress [9]. Academic difficulties, learning disabilities [10], shyness and social difficulties, anxiety disorder, behavioural problems [11], [12], [13] and ADHD [14] are common detrimental consequences.

Acquisition of early motor skills is known to enhance the child's cognitive, language and social development. However, the positive association between motor and language development has been considerably investigated [6], [15], [16]. Libertus and Violi et al., have found that the onset of independent sitting may initiate a developmental cascade that results in increased language learning opportunities [17]. On the other hand, a link between motor impairment and language impairment has been proved [18], [19]. The presence of subtle motor impairment may explain the unresponsiveness of children with language impairment to speech therapy.

Language development is also influenced by other factors. Environmental factors as perinatal problems, poor socioeconomic conditions, lack of parental interaction and improper nutrition may precipitate delay in all developmental domains, including language domain [8]. Speech and language delay may be symptoms of a global developmental disorder such as autistic spectrum disorder, or a genetic disorder as Down syndrome or may be an indicator of hearing impairment. The tendency to speech and language disorders is mostly believed to be multifactorial, involving complex interactions between some common genetic variants and environmental factors [20].

Screening procedures and regular surveillance to recognise factors affecting infant development are crucial for early detection of potential developmental delays and hence, choosing proper management approaches. Therefore, the purposes of the present study were:

- To investigate a group of Egyptian infants for potential risk factors that can influence language development as socioeconomic factors, nutritional factors and perinatal maternal and infant medical conditions.

- To estimate the proportion of infants with subtle motor impairment among a group of infants presented with language impairment and among another matched group with normal language development

- To explore to what extent can the involvement of impaired motor development potentiate the risk of developmental language impairment.

Subjects and Methods

Study design and setting

This cross-sectional case-control study involved male and female Egyptian children aged from 18 to 36 months. They were recruited from Developmental and Behavioral Paediatrics Clinic at the National Research Centre (NRC) and the Pediatrics Outpatient Clinic of Ain Shams University (ASU) in the period from September 2016 to September 2018. A child was enrolled if he belonged to the middle socioeconomic class, the parents' main complaint was the child's delayed speech, and if they consented to participate in the study. Children were excluded if they demonstrated any obvious congenital anomalies, features of genetic diseases, or had a history of any metabolic or physical problems.

Sample Size

Previous research had found that delayed motor milestones were documented in about 70% of children with developmental language impairment (LI) and only in 22% of the control children [21].

In this study, it was planned to use two-sided confidence intervals for the difference in proportions to calculate sample size. The used confidence interval method was the Yates chi-square simple asymptotic method with continuity correction (Newcombe, 1998) [22]. The proportion estimates to be used 0.70 for Group 1, and 0.22 for Group 2.

Calculated group sample sizes of 353 (to be rounded to 360) for group 1 and of 293 (To be rounded to 295) for group 2 produce a two-sided 85% confidence interval for the difference in population proportions with a width that is equal to 0.100

Subjects

Enrolled children were classified according to their performance on language domain of Bayley Scales of Infant and Toddler Development (Bayley-III) into two groups: infants having a below-average composite score (impaired development) and those having average or above-average scores (normal development). Three hundred and sixty children with below-average language composite score were recruited as cases, and 295 children of the same age and sex with average and above-average languages composite score served as a control group.

Methods

Socio-demographic assessment: For this special assessment questionnaire was used including questions about maternal age, maternal and paternal education and occupation, marital status, family income, and child order of birth [23]. Family income was classified according to father's occupation into two categories; lower-middle-income, if the father is unemployed, day-by-day worker, farmer, or manual labourer; upper-middle-income, if the father is employee, professional and employer, or a dealer. Mother education was classified into 3 categories; illiterate to preparatory school, secondary school, and higher education.

Assessment of maternal and prenatal history: This included parity, history of maternal chronic diseases as hypertension, diabetes or hypothyroidism, and diseases acquired during pregnancy as gestational diabetes or preeclampsia. The infant's data about gestational age, mode of delivery, history of complicated labour such as premature rupture of membranes, fetal asphyxia or umbilical cord prolapse were recorded. History of postnatal problems as cyanosis, jaundice or convulsions and admission to NICU was enquired.

Infant Feeding Practices in the first six months of life: was assessed to identify infants who were predominately breastfed, artificially-fed (who were consuming other milk including fresh, tinned, and powdered milk from cows or other animals) or mixed fed (artificial plus breast milk). The time of introduction of complementary feeding was recorded whether before or after the sixth month of age.

Thorough physical examination and anthropometric measurements: All measurements were made according to techniques described in the Anthropometric Standardization Reference Manual [24]. Physical examination and assessment of growth were performed for cases and control subjects.

Assessment of language and motor development: using the Bayley Scales of Infant and Toddler Development (Bayley-III): These scales were developed by Nancy Bayley [25] to assess the development of infants and toddlers between the age of 1 month to 42 months. Bayley-III consists of 5 subscales, i.e. Cognitive Scale, Language Scale (Receptive Communication and Expressive Communication), Motor Scale (Fine Motor and Gross Motor), Social-Emotional Scale, and Adaptive Behavior Scale. In this study, only the language and motor domains were being measured. The test was administered according to the infant's age-specific start point. Each correct response is given a score of 1, and the total raw score is then converted into its composite score.

Ethical Considerations: The study complies with the International Ethical Guidelines for Biomedical Research Involving Human Subjects [26]. The Research and Ethical Committee of NRC cleared the study protocol. The ethical approval number was 11020. Informed consent was obtained from the parents of enrolled children.

Confidentiality: Mothers and children were identified by a serial number, and the information at the individual level was kept strictly confidential.

Results

The included children were divided according to the language composite score on Bayley scale into two groups; below-average group (n = 360) who were considered the cases, and average and above-average group (n = 295) who were the controls. Tables 1, 2 and 3 show the results of the univariate analysis of factors affecting language composite score.

Table 1: The risk of impaired language development according to different feeding practices

	N	Children with impaired language development (Cases) (n = 360)	Children with normal language development (Control) (n = 295)	P	OR (95%CI)
Type of feeding					
Breast fed	321	160 (49.8)	161 (50.2)		1
Bottle fed	241	146 (60.6)	95 (39.4)	0.012	1.6 (1.1-2.2)
Mixed fed	93	54 (58.1)	39 (41.9)	0.16	1.4 (0.9-2.2)
Time to add complementary food					
Before six months	201	128 (63.7)	73 (36.3)	0.003	1.7 (1.2-2.4)
After six months	454	232 (51.0)	222 (49.0)		

OR: Odds ratio; CI: Confidence interval.

Comparison of cases and control groups revealed the independent factors lowering the language composite score and present children who are at more risk of impaired language development. Early introduction of complementary food before the age of six months carries a highly significant risk of impaired language development (OR = 1.7, P = 0.03) Table 1, the lower family income and low maternal education (illiteracy up to preparatory schools vs high education) represent highly significant social risk factors (OR = 1.7, p = 0.001 and OR = 1.9, p = 0.001 respectively) Table 2.

Table 2: The risk of impaired language development according to socioeconomic factors

	N	Impaired language development (n = 360)	Normal language development (n = 295)	P	OR (95%CI)
Childbirth order					
> 3	213	120 (56.3)	93 (43.7)	0.623	1.1 (0.8-1.5)
≤ 3	442	240 (54.3)	202 (45.7)		
Maternal age					
≤ 25 years	241	136 (56.4)	105 (43.6)	0.564	1.1 (0.8-1.5)
> 25 years	414	224 (54.1)	190 (45.9)		
Family Income					
Lower Middle	314	194 (61.8)	120 (38.2)	0.001	1.7 (1.3-2.3)
Upper Middle	341	166 (48.7)	175 (51.3)		
Mother education					
Illiterate to prep	194	124 (63.9)	70 (36.0)	0.001	1.9 (1.3-2.9)
Secondary	254	137 (53.9)	117 (46.1)	0.192	1.3 (0.9-1.9)
High education	207	99 (47.8)	108 (52.2)		1
Occupation					
House wife	513	278 (54.2)	235 (45.8)	0.451	0.9 (0.6-1.3)
Working	142	82 (57.7)	60 (42.3)		

Children subjected to delivery problems are at most risk for impaired language development (OR = 7.6, p < 0.001) and pregnancy-related diseases of the mother increases the risk of impaired language development significantly (OR = 2.5, p < 0.001), weight and height for age expressed without statistically significant difference (Table 3).

Table 3: The risk of impaired language development according to maternal and child medical history

	N	Children with impaired language development (n = 360)	Children with normal language development (n = 295)	P	OR (95%CI)
Maternal related Factors					
Chronic diseases					
Yes	124	74 (59.7)	50 (40.3)	0.24	1.3 (0.8-1.9)
No	531	286 (53.8)	245 (46.2)		
Pregnancy-related diseases					
Yes	89	65 (73.0)	24 (27.0)	< 0.001	2.5 (1.5-4.1)
No	566	295 (52.1)	271 (47.9)		
Iron deficiency anaemia					
Yes	312	163 (52.2)	149 (47.8)	0.18	0.8 (0.6-1.1)
No	343	197 (57.4)	146 (42.6)		
Nutritional status					
Malnourished	529	295 (55.8)	234 (44.2)	0.39	1.2 (0.8-1.8)
Normal	126	65 (51.6)	61 (48.4)		
Infant related Factors					
Gestational age					
Preterm	49	32 (65.3)	17 (34.7)	0.13	1.6 (0.8-2.9)
Full term	606	328 (54.1)	278 (45.9)		
Type of labour					
Cesarean	372	198 (53.2)	174 (46.8)	0.31	0.9 (0.6-1.2)
Normal	283	162 (57.2)	121 (42.8)		
Delivery problems					
Yes	86	76 (88.4)	10 (11.6)	<0.001	7.6 (3.9-15.1)
No	569	284 (49.9)	285 (50.1)		
Weight for age					
Underweight	49	28(57.1)	21(42.9)	0.865	1.1(0.6 -1.9)
Normal weight	606	332(54.8)	274(45.2)		
Height for age					
Stunted	82	47(57.3)	35(42.7)	0.734	1.1(0.7 -1.8)
Normal height	573	313(54.6)	260(45.4)		

OR: Odds ratio; CI: Confidence interval.

The proportion of infants with impaired motor development among cases with language impairment is high (69%) if compared with that (38%) among the control group, as shown in Figure 1.

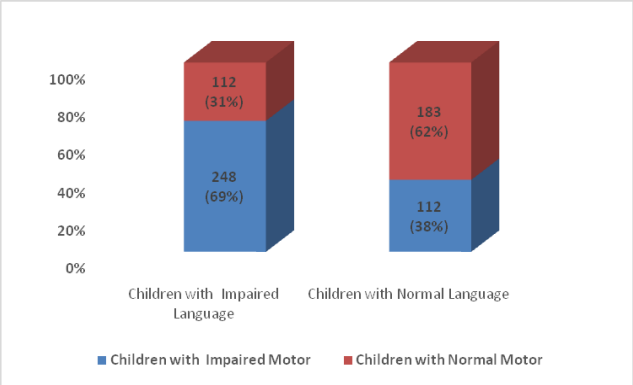

Figure 1: The proportion of children with impaired motor development among cases with impaired language development and controls with normal language development

Table 4 shows the most important predictors of impaired language development in the studied sample. Impaired motor development appears as a further highly significant risk factor to the previously mentioned factors, as shown in logistic regression analysis in Table 4.

Table 4: Logistic regression of factors affecting language composite score

	B	p	OR	95%CI of OR	
				Lower	Upper
Early introduction of complementary food	0.771	< 0.001	2.162	1.457	3.207
Lower middle family income	1.176	0.002	3.242	1.719	6.115
Delivery problems	2.484	< 0.001	11.986	4.906	29.284
Pregnancy related diseases	2.837	< 0.001	17.070	3.869	75.312
Mother education (illiteracy vs. high education)	0.511	0.013	1.667	1.113	7.497
Impaired motor development	1.613	< 0.001	5.016	3.068	8.199

B: Regression co-efficient; OR: Odds ratio; CI: Confidence interval.

Discussion

Child development is influenced by genetic and environmental factors. Environmental issues appear to largely influence young children's attainment of language skills [27], [28]. In Egypt as a developing country, investigation and consequently, prevention of potential environmental risks is crucial.

This study demonstrated that factors that are independently associated with delayed language development in Egyptian children were early introduction of complementary food before the age of six months, low maternal education, low family income, delivery problems, pregnancy-related diseases of the mother and subtle delayed motor development.

The beneficial effect of breastfeeding on general growth and development of children has long been well recognised [29]. In the current study, history of feeding practices in the first six months of life revealed that bottle feeding and early weaning before the age of six months were associated with a significant risk of DLD. Many studies linked improved cognitive development rather than language development in infancy to breastfeeding. Exclusive breastfeeding was reported to positively affect cognitive development of children in early infancy [30], even after adjustment for other key cofactors [31]. In previous Egyptian studies, exclusive breastfeeding versus bottle-feeding during the first six months of life was correlated with above-average cognitive [32] and socio-emotional development of infants [33].

Few studies tested the link between breastfeeding and the language development of children. Leventakou et al. reported that a longer period of breastfeeding was independently associated with higher scores of language and motor development at 18 months of age [34]. A more recent study confirmed these findings and recommended the promotion of breastfeeding for more than 12 months to attain the maximum benefit in the cognitive and language development of children [35].

Many theories are proposed to explain the effect of feeding on language development. It was proposed that human milk contains a group of nutrients including the characteristic essential fatty acids such as docosahexaenoic acid (DHA) and

choline, hormones, and growth factors that stimulate optimal development of brain structure and function [36].

Another theory is, the effect of breastfeeding on immune system function was supposed to influence learning and memory [37]. Also, the act of breastfeeding that enhances mother-infant relationship is thought to be important for cognitive, socio-emotional and language development [38].

Another independent factor that negatively affects language scores in the current study was a low socio-economic status (SES). The socioeconomic standard is usually ruled on the level of parental education, parental occupation and monthly family income [23]. This study showed that lower language composite scores were associated with lower family income and lower mother education. As language is a social act that progresses under social stimuli, it has been shown that children from lower-SES background show slower growth of vocabulary compared to higher-SES children from infancy up to school years [39], [40]. One pathway for explanation of this difference is the availability of learning resources in high-income families whether inside home (as books and toys) or outside home (as high-quality daycare centres or outdoor activities) [41]. Another pathway for this difference is the variation of parents' speech to their children. Hart and Risley estimated that children from professional families hear an average of 45 million words by age four compared to 13 million words in children from low-income families [42]. Other investigators found that the quality of speech and not the total amount of speech plays a more critical role in language development. In a large sample of low-income families, language development was positively affected by maternal vocabulary input and maternal language and literacy skills [43]. It was found that variation in the quality of nonverbal and verbal interactions were more powerful predictors of language development rather than the number of mothers' words during the communication with the infant [44], [45]. Mothers' level of education appeared to have a major effect on early language development not only in normal children but also in children with autism spectrum disorder (ASD) [28]. We thought that maternal education not only can affect language development directly through maternal vocabulary input but also indirectly through choosing appropriate feeding practices [46] and providing a health care and a safe environment for their children [47].

Another important factor affecting language development is the perinatal risk factors. In the current study, pregnancy-related complications (most commonly gestational hypertension, preeclampsia, eclampsia and gestational diabetes) and delivery problems (mostly birth trauma and asphyxia) were the main perinatal risk factors recorded in children with DLD. This could be explained based on placental insufficiency, oxygen deprivation in-utero, birth asphyxia, and neonatal hypoglycaemia which could affect neurocognitive functions and increase the risk of developmental disability [48], [49], [50].

In contrast to our findings, variables as first-minute Apgar scores < 7, mother's age, emergency caesarean section, maternal haemorrhage, and threatened abortion were significant factors for delayed cognitive and communication skills, while factors such as preeclampsia and premature rupture of the membrane had no significant relationship [51]. Other studies linked developmental disorders to prematurity, low birth weight, maternal difficulties during pregnancy, and congenital malformations [52]. In the current study, variables as maternal malnutrition, maternal chronic diseases, prematurity and caesarean delivery seemed irrelevant to developmental delay. Thus, the relationship between specific perinatal risk factors and subsequent developmental delay has not reached a consensus.

Nutritional deficiencies during infancy are likely to affect cognition, communication, behaviour, and productivity throughout childhood and adulthood [53].

In the current study, though the prevalence of underweight children and that of stunted children were higher in cases than in controls, the differences didn't reach a significant level (7.8% of cases were underweight vs 7.1% in controls; 13% of cases were stunted vs 11.9% in controls). Thus, malnutrition was not a significant risk factor for DLD in this study. This finding is in agreement with that of Mendes et al., 2012 [54]. However, other studies found that both malnutrition and anaemia early in life might lead to problems in cognitive development and language acquisition [55], [56]. We think that the severity of malnutrition and the association of anaemia are important variables controlling the impact on language development.

Language development depends on other developmental domains. The influence of cognitive and social-emotional domains on language development is completely supported [57]. Some studies support language and motor skills as separate domains, while others suggest that motor skills are a prerequisite for language development [58]. Some research recognised the relationship between motor and cognitive development, and consequently between motor and language development as a sub-domain of cognition. This relationship is a logical consequence in the context of bodily interaction with the physical and social environment [59]. Neuroimaging techniques have shown that areas of the brain implicated in language functions are activated during motor tasks [60], and the activation of motor areas was detected during language tasks [61]. Behavioural studies revealed associations between infant motor maturity and language development [42]. Also, other studies have shown a link between motor performance and between motor performance and language [62], [63].

In the current study, the interrelationship between language and motor development was evident. The percentage of children have got below-average score on Bayley-motor scale, was significantly higher among children with DLD than in normal children (69% vs 38%). Also, delayed motor development appeared as a highly significant predictor of language development in logistic regression analysis. The application of these findings is very important. It denotes the significance of careful evaluation of all developmental domains even the child is presented with DLD only. Non-responding children to intensive speech therapy may benefit if they attend physiotherapy sessions in parallel.

Logistic regression analysis in this study approved the independent predictors of DLD according to their contribution and their level of significance as follows: maternal health problems during pregnancy and child-birth difficulties, followed by impaired motor development, low family income, early introduction of complementary food and the least significant predictor was maternal education.

Limitations: Details of parental-child interaction and whether the child attended childcare centre were not included in the questionnaire. Neither language impairment was not classified into receptive communication disorders and expressive communication disorders, nor was motor impairment not classified into fine motor and gross motor disorders. This is because estimation of composite score of Bayley-scales depends on sum of both functions.

In conclusion, a group of social, medical and nutritional factors are interacting to affect language development in Egyptian children. The most significant risk factors were pregnancy-related diseases and labour-associated problems. This denotes inadequate access of Egyptian mothers to maternal health care during pregnancy and childbirth. Low family income and the level of maternal education were the predominant social risk factors. Child developmental screening should include all developmental domains. Subtle motor impairments common among children with DLD and can potentiate its risk.

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Clinical Science

Survey of Current Difficult Airway Management Practice

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Keywords: survey; anticipated difficult airway management; non-anticipated difficult airway management

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Abstract

BACKGROUND: Even for the most experienced anesthesiologists "can't ventilate can't intubate" scenario in difficult airway management is challenging, and although rare it is life-threatening.

AIM: The aim of this survey was to analyse the current practice of difficult airway management at our University teaching hospital.

MATERIAL AND METHODS: A ten-question-survey was conducted in the Tertiary University Teaching Hospital "Mother Theresa", Clinic for Anesthesia, Reanimation and Intensive Care. The survey included demographic data, experience in training anaesthesia, practice in management of anticipated and non-anticipated difficult airway scenario, preferable equipment and knowledge of guidelines and protocols. Responses were noted, evaluated and analysed with the SPSS statistical program.

RESULTS: The overall response rate was very good; 94.5% answered the survey. During the assessment of the level of comfort with diverse airway equipment, there was diversity of answers due the experience of anaesthesia training, although the most frequent technique among all responders for anticipated difficult intubation was video laryngoscopy (48%). As for non-anticipated difficult intubation when conventional techniques failed to secure the airway most of the responders answered that they used supra-gothic airway device – laryngeal mask (38%) as a rescue measure.

CONCLUSION: Airway assessment, adequate training, experience, and availability of essential equipment are the pillars of successful airway management.

Introduction

Airway management is the fundamental skill of an anesthesiologist. Every adverse event in airway management is unique, where the outcome depends on the emergency of the procedure, knowledge, skills and practice of the anesthesiologist, affected by the patient co-morbidities and accessible resources [1], [2].

Therefore, national guidelines, recommendations, clinical consensus on difficult airway management are available, published in the United Kingdom, in the USA, and western European countries. In them several techniques and protocols have been described and recommended [1], [3], [4], [5], [6].

These declarations reflect common thinking and evidence on an appropriate reaction to difficult airway management when encountering an unconscious/induced patient. The importance of the appropriate applications from the statements is a simplified response to a ''can not intubate, cannot ventilate'' situation that is challenging even for the most experienced anesthesiologists [6], [7].

The aim of this survey study was to evaluate the current practice of difficult airway management in anticipated and non-anticipated circumstances at our Tertiary University Teaching hospital.

Material and Methods

A questionnaire was delivered among anaesthesia residents of the first to the fifth last year of residency, young specialists and experienced anesthesiologists at the Tertiary University Teaching Hospital “Mother Theresa”, Clinic for Anesthesia, Reanimation and Intensive Care, University Ss. "Cyril and Methodius" of Skopje, Medical Faculty - Skopje.

The questionnaire contained 10 questions including: demographic data, experience in training anesthesia, preferred equipment for anticipated and non-anticipated difficult intubation, current practice, available resources and equipment, clinical examination and preferred test for predicting difficult intubation, the day / night shift influence on the usage of additional equipment, and the awareness of the current available guidelines. The questionnaire was anonymous, and the responders answered it voluntarily.

At the beginning of the questionnaire, mainly we collected demographic data, including gender, age, and years of experience.

The rest of the questionnaire assessed the practice for clinical examination and preferred test for predicting difficult intubation, availability of the resources, techniques and equipment, and the preferred choice for management of anticipated and non-anticipated difficult intubation scenario or the comfort with the usage of the equipment like fibreoptic bronchoscopy, video laryngoscopy, McCoy conventional laryngoscope, stylet or Boogie.

Also, the familiarity with protocols and guidelines for difficult airway was assessed.

The examiners were assessed for their preferred choice when managing anticipated and unanticipated difficult airway scenarios and their strategy.

The last question was: what is most important during difficulty airway scenario - the experience or the resources?

Statistical analysis was performed with SPSS (20.0) program. Categorical variables were expressed as percentage and data were reported as median and ranges.

Results

We distributed 55 questionnaires; 52 were returned. Thirty-two residents and 20 specialists (94.5%) in Anesthesia and Intensive Care responded to the survey.

Summarised demographic data are presented in Table 1. About 59% of responders were at the age ranging from 25 to 34 years, all of the residents, whereas 26% of responders were aged between 35-44 years, only one of them was resident. Gender distribution was: 31% males and 59% females. As for the experience 61% were residents, 15% with small experience in the field of anaesthesia (< 5 years), 11% had experienced between 5 and 10 years and only 5.7% had experience of 20 and > 20 years.

Table 1: Demographic data

Age Number (n) Percentage (%)		
25 – 34	31	59%
35 – 44	14	26%
45 – 54	6	11%
55 – 64	1	2%
Gender Number (n) Percentage (%)		
Male	16	31%
Female	36	69%
Experience Number (n) Percentage (%)		
Residents	32	61%
0 - 4	8	15%
5 – 9	6	11%
10 – 19	3	5.7%
>20	3	5.7%

All of the responders answered that they perform a clinical examination and have a preferred test for predicting difficult intubation. Both residents and specialists, selected Mallampati score as a preferred choice-38%, thyromental distance-25%, 5.7% use the 3-3-2 test, and 29% answered that they combined the clinical tests.

During the assessment of the level of comfort with diverse airway equipment, there was diversity of answers due to their experience although the most frequent technique among all responders for anticipated difficult intubation was video laryngoscopy (48%). As for non-anticipated difficult intubation when conventional techniques failed to secure the airway most of the responders answered that they used supra-gothic airway device – laryngeal mask (38%) as a rescue measure. Airway management technique of all responders in anticipated and non-anticipated difficult airway scenario is summarised and presented in Figure 1 and Figure 2.

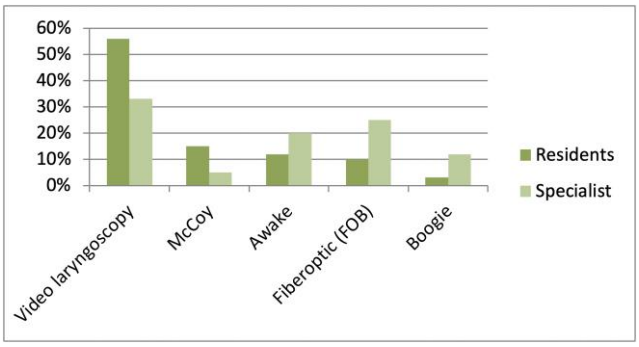

Figure 1: Number of responders comfortable with alternative airway devices in anticipated difficult airway management

When questioned about how many attempts they have to secure the airway with endotracheal tube before they request additional equipment, the answer

was after two attempts-55% of the specialists and 65% of the residents. 25% of the specialists and 34% of the residents require additional equipment after three attempts. None of the residents attempted more than three times. Only 5% of the specialists made four attempts before requesting additional equipment. As for the time of the day/ night shift for the usage of additional equipment, most of the responders used additional equipment in the day shift.

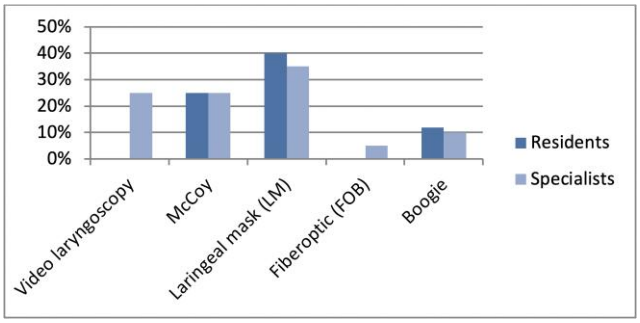

Figure 2: Number of responders comfortable with alternative airway devices in non-anticipated difficult airway management

Our survey found that only 12% of the residents and 10% of all responders are familiar with the protocols for difficult airway management and more of them consider experience as the most important in the management of difficult airway regardless the equipment and techniques available.

Discussion

This questionnaire addressed the common practice in our everyday clinical practice, the preferred equipment for anticipated and non-anticipated difficult airway management, available resources and equipment and the awareness of the currently present available guidelines. This is the first survey conducted at our Clinic and reported in our country. The response rate is excellent (95.4%) and comparable with other reports from eastern European countries, and the USA [8], [9], [10], [11].

All responders in our survey answered that they perform a clinical examination and have preferred test for predicting difficult intubation, which is very much similar to many other reports from all over the world [9], [12], [13].

A limiting factor in developing countries like ours is the resources restriction. In our University Teaching Hospital from recently, we have new airway management devices, including video laryngoscope, fibre optic stylet, and fibre optic bronchoscope. Availability of equipment is different between non-teaching and teaching hospitals according to the literature. There are similar findings in the report of Jenkins and colleagues where availability of fibre optic bronchoscope was 99% among anesthesiologists in Canada [9], [14], [15], [16].

The level of comfort of the anesthesiologists in the management of difficult airway is related to the experience, practice and knowledge. In this survey, the majority of the experienced and not experienced anesthesiologists have reported usage of laryngeal mask as rescue measure in non-anticipated difficult airway management. Regarding anticipated airway management most of the experienced anesthesiologists use video laryngoscope technique. On the other hand, less experienced anesthesiologists always prefer awaken intubation [17]. More experienced anesthesiologists are still trying the conventional methods at least once or twice before requesting additional equipment. These findings are similar to others reported in the literature as those of Bokhari and coauthors [10].

Fibre optic bronchoscope is relatively new equipment available in our Hospital. Our medical staff doesn't have too much experience with its use, and therefore the fibre optic bronchoscope is not the preferred choice for management of difficult airway scenarios in our everyday clinical practice. In their observation, Jenkin and Wong report 59 % of the experienced and 22% of the not experienced residents used fibre optic bronchoscope. In our report only 25% of the specialists and none of the residents will lay hands-on fibre optic bronchoscope for managing difficult airway situation [14], [17]. It can be concluded that additional practising and training in the use of FOB is needed.

In contrast to fibre optic bronchoscope, video laryngoscope is a new gadget available at our Hospital, and although our experience is also limited concerning its use, still most of the responders answered that the first and preferred choice in management of difficult airway scenario is video laryngoscope. This finding is similar to the survey presented in a report from India [18].

Recently, video laryngoscope has been included in the algorithm of the difficult airway society, but rapidly it is gaining attention and is very promising due to its brief learning curve [4].

In a non-anticipated difficult airway scenario, most of the experienced specialists in anaesthesia will try the conventional method and afterwards will choose laryngeal mask 35% and video laryngoscope 25% of specialists. On the contrary, 40% of residents will choose laryngeal mask, and 25% will choose McCoy Only 6% will pick video laryngoscope. Similar reports were published by Dimitriou and colleagues and Ezri and colleagues. The probability of this choice is the availability and the easiness of its use [12], [16].

Regarding the low knowledge of the currently present guidelines, protocols and consensuses, our survey has different results from all other reported. This is maybe due to the restricted resources and

fund which are limiting our opportunities for continuing medical education, improving skills and practice outside the borders of our country.

It is confirmed that training, simulations, and practice are some of the tools for improving the skills and knowledge in anaesthesia. In this era of technology, computers and simulators can help us to improve that [19], [20].

In conclusion, guidelines are directed to special circumstances, and therefore knowledge in the field of difficult airway management can improve our practice and provide better care for our patients. Airway assessment, adequate training, experience, and availability of essential equipment are the pillars of successful airway management.

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Appendix 1: Survey Questions

1. Age _____
2. Gender _____
3. Anesthesia experience:
   a) Resident
   b) Consultant
   - 0 - 4 years
   - 5 - 9 years
   - 10 – 20 years
   - > 20 years
4. Your first choice in anticipated difficult airway scenario?
   a) Awake intubation
   b) Direct laryngoscopy with Mccoy laryngoscope
   c) Bougie
   d) Video laryngoscopy
   e) Video stylet
   f) Fiberoptic laryngoscope
5. Your preferred choice in unanticipated difficult intubation if conventional method fails?
   a) Supraglottic devices (Laryngeal mask)
   b) Direct laryngoscopy with Mccoy laryngoscope
   c) Bougie
   d) Video laryngoscopy
   e) Video stylet
   f) Fiberoptic laryngoscope
6. During emergency intubation, which one of the following is your preferred test for predicting difficult intubation?
   a) Mallampati score
   b) Thyromental distance
   c) 3-3-2-test
   d) Upper lip bite test (if applicable)
   e) Other _____
7. After how many failed attempts of intubation you require additional equipment (Mccoy laryngoscope, Video laryngoscopy, Video stylet, Fiberoptic laryngoscope)?
   a) 2
   b) 3
   c) 4
   d) > 5
8. Do the time frames or day/ night shift has influence on the usage of additional equipment?
   a) Often in day shift
   b) Often in night shift
9. Are you familiar with the guidelines and protocols for difficult airway in emergency critical settings?
   a) Yes
   b) No
   If you answer is Yes, please specified which one is it:_____
10. Which of the following you consider most important in difficult airway scenario?
   a) Experience of the doctor
   b) Equipment for difficult intubation

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Clinical Science

Relation of Asthma Control with Quality of Life among a Sample of Egyptian Asthmatic School Children

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Keywords: Asthma; Quality; Children; Asthma Control; Emotional function

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Abstract

BACKGROUND: Asthma is considered a chronic health illness that not only resulted in physical symptoms but also emotional effects. It is; therefore, so important to assess the quality of life of asthmatic patients besides their level of disease control.

AIM: To determine the correlation of asthma control with the health-related quality of life (HRQOL) of asthmatic children in Egypt.

METHODS: One hundred and twenty-eight asthmatic Egyptian children were enrolled in the study. They were subjected to asthma severity grading, asthma control questionnaire (ACQ) and pediatric asthma quality of life questionnaire (PAQLQ). Studied cases were taken from 6 primary and preparatory schools, Giza governorate.

RESULTS: The mean child control score was significantly higher in not well-controlled asthmatics compared to well-controlled asthmatics ($p < 0.005$). The not well controlled asthmatic children showed significantly lower activity limitation score, symptoms score, and overall asthmatic score compared to controlled asthmatic children ($p < 0.05$). The severity of asthma shows significant positive correlation with symptoms score, emotional function score and overall asthmatic score ($p < 0.05$).

CONCLUSION: The quality of life for the asthmatic children is strongly correlated with the level of asthma control and severity.

Introduction

Asthma is a diverse disease characterized by chronic airway inflammation. It is known by history of respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity, together with variable expiratory airflow limitation [1]. This disease can spontaneously remit or improve with treatment [2].

It is considered a very common disease in children and adolescents [3]. It becomes a widespread health problem that affects not only high-income countries; but also, all countries regardless the level of development, on the other hand, most asthma-related deaths occur in low- and lower-middle-income countries [4]. The burden of asthma to governments, healthcare systems, families, and patients is increasing worldwide [5]. It is common in Egypt, and probably underdiagnosed and undertreated, particularly among children from less wealthy families [6].

Asthma is considered to have a negative impact on the daily life activity of children. Asthmatic children, especially those suffering from less disease control, show lower activity than normal children [7].

Asthma puts a serious burden on the child's health-related quality of life (HRQOL), despite the availability of effective and safe treatment [8]. Quality of Life (QOL) is a concept widely used to refer to the subjective wellbeing of individuals. This term is used to refer to individuals' subjective satisfaction with important aspects of their life as their physical and mental well-being, social relationships and individual activities [9]. The Pediatric Asthma Quality of Life Questionnaire (PAQLQ) is one of the most widely used instruments for measuring health-related QoL in children with asthma. The standardised version of PAQLQ contains 23 questions in three domains, i.e., activity limitation, symptoms and emotional function [10].

How bronchial asthma affects the quality of life has been the focus of many published types of research. There was a study which had been done in Nigeria concluded that around a quarter of the children attending asthma clinic were psychologically affected beside the interference of asthma with the daily activities [11]. In the Arab world, many studies revealed that psychosocial aspects of asthma had a significant adverse impact on the quality of life of children [12].

In Egypt, Nowadays, it's of utmost importance to study bronchial asthma effects on patients' quality of life, but unfortunately there is still a lack of this type of studies. Therefore, our aim comes to assess asthma control level and its impact on quality of life of asthmatic children.

Methods

Study Population

This study was a cross-sectional study. It was conducted on 128 asthmatic children and adolescents, 7-16 years old, from six primary and preparatory schools, Giza governorate as a part of research project, funded by National Research Centre 10th research plan, entitled " Effectiveness of health education program on pulmonary functions and quality of life in Egyptian asthmatic children" during the period from September 2016 to April 2018.

Inclusion Criteria

Both sexes were represented (males and females).

Registered asthmatic and susceptible cases (who had a family history of atopy and the child had history of atopy and recurrent respiratory tract infections then diagnosed by us as bronchial asthma) were picked up from the school medical records with the help of the school medical staff (school doctors and nurses).

Exclusion criteria

- The child with an acute disease including upper or lower respiratory tract infections.

- The child with history of congenital heart disease, documented immunodeficiency, rheumatologic disease, cystic fibrosis, renal dysfunction or congenital anomaly.

- Use of systemic steroids for an indication other than asthma.

- Children with mental disease or neurological disability.

- Any child whose parent refuse to give informed consent.

Site of study

The Pediatric Pulmonary Function Testing Clinic at the Medical Research Centre of Excellence (MRCE), National Research Centre (NRC).

Each child was subjected to:

- Full medical history and examination are laying stress on sociodemographic characteristics of the children.

- Anthropometric measurements [13] in the form of:

- Height: was measured using Harpenden stadiometer.

- Weight: was measured by the Tanita scale.

- BMI: was calculated according to the equation:

$$BMI = Wt\ (kg) / Ht^2 (cm^2)$$

- Evaluation of Asthma Control: Validated Arabic version of the asthma control questionnaire (ACQ) was used [14].

Asthma control questionnaire (ACQ)

The 6-item Asthma Control Questionnaire (ACQ-6) had been used to measure the objectives of asthma treatment as defined by international guidelines (minimisation of day- and night-time symptoms, activity limitation, β2 agonist use and bronchoconstriction). Responses are given on a 7-point scale, and the overall score is the mean of the responses (0 = totally controlled, 6 = severely

uncontrolled) [15].

Developmental studies have established the cutoff points for controlled asthma ACQ ≤ 1.5 and not well controlled asthma ACQ ≥ 1.5 [16]

- Assessment of Quality of Life: Validated Arabic version of Pediatric asthma quality of life questionnaire (PAQLQ) [17].

Asthma Severity [18]

Asthma severity is determined before initiating therapy retrospectively by history, spirometer and peak flow by analysis of the following points: symptoms; nocturnal awakenings; rescue medication use; activity limitation; and FEV_1.

According to EPR-3 divides asthma severity into four groups: intermittent, mild persistent, moderate persistent, and severe persistent.

Intermittent asthma is characterised as follows:

- Symptoms of cough, wheezing, chest tightness, or difficulty breathing less than twice a week;

- Flare-ups are brief, but the intensity may vary with no symptoms in between;

- Nighttime symptoms less than twice a month;

- Lung function test FEV_1 is 80% or more above normal values;

- Peak flow has less than 20% variability.

Mild persistent asthma:

- Symptoms of cough, wheezing, chest tightness, or difficulty breathing 3-6 times a week

- Flare-ups may affect the activity level

- Nighttime symptoms 3-4 times a month

- Lung function test FEV_1 is 80% or more above normal values

- Peak flow has less than 20-30% variability

Moderate persistent asthma:

- Daily symptoms of cough, wheezing, chest tightness, or difficulty breathing

- Flare-ups may affect child activity

- Nighttime symptoms five or more times a month

- Lung function test FEV_1 is above 60% but below 80% of normal values

- Peak flow has more than 30% variability

- Severe persistent asthma:

- Continues symptoms of cough, wheezing, chest tightness, or difficulty breathing

- Frequent nighttime symptoms

- Lung function test FEV_1 is 60% or less of normal values

- Peak flow has more than 30% variability

Pediatric asthma quality of life questionnaire (PAQLQ)

It measures the functional (physical, emotional, occupational and social) problems that are most troublesome to children with asthma. PAQLQ has 23 questions in three domains (symptoms, activity limitation and emotional function). Each question had a 7-point scale (7 = no impairment, 1 = severe impairment). The number of questions in each domain is as follow Activity limitation: 5 (2 generics, 3 patient-specific), Symptoms: 10 and Emotional function: 8. The 23 questions in the PAQLQ are divided into 3 domains: Activity limitation: in questions 1, 2, 3, 19, 22. Symptoms: in questions 4, 6, 8, 10, 12, 14, 16, 18, 20, 23. Emotional function: in questions 5, 7, 9, 11, 13, 15, 17, 21. Individual questions were equally weighted. The overall PAQLQ score was the mean of the responses to each of the 23 questions. The resultant overall score would be between 1 and 7. The domains were analysed in the same way (the domain scores were also the mean values for the items in each domain). So that the score of each domain would also be between 1 and 7 [19]

Statistical analysis

Collected data were coded, tabulated, and statistically analysed using statistical package for social sciences software, SPSS version 24. Descriptive statistics were performed for quantitative parametric data as mean ± SD, whereas they were performed for qualitative data as number and percentage. Independent sample T-test was used to compare two means. Pearson's correlation was used to correlate variables. The level of statistical significance for all tests was set at $p < 0.05$. Otherwise, the tests were considered insignificant.

Compliance with ethical standards

- This study was approved by the Medical Research Ethical Committee of NRC with approval number 16/381. Written informed consent from both parents and oral informed consent from children were taken after full explanation of the study protocol.

- There is no conflict of interest to disclose.

Our study was funded by the National Research Centre as a part of research project 10th research plan, entitled "Effectiveness of health education program on pulmonary functions and quality

of life in Egyptian asthmatic children".

Results

The total number of asthmatic children was 128. Fifty-eight (45.3%) were males, and seventy (54.7%) were females. They were referred from 6 primary and preparatory schools in Giza governorate and followed up in the Pediatric Pulmonary Function Testing Clinic at the Medical Research Centre of Excellence (MRCE), National Research Centre (NRC).

Table 1 shows the clinical data of asthmatic children. There is a high percentage of potential exposures to smoke (82.8%) and artificial feeding (71.1%) among asthmatic children.

Table 1: Clinical data of asthmatic cases

	No	%
Gender		
Male	58	45.3
Female	70	54.7
History of allergy	44	34.4
Potential exposures to smoke	106	82.8
Positive consanguinity	10	7.8
Household pets	0	0
Mode of delivery		
Normal	56	43.7
CS	72	56.3
Type of feeding		
Breastfeeding	10	7.8
Artificial feeding	91	71.1
Mixed	27	21.1

According to the asthma control questionnaire (ACQ), 76 (59.4%) of cases were classified as not well-controlled asthmatics and 52 (40.6%) as well-controlled asthmatic children.

Anthropometric data and quality of life scores in well-controlled asthmatic versus not well controlled asthmatic children are shown in Table 2. No significant difference was found between well-controlled asthmatic and not well controlled asthmatic children in age, weight, height and BMI.

Table 2: Quality of life scores in well-controlled asthmatic versus not well controlled asthmatic children

	Well Controlled asthmatic (No = 52)	Not well-controlled asthmatic (No = 76)	t	p
Age in years	9.86 ± 3.51	9.92 ± 3.43	-0.057	0.955
Weight in Kg	39.90 ± 14.66	38.42 ± 14.63	0.382	0.704
Height in cm	140.33 ± 17.92	137.78 ± 19.57	0.517	0.607
BMI	19.31 ± 3.97	19.48 ± 3.74	-0.163	0.871
mean child control score	0.96 ± 0.74	2.83 ± 1.08	2.877	0.000**
Activity Limitation Score	5.48 ± 1.53	4.17 ± 1.06	1.994	0.042*
Symptoms Score	5.28 ± 1.31	4.13 ± 1.69	2.761	0.008*
Emotional Function Score	5.48 ± 1.43	4.61 ± 1.85	1.921	0.060
Overall asthmatic child Score	5.34 ± 1.37	4.26 ± 1.63	2.606	0.012*

*Significant at $p < 0.05$; **highly significant at $p < 0.005$.

The mean child control score was found significantly higher in not well-controlled asthmatics compared to well-controlled asthmatics ($p < 0.005$). The not well controlled asthmatic children showed significantly lower activity limitation score, symptoms score, and overall asthmatic score compared to controlled asthmatic children ($p < 0.05$).

Table 3 shows the correlations between asthma severity and quality of life scores in asthmatic children. The severity of asthma shows a significant negative correlation with symptoms score, emotional function score and overall asthmatic score ($p < 0.05$).

Table 3: Correlations between asthma severity and quality of life in asthmatic children

		Activity limitation score	symptoms score	Emotional function score	overall asthma score
Intermittent Vs. Mild / Moderate persistent	Pearson Correlation	-0.360	-0.275*	-0.434*	-0.423*
	Sig. (2-tailed)	0.091	0.042	0.039	0.044

Table 4 shows the correlations between asthma severity and history of allergy of asthmatic children. The severity of asthma shows a significant positive correlation with history of allergy ($p < 0.05$).

Discussion

Bronchial asthma prevalence worldwide among children is having a steadily increase during the last two decades. Asthma prevalence among Egyptian children aged 3-15 years was estimated to be 8.2% [20]. It is considered as the most common chronic inflammatory disease in childhood. It is estimated that about 40% of all young children have at least one episode of asthmatic symptoms like wheezing, coughing, and dyspnea [21]. People with asthma report impact on the physical, psychological and social domains of quality of life [22]. The Pediatric asthma quality of life questionnaire (PAQLQ) is fully validated to be used in both clinical trials and clinical practice. It is composed of the daily problems and limitations, which the majority of asthmatic children are suffered from. It contains both discriminative properties (reliability and cross-sectional validity) and strong evaluative properties (responsiveness and longitudinal validity) [23].

In this study, we had found that the not well controlled asthmatic children showed significantly higher mean child control score and lower activity limitation score, symptoms score, and overall asthmatic score compared to controlled asthmatic children. This means that Health-related quality of life (HRQOL) was affected by the level of asthma control especially the physical activity as engagement in sports and exercises. This agrees with El Gendi et al., [24], which was done in Benha, Egypt with a sample size of 125 pair (asthmatic child and his caregiver). On the other hand, we didn't find any significant difference between the two asthmatic groups about the emotional function score which may be due to small sample size this is opposite to what had been found in study results of La Scala et al., [25] despite their sample was 56 but they were assesses at least twice at different times, their study took place at

Department of Pediatrics of Universidad Federal de São Paulo, Brazil. They found a significant difference in the emotional score that means an asthmatic child is in need not only for medical treatment but also needs psychological consultation.

Our study revealed that there was a significant negative correlation between asthma severity and symptoms score, emotional function score and overall asthmatic score ($p < 0.05$) which implies that when asthmatic children suffering from high grades of severity, they had a negative impact on their HRQOL, according to our results, specifically on their emotions and symptoms. Those findings go with Sawyer et al., the study [26] which besides our study they included children with severe asthma then recorded that children in the moderate/severe asthma group had a worse level of functioning in many domains of their HRQL than children in the mild asthma group. This gives a clue that there may be a dose ± response' relationship between the frequency and intensity of children's asthma symptoms and their level of functioning in several areas of their HRQL.

This study had shown a significant positive correlation between asthma severity and history of allergy; really this combination has become a common trouble nowadays as most of the studies had confirmed that allergy is always strongly connected with the grade of asthma severity [27], [28]. One of the limitations of this study was the length of our questionnaires which were time-consuming for students; in turn, this affects the number of patients in the present study. Another limitation is related to its cross-sectional design, and hence the long-term effects of asthma on QOL of asthmatic children could not be evaluated.

In conclusion, this study showed that the most affected quality of life scores for asthmatic children was the activity limitation score and symptoms score. Recently, we need to use Pediatric asthma quality of life questionnaire (PAQLQ) more in regular treatment and follow up of children with asthma.

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Clinical Science

Comparison of the Use of Hypnotic in Psychiatric Patients with Insomnia at the Mental Health Centre Prolet in Skopje

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Abstract

BACKGROUND: Insomnia is a symptom complex that comprises difficulties falling asleep, staying asleep or non-refreshing sleep in combination with daytime dysfunction or distress. Most people experience insomnia at some time during their lives. Because of its high incidence, and also because its symptoms are usually mild and transient, the importance of insomnia is frequently underestimated. Various conditions are associated with insomnia and can contribute to its development. They can be related to neurological or psychiatric disorders, which in turn may be aggravated by a deficiency of restorative sleep and daytime fatigue.

AIM: In this study, the authors compare the hypnotically effect of Flurazepam and Zolpidem applied on psychiatric cases treated in the Mental Health Centre "Prolet" in Skopje, Republic of Macedonia.

METHODS: The investigation covers 45 patients who have insomnia, in addition to their primary mental illness. The examination took six weeks, and it was divided into 3 equal phases. In the first phase of three weeks, Zolpidem was used, and in the third phases, Flurazepam was administrated. We used a self-estimating scale of 13 items and methods of global clinical estimation in the evaluation of received effects.

RESULTS: The results show that referring to the induction of the sleeping period, its duration and quality and the number of awakenings, there are no significant differences between the two medicaments used, but there was a significant difference between hypnotic medicaments and placebo.

CONCLUSION: The termination with the therapy, didn`t lead to the appearance of abstinential symptoms.

Introduction

Sleep medicine has been emerging with more public concerns over the past quarter-century, which involves multidisciplinary fields of specialists, including pulmonology, neurology, cardiology, otolaryngology, psychology, psychiatry, endocrinology, geriatrics, paediatrics, dentistry, physiology, pharmacology, and even alternative medicine. Among a wide variety of sleep disorders, insomnia is a particular example that heavily involves multidisciplinary efforts. Insomnia is highly prevalent in clinical practice, independently or comorbidity with another medical or psychiatric disorder [1], [2], and its management usually involves clinicians or specialists from various academic backgrounds.

Insomnia is a symptom complex that comprises difficulties falling asleep, staying asleep or non-refreshing sleep in combination with daytime dysfunction or distress. The symptom complex can be an independent disorder (primary insomnia) or the result of another condition (secondary insomnia) [3]. Insomnia is commonly divided into 3 types based on duration. Transient insomnia lasts up to 1 week and is often referred to as adjustment sleep disorder because it is caused most often by acute situational stress, such as a test or deadline. It is often recurrent with the same or similar stresses. The second type, short-term insomnia, by definition, lasts 1 to 6 months and is usually associated with more persistent stressful situational (death or illness) or environmental (noise) factors. Finally, chronic insomnia is insomnia lasting more than 6 months.

Most people experience insomnia at some time during their lives. Because of its high incidence, and also because its symptoms are usually mild and transient, the importance of insomnia is frequently

underestimated. However, as a chronic disorder, which affects about 10% of the population, its treatment is often challenging and, moreover, it is associated with a substantial number of comorbid symptoms [4], [5], [6]. Various conditions are associated with insomnia and can contribute to its development. They can be related to neurological or psychiatric disorders, which in turn may be aggravated by a deficiency of restorative sleep and daytime fatigue. Insomnia can also result from a primary dysfunction or an age-related decline in the circadian system.

Insomnia is characterized by one or more of the following: difficulty falling asleep [e.g. sleep onset latency (SOL) of more than 30 minutes], insufficient sleep [e.g. total sleep time (TST) of less than 5.5 – 6 hours], numerous nocturnal awakenings, early morning awakenings with inability to resume sleep, or non-restorative sleep. Common daytime complaints include somnolence, fatigue, irritability, and difficulty concentrating and performing everyday tasks. Because insomnia is associated with reductions in attention span, affected individuals can often be impulsive and experience impaired judgment, and thus are at an increased risk for having injuries at home or work, or involvement in accidents while driving. Psychiatric and other medical illnesses, including cardiovascular diseases, weight gain and glucose intolerance, are other conditions which include insomnia in their overall symptom complex [7].

The International Classification of Sleep Disorders (ICSD-2) [8] considers severity criteria as a guide to be applied in conjunction with consideration of the patient's clinical status. Mild insomnia refers to complaints of an insufficient amount of sleep almost every night or of not feeling rested the following day. There is little or no impairment in social and / or occupational functioning. Moderate and severe insomnia refers to complaints of experiencing an insufficient amount of sleep every night or of not being rested after the impaired sleep episode, accompanied by moderate and severe impairment of social and/or occupational functioning, respectively. The challenge for clinical treatment is to select the therapy, which is most appropriate for these differing degrees of severity.

The last several decades have seen an evolution in thinking about the classes of medications which are to be preferred for treating insomnia. The benzodiazepines (BZDs) were introduced in the 1970s and rapidly increased in popularity because of their efficacy and better safety compared to the barbiturates, carbamates, chloral derivatives and methaqualone [9]. In recent years, however, prescriptions for BZDs have progressively declined, especially because of their associated side effect profile, including their tendency to promote dependence, the occurrence of rebound insomnia following the withdrawal of short- and intermediate-acting derivatives, and the loss of efficacy after several weeks of treatment. The clinical need for medications which did not have these side effects was an important factor leading to the development of structurally dissimilar non-BZD hypnotics. These included the sedating antihistamines, the melatonin receptor agonists ramelteon and tasimelteon, certain antidepressants, and the so-called z-drugs, i.e. the cyclopyrrolones zopiclone and eszopiclone, the pyrazolopyrimidine zaleplon, and the imidazopyridine derivative, zolpidem, imidazole.

Flurazepam (flurazepam hydrochloride), a benzodiazepine derivative, is a hypnotic agent which does not appear to decrease dream time as measured by rapid eye movements (REM). Flurazepam decreases sleep latency and several awakenings for a consequent increase in total sleep time.

The duration of hypnotic effect and the profile of unwanted effects may be influenced by the alpha (distribution) and beta (elimination) half-lives of the administered drug and any active metabolites formed. When half-lives are long, the drug or metabolite may accumulate during periods of nightly administration and be associated with impairments of cognitive and motor performance during waking hours. If half-lives are short, the arid drug metabolites will be cleared before the next dose is ingested, and carry-over effects related to sedation or CNS depression should be minimal or absent. However, during nightly use and for an extended period, pharmacodynamic tolerance or adaptation to some effects of benzodiazepine hypnotics may develop. If the drug has a very short elimination half-life, it is possible that a relative deficiency (i.e., about the receptor site) may occur at some point in the interval between each night's use. This sequence of events may account for two clinical findings reported to occur after several weeks of nightly use of rapidly eliminated benzodiazepine hypnotics: 1) increased wakefulness during the last third of the night; and 2) the appearance of increased daytime anxiety. Flurazepam is a benzodiazepine with a long half-life [10].

Zolpidem has proved to be a suitable hypnotic, especially about efficacy in sleep initiation. As will be discussed below, it is relatively well tolerable and almost devoid of the side effects typically associated with BDZs. The low incidence of side effects is, in part, a consequence of a relatively short half-life in the circulation. At usual doses of immediate-release (IR) Zolpidem, the peak plasma concentrations are attained between about 45 min and 2 h after intake [11], a kinetics profile that corresponds well with the time course of psychomotor tests, in which the maximum efficacy was found around 1.5 h followed by a rapid decline [12]. The pharmacokinetics of such a short-acting drug, when given as an IR formulation, maybe not ideal in terms of promoting sleep maintenance. To respond to the clinical need for an agent which could reduce the number of nocturnal awakenings, Zolpidem extended-release was developed.

In this study, we want to compared the effects of two drugs Flurazepam-15 mg and Zolpidem -10 mg at psychiatric patients with sleeping disturbance.

Material and Methods

A blind, placebo-controlled, comparative study was made. The study lasted for six weeks.

The study involved 45 patients, divided into three equals phase (two weeks each), in the first phase receiving zolpidem, in the second phase-placebo only, and in the third phase was administrated-flurazepam only as an evening therapy. 35 (77.8%) of them had diagnosis schizophrenia, and 10 (22.2%) had psychosis. Thirty (66.7%) were men, and 15 (33.3%) were women. The average age is 42.5 y., ranging from 36 to 56 (minimum 36 y and a maximum of 56 y). The drugs are taken at night before bedtime.

The patients were informed about the study, and they had to follow the investigator instructions. Patients were instructed not to nap or drink alcohol during the weeks of the study, or to consume food or caffeinated beverages after 7:00 p.m.

We used self-administrative questioner to evaluation the effects of 13 items. Every day the quality of sleeping was estimated by the authors and patients helped by standardised questionnaires. The question referred to sleeping induction; it`s duration and quality, several awakenings through the night, subjective fill in-of “good rest”, hangover, tiredness through the day. One week after the third phase patients were observed whether should appear any abstinential symptoms.

Answers were summarised and statistically evaluated. Statistical analysis of quantitative data was performed by an analysis of variance (ANOVA) for repeated measures. In those cases, in which the ANOVA showed a significant drug effect, comparisons between individual treatments were performed by a post hoc least significant difference test. Categorical data were assessed by the use of chi-square tests and Fisher exact 2 tailed.

There were no reports of amnesia, disorientation, hallucinations or other major side effects.

Results

Results showed that the active drugs were efficient in controlling insomnia.

Both drugs improved a measure of "how good a night's sleep", and zolpidem improved the score on a question of "how rested do you feel". There was a trend for both compounds to reduce "difficulty getting to sleep" (Table 1).

TABLE 1: Drug-induced changes in subjective measures of sleep over the entire night as seen on the Morning Sleep Questionnaire

	Placebo	Flurazepam	Zolpidem	P
How good a night's sleep?[a]	2.3 ± 0.4	3.2 ±0.3*	3.2 ± 0.3*	< 0.05
How rested do you feel?[b]	1.2 ± 0.2	1.5 ± 0.2	1.8 ± 0.2*	< 0.05
Difficulty getting to sleep?[c]	3.0 ± 0.2	2.2 ± 0.3	2.1 ± 0.4	> 0.05

"p" values in the right-hand column refer to the results of an analysis of variance; [a] = very poor; 2 = poor; 3 = usual quality; 4 = excellent; [b] = much less rested; 2 = rested as usual; 3 = much more rested; [c] = less difficult; 2 = usual amount; 3 = more difficult; 4 = didn't sleep; * Differs from placebo by $p < 0.05$ by post hoc least significant difference test.

The results of this trial/study show that there is no statistically significant difference between Zolpidem and Flurazepam referred to the induction of sleep. Significant differences appear between the hypnotics and placebo (Table 2).

Table 2: Comparison between periods of investigation

		Placebo	Fluraz epam	Zolpidem	F/P p	Z/P p	F/Z p
1. How much time you need to fall asleep	≤ 30min	10	37	36	< 0.05	< 0.05	> 0.05
	> 30min.	35	8	9			
2. How you fall asleep	fast	10	37	36	< 0.05	< 0.05	> 0.05
	slow	35	8	9			
3. Habitual total sleep time (hours)	> 5h	11	39	35	< 0.05	< 0.05	> 0.05
	< 5h	34	6	10			
4. Do you feel sleepy enough	yes	11	39	35	< 0.05	< 0.05	> 0.05
	no	34	6	10			
5. How many times did you wake during the night	0 - 1	12	39	36	< 0.05	< 0.05	> 0.05
	> 2	33	6	9			
6. How you slept	well	10	40	37	< 0.05	< 0.05	> 0.05
	bad	35	5	8			
7. You wake up	easy	36	41	40	> 0.05	> 0.05	> 0.05
	difficult	9	4	5			
8. How do you feel after waking up	good	29	39	31	< 0.05	> 0.05	< 0.05
	bad	16	6	14			
9. Do you dream	yes	32	37	35	> 0.05	> 0.05	> 0.05
	no	13	8	10			
10. Do you feel tired through the day	yes	18	9	15	< 0.05	> 0.05	> 0.05

Significant differences appear between the Flurazepam and placebo at Q1-6 and Q8, not significant at Q7 and Q9-10(SS Q1 and Q2: chi square = 32.4641, p = 0.000000; Q3 and Q4: chi square = 35.2800, p = 0.000000; Q5: chi square = 32.4641, p = 0.000000; Q6: chi square = 40.500, p = 0.000000; Q8: chi square = 6.0160, p = 0.014176; Q10: chi square = 4.2857, p = 0.0384339; NS Q7: chi square = 2.2478, p = 0.13.3808; Q9: chi square = 1.5528, p = 0.212723;).

Significant differences appear between the Zolpidem and placebo at Q1-6, not significant at Q7-10(SS Q1 and Q2: chi square = 30.0593, p = 0.000000; Q3 and Q4: chi square = 26.9720, p = 0.000000; Q5: chi square = 21,7143, p = 0.000000; Q6: chi square = 32.4641, p = 0.000000; NS Q7: chi square = 1.3534, p = 0.244680; Q8: chi square = 0.2000, p = 0.654720; Q9: chi square = 0.5256, p = 0.468448; Q10: chi square = 4.2857, p = 0.511683).

Significant differences appear between the Zolpidem and Flurazepam at Q8 (SS Q8: chi square = 4.1143, p = 0.042522), Not significant at Q1-7 and

Q9-10 (NS Q1 and Q2: chi square = 0,0725, p = 0.787699; Q3 and Q4: chi square =1,2162, p = 0.270104; Q5: chi square = 0.7200, p = 0.396143; Q6: chi square = 0.8092, p = 0.368595; Q7: Fisher exact 2 tailed p = 0.5; Q9: chi square = 0.2778, p = 0.598161; Q10: chi square = 2.0455, p = 0.152661).

Total sleeping time for both tested hypnotic drugs is approximately equal but significantly longer than the time when placebo administrated. The subjective judgment of the quality of sleep did not show a significant difference between the two hypnotics as well. Experience of “sleeping well” (pleasant dream) was present in both cases. Dreaming was also present.

Several “wakenings” was significantly lower when the hypnotic was administrated compared to placebo, which corresponds with the subjective judgment of the quality of the sleep.

Difficulties with morning wake up described as dizziness, hangover, drowsiness frequently present when Zolpidem was used, which was statistically as a not significant difference.

Daily tiredness and drowsiness particularly in the afternoon as a side effect appeared with Zolpidem, which was statistically as a not significant difference.

During the administration of both drugs, worsening of the principal disorder did not occur, as well as any somatic complication. Interruption of Flurazepam treatment did not cause abstinential difficulties.

Discussion

Individuals have different opinions they sleep well or not. Sleeping well usually means short time “to fall asleep” few times wreaking up through the night, filling for “good rest” in the morning, opposite to “sense of tiredness” in lack of sleeping [13], [14]. The “ideal hypnotic drug” has to ensure quick falling asleep, permanent sleeping for 6-8h, without side effects in the next day [2], [15]. In the psychiatric population, we often meet insomnia as initial one, in introductory and developed form of psychosis, such, also as terminal in depression.

At the beginning of treatment, hypnotics are commonly administrated besides other antipsychotic or antidepressant therapy.

Insomnia is a frequent symptom in everyday psychiatric practise and prescribing hypnotic medicines as well. Because of this, there are specific risks for developing psychophysical addiction and possibility for suicide abuse, before prescribing priority, it is necessary to estimate ethyology and possibility for correction of the causes.

Global statistical analysis regarding all parameters shows the equivalence of both of the hypnotics. Basic qualities of the used drugs, efficiency, tolerance and compatibility are present in both of them, but there is a difference in favour of Flurazepam in terms of better tolerance.

About recommendations in the treatment of insomnia, one should have to distinguish between the different causes and clinical phenomenology of the various forms of this disorder. If sleep disturbances are primarily associated with psychiatric disorders, in particular, depression, the usefulness of hypnotics needs to be carefully monitored, and interference with antidepressants has to be taken into consideration. Zolpidem may be used and has already been successfully tested in MDD [16], [17], [18] and GAD [19], but due caution is still recommended for long-term treatment [20], [21]. In these cases, a drug like agomelatine may be an alternative medication of choice, in as much as it combines sleep-inducing, melatoninergic properties with actions of an antidepressant [22], [23], [24].

Most of the patients chronically use hypnotics together with other psychotropic drugs, so this examination does not fill full all requirements from a pharmacological point of view which we should consider and also commonly used a combination of medicaments should be considered as well.

The perceived and measured effectiveness of Zolpidem and Fluarezepam in decreasing sleep latency, increasing and maintaining sleep duration, and improving sleep quality without causing significant side effects or affecting next day performance suggests that Zolpidem and Fluarezepam are important in the treatment of insomnia.

To conclude Flurazepam beside its clinically equal efficiency as Zolpidem, most common hypnotic drug, has an advantage with good tolerance and the possibility of safe combination with antipsychotic or antidepressant medicines. But still, psychiatric observation and control are often necessary also reduction or discontinuing the usage as prevention to a possible threat of addiction particularity in risk cases. Adverse effects are moderate, frequently in the incidence range of placebos, and certainly less frequent and severe.

We had a very similar conclusion with Montie et al. [16]. The evaluation and management of insomnia are often challenging. Insomnia is a multidimensional disorder, and consequently, any approach to its management should consider a combination of both pharmacological and non-pharmacological measures. In practice, pharmacological approaches tend to be used with greater frequency than psychotherapy and other treatment methods. In patients with chronic insomnia and a coexisting psychiatric, neurologic or medical

condition, the underlying disorder needs to be treated appropriately [16].

Insomnia involves multidisciplinary fields of research. In recent years, advances have been made in the understanding of insomnia and its treatment options [2], [25]. However, the breakdown of disciplinary boundaries makes it more difficult for scientists or clinicians to reconcile all of the publications relevant to their research [2], [26]. We recommend that both pharmacological and nonpharmacological treatment for insomnia can have great potential for advancement in future years. Although sedative and hypnotic drugs dominated insomnia treatment for a long time, nonpharmacological therapies such as cognitive behavioural therapy have attracted considerable attention in recent years, for the benefits of reducing dosage and side effects of medication and providing alternative options. Also, the treatment efficacy and clinical outcome were not equally established for insomnia treatment modalities.

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Clinical Science

Clinical Outcome of Arthroscopic Posterior Cruciate Ligament Reconstruction with Adjustable-Loop Femoral Cortical Suspension Devices

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Abstract

BACKGROUND: Incidence of isolated posterior cruciate ligament (PCL) injury is lower than PCL rupture is associated with other knee injuries. Adjustable loop femoral cortical suspension device is commonly used for femoral graft fixation during PCL reconstruction.

AIM: This study purpose is to describe the functional outcome of PCL reconstruction using an adjustable loop femoral cortical suspension device.

METHODS: This study used prospective design with consecutive sampling. All patients underwent PCL reconstruction with adjustable loop femoral cortical suspension devices using peroneus longus tendon autograft. Patients were evaluated at 6 months after surgery using posterior drawer test and functional outcome scoring system (Lysholm knee score, Cincinnati Score and International Knee Documentation Committee (IKDC) score).

RESULTS: 20 patients were enrolled in this study with a mean age of 27.65 ± 9.78. Lysholm knee means the score was improved from 59.80 ± 18.73 pre-operative and 80.55 ± 11.72 post-operative ($p < 0.05$). Cincinnati mean score was improved from 52.01 ± 20.29 pre-operative to 72.95 ± 15.26 post-operative ($p < 0.05$). IKDC mean score was improved from 48.36 ± 13.18 at pre-operative to 72.5 ± 13.13 post-operative ($p < 0.05$).

CONCLUSION: PCL reconstruction using adjustable loop femoral cortical suspension device using peroneus longus tendon autograft showed good clinical outcome and knee functional outcome (Lysholm, Cincinnati, and IKDC score) at 6 months follow-up.

Introduction

Posterior cruciate ligament (PCL) injury is a rare case. Shelbourne et al. reported that PCL tears occurred in 1%-44% of all acute knee injuries and presented concomitant with complex knee trauma [1]. PCL reconstruction purpose is to restore knee stability and to prevent the development of osteoarthritic changes in knee joint [2]. The principles of PCL reconstruction are identifying and treating the pathology, placing tunnels accurately to produce anatomical graft insertion sites, utilising strong graft material, mechanical – tensioning of the graft, fixating the graft and giving the optimal post-operative rehabilitation program [3].

The methods of femoral graft fixation for PCL

reconstruction are interference screw, cortical suspension devices and cross-pins [4]. There are 2 common types of cortical suspension devices; fixed loop and adjustable loop. The fixed loop cortical suspension device is a graft fixation device which the graft is attached to a continuous suture loop that is connected to a button. This device is fixed at the distal femoral cortex, and the tunnel is filled with the graft without any implants needed. The fixed loop button demonstrates desirable biomechanical properties when it fixes the hamstring graft. The newest study had shown that the use of suspensory devices in PCL reconstruction has advantaged in the length of the graft used and provided stable fixation [5], [6].

In contrast, an adjustable loop cortical suspension device has a button that is attached to the graft through the adjustable loop. Its loop is tightened to pull the graft through to the proximal of the femoral tunnel, which eliminated the additional tunnel length to flip the button [7]. Adjustable loop button allows the surgeon to adapt tunnel length difference intra-operatively. It can avoid the necessity for drilling a longer tunnel and maximise the amount of graft within the tunnel by fulfilling the bone tunnel. An additional advantage of the adjustable loop button includes the ability for graft retention on the femoral side after tibial fixation. However, the flexibility of the loop length of the adjustable loop button is the need to concern, because it can increase post-operative graft slippage [8], [9].

This study purpose is to evaluate the functional outcome after PCL reconstruction with adjustable loop cortical suspension device using peroneus longus tendon autograft at 6 months follow-up.

Methods

This study was a prospective design with consecutive sampling. Twenty patients underwent PCL reconstruction from December 2016 until August 2018. Inclusion criteria were PCL rupture patient with the age range between 18-45 years old, diagnosed with positive posterior drawer test grade 3 and confirmed with Magnetic Resonance Imaging (MRI). Exclusion criteria were chondral damage, fracture at knee region, and pathologic condition in the lower extremity. All patients underwent PCL reconstruction with peroneus longus tendon autograft using adjustable loop femoral cortical suspension device (GraftMax^{TM} Button, Conmed, USA). All patients were followed up at minimum 6 months post-operative. This study evaluated posterior drawer test and kneed functional score Lysholm knee score, Cincinnati score, and International Knee Documentation Committee (IKDC) score. JAH performed clinical outcome evaluation. This study was reviewed and approved by the Medical and Health Research Ethics Committee at the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada (IRB number KE/FK/0258/EC/2019).

Surgical Technique

Under spinal anaesthesia, the patient was in the supine position, and the patient's thigh has applied the tourniquet over the cast padding. SR did all of PCL reconstruction procedure. Using a distal foot stop and lateral support, the knee was retained in 90° of flexion, varus or valgus stress manoeuvres allowed and full passive range of motion performed easily. We used standard arthroscopic examination with a 30° arthroscope using standard anteromedial (AM) and anterolateral (AL) portals to evaluate any pathology. PCL rupture was confirmed. The minimal amount of PCL remnant was excised with 4.2 mm shaver from the AM portal to improve visualisation. The arthroscope can be easier introduced into the posteromedial compartment.

By the AL portal through the intercondylar notch, the 30° arthroscope was passed between the medial femoral condyle and the PCL remnant to achieve the posteromedial compartment. A spinal needle was inserted with an arthroscopic guide to making a posteromedial (PM) portal with a number 11 blade approximately 5-10 mm above the tibial surface and posterior to the medial femoral condyle. The arthroscope was moved to the AM portal and placed in the posterolateral compartment through the intercondylar notch, lateral to ACL fibres. The knee should in 90° of flexion position during PL and PM portals creation to prevent any damage to the vessels and nerves. The distance between the PM portal and 2 branches of the saphenous nerve is approximately 17 – 20 mm and between the PL portal and common peroneal nerve is 25 mm in 90° flexion position.

Graft Preparation

The peroneus longus tendon autograft was harvested using an open tendon stripper with 1.5 cm skin incision about 2 cm above the lateral malleolus. The distal insertion of peroneus longus was sutured with the peroneus brevis tendon. The surgeon was cut the tendon above the sutured site. Peroneus longus tendon length was obtained maximal length approximately 3 fingers below the fibular head to prevent injury to the common peroneal nerve.

Femoral Tunnel Preparation

The femoral footprint was visualised and cartilage border was identified with the radiofrequency probe. The knee was flexed 90°. The femoral PCL guide was positioned at the condyle's articular surface using 2.4 mm guide passing pin until penetrate the

medial femoral cortex (PCL femoral origin). A 4.5 mm cannulated drill was used to create the first full-length passing tunnel. Cannulated drill which matches the diameter of the harvested graft was made through that tunnel. The depth of the socket was calculated based on the length of the prepared graft (usually 25-30 mm). The free end of number 2 Vicryl suture loop is advanced out the AM thigh using the guide passing pin. The arthroscope was moved to the AL portal, and the femoral blue Vicryl was taken from the AM portal.

Tibial Tunnel Preparation

The surgeon made an accessory portal with needle guide at medial to the lateral part of the medial femoral condyle (usually passed through the patellar tendon). The surgeon cleaned this side to make a better visualisation of PCL remnant. PM portal was made with transillumination guide, and the needle was kept in line with posterior plateau. The shoulder trochar was put in PM portal. The PCL tibial guide that was set at 65 was placed through the AM portal at the anatomic position of the PCL insertion (middle of the PCL remnant). The surgeon made an incision about 2 cm at the proximal medial tibia and placed the drill sleeve. With the 30 arthroscopes in the PM portal, the surgeon drilled a 2.4 mm guide pin carefully into the tibia to avoid the posterior neurovascular structures damage. To confirm sagittal plane of guidewire placement was right, the surgeon used assisted fluoroscopy. The protection curettage was inserted from the AM portal and placed over the 2.4 mm guidewire. A cannulated reamer that matches to graft diameter is used for the final tibial tunnel preparation. Soft tissue remnant was removed at the posterior end of the tibial tunnel by shaver or radiofrequency probe through the tibial tunnel. By keeping the arthroscope in the PM portal, a looped number 3 nonabsorbable suture was inserted into the tibial tunnel using a suture passer with an eyelet. This suture was retrieved from the AM portal through the intercondylar notch with an arthroscopic grasper. The suture was tied with the femoral tunnel suture and was pulled through the tibial tunnel. The knot between the sutures was opened and removed (tibial tunnel suture).

Graft Passage and Fixation

The graft was passed through the tibial tunnel. The difficult part of the procedure was passing the graft gradually through both the tibial and femoral tunnel, which was in the opposite direction. The surgeon tried to reduce as much as possible of excessive friction between the graft and the tunnel that may lead to entrapment or even rupture of the graft. For this reason, we divided the procedure into 2 steps: first, the sutures of the normal pull-up (femoral side of the graft) was shuttled through the tibial tunnel and was taken through the AM portal by the number 3 nonabsorbable (green) Mersuture. The killer turn angle at the posterior exit of the tibial tunnel was the most dangerous step because of the severity of the reflexing angle and the difficulty of controlling the graft progression of the hidden and narrow compartment, especially when used anterior viewing portal. Therefore, the arthroscope can be placed in the PM portal. While the assistant was pulling the sutures of the pull-up through the AM portal, the surgeon was using the switching stick from the PL portal as a pulley to help the progressive graft passage until the tibial side mark appeared posteriorly (a 2 cm length was left in the tibial tunnel). Second, the loop of the number 3 non-absorbable Mersuture (tibial tunnel) was passed through the loop of the number 2 Vicryl (femoral tunnel).

Consequently, the traction sutures of the normal-sized pull-up were passed through the number 2 Vicryl suture and shuttled directly through the femoral tunnel. The femoral pull-up was flipped over the medial femoral cortex and was secured into the prepared socket by pulling its adjustable loop suture the graft. The surgeon performed full ROM. Final PCL tensioning was performed by pulling the sutures and securing the suture with a bio-absorbable screw at the tibial side. During final fixation, the knee is retained in 70° of flexion, and an anterior drawer was applied.

Single bundle arthroscopic PCL reconstruction with adjustable femoral cortical suspension device

The graft was passed through the tibial tunnel, killer turn angle and femoral tunnel with suture guide. The suture guide was pulled until all the graft suture had passed the femoral skin. The grey suture (the button suture) was pulled until slipped with the blue Vicryl. The blue-white suture was pulled until it had passed the femoral tunnel. The graft was fastened with bio-absorbable screw in the tibial tunnel with 90° knee flexion and anterior drawer of the tibia. The remaining graft was sutured with the fascia. The surgeon closed the skin, and the operation was done.

Results

During the period of the study, twenty patients fulfilled the inclusion criteria and underwent PCL reconstruction with adjustable loop cortical suspension device using peroneus longus tendon. There were twenty patients which consist of 15 males and 5 females. The patient's mean age was 27.65 ± 9.78 range from 16 until 55 years old. Site of injury was 13 at the right knee and 7 in the left knee. Injury mechanism occurred 6 in sport, 9 in a vehicle accident and 5 in another injury mechanism. Peroneus longus tendon means diameter was 8.35 ± 0.58 ranges from

7.50 to 10.00. Subjects' characteristics were shown in Table 1.

Follow-up evaluation using posterior drawer test at 6 months post-operative showed positive drawer test grade 1.

Table 1: Subjects' characteristics

Characteristics	Mean	SD	Min	Max	N (%)
Age	27.65	9.78	16.00	55.00	
Sex					
Male					15(75.0)
Female					5(25.0)
Injury site					
Right					13 (65.0)
Left					7 (35.0)
Injury mechanism					
Sport					6 (30.0)
Vehicle accident					9 (45.0)
Others					5 (25.0)
Graft diameter	8.35	0.58	7.50	10.00	

Abbreviations: SD: Standard Deviation; Min: Minimum; Max: Maximum; N: Number of Subjects

There were significant differences between the preoperative and 2-year postoperative score in Lysholm knee score, Cincinnati score, and IKDC score ($p < 0.05$), as shown in Table 2. Lysholm knee means the score was improved from 59.80 ± 18.73 pre-operatively to 80.55 ± 11.72 at 6 months follow-up. Cincinnati mean score was improved from 52.01 ± 20.29 pre-operatively to 72.95 ± 15.26 at 6 months follow-up. IKDC mean score was improved from 48.36 ± 13.18 pre-operatively to 72.5 ± 13.13 at 6 months follow-up.

Table 2: Functional outcome

Scoring assessment	Pre-operative		Post-operative		Significance
	Mean	SD	Mean	SD	
Lysholm	59.80	18.73	80.55	11.72	0.000
Cincinnati	52.01	20.29	72.95	15.26	0.000
Ikdc	48.36	13.18	72.50	13.13	0.000

Abbreviations: SD: standard deviation.

Discussion

Our main finding in this study was that PCL reconstruction using adjustable loop femoral cortical suspension had satisfactory clinical outcomes. There were only two studies which reported clinical outcomes of PCL reconstruction with adjustable loop suspension device (Freychet et al., and Setyawan et al.,). Our study emphasised their findings that the PCL reconstruction technique would yield favourable results [10], [11].

Freychet et al. found that the mean postoperative IKDC and Lysholm score were 85.0 (SD 13.5) and 87.4 (SD 13.1), respectively, meanwhile, in our study, the mean was 72.5 and 80.55. These differences might not be significant. It might cause by the different duration of the follow-up (24versus 6 months), different operation technique (double-bundle versus single-bundle technique). Setyawan et al. found that the mean postoperative IKDC, Cincinnati, and Lysholm scores were 78.17, 79.00 and 80.20, respectively, and the scores were improved significantly in 2 years follow-up. These findings may be attributable to the difference in the duration of follow-up (2 years versus 6 months), but might not be significant statistically [10], [11].

Peroneus longus tendon autograft has several advantages, including no anterior knee pain, no kneeling pain, and reduce the incidence of postoperative thigh hypotrophy [11]. Setyawan et al. explained that the usage of peroneus longus tendon gave excellent ankle functional score based on FADI and AOFAS score [11]. However, some disadvantages of peroneus longus usage are still debatable. A biomechanical study that explained tensile strength comparison between peroneus longus tendon, hamstring tendon, patellar tendon, and quadriceps tendon showed that the tensile strength of peroneus longus was comparable to hamstring tendon, and was significantly stronger than patellar tendon and quadriceps tendon [12].

Adjustable loop suspension device has an advantage including reducing tunnel widening because it can reduce the distance between the button and the proximal end of the graft [7]. However, there were still few studies that described the usage of adjustable loop suspension in PCL reconstruction.

Recent systematic review and meta-analysis by Lee et al. concluded that biomechanically double-bundle is more superior to single-bundle PCL reconstruction in terms of anteroposterior stability [13]. A recent systematic review by Qi et al., and Chahla et al., found no differences in patient-reported outcomes [14], [15]. Following recent evidence, we would prefer using single-bundle PCL reconstruction due to simpler surgical techniques and similar outcomes.

In our study, we only included patients with isolated PCL injuries, excluding multi ligamentous knee injury. Interestingly, Freychet et al., found that there was no significant difference in outcome scores when the injury was stratified by Knee Dislocation classification in 2 years of follow-up [10]. Mygind-Klavsen et al. found that patients with a multi ligamentous knee injury and isolated PCL injury would have identical functional and objective outcomes with a mean follow-up of 5.9 years [16]. Spiridonov et al., also reported that there was increased significantly in Cincinnati and IKDC score in both isolated and multi ligamentous knee injury [17].

PCL reconstruction is rare and technically challenging than ACL reconstruction. Limitation to visualise posterior compartment with standard AM and AL portals and the risk of neurovascular injuries may lead to limb-threatening complication. Adjustable loop suspension device may accomplish a satisfactory size of PCL graft, and the peroneus longus tendon length restriction can be avoided. This study has several limitations. There is no long-term result and no control group. We also used prospective design and limited

sample size, which was because of a small number of isolated PCL injuries. We would recommend more extensive studies with bigger sample size, control group and randomised controlled trial study design usage. Despite these limitations, the procedure was shown favourable results.

In conclusion, PCL reconstruction with adjustable loop femoral fixation device using peroneus longus tendon autograft was shown good knee functional outcome score at 6 months follow-up.

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Clinical Science

The Outcome of Diabetic Patients with Cardiomyopathy in Critical Care Unit: Hospital and Short-Term Outcome in a Period of Six Months to One Year

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Abstract

BACKGROUND: Diabetes mellitus (DM) is a major risk factor for heart failure (HF) and coronary artery disease (CAD). DM may cause structural changes involving the left ventricle (LV) systolic and diastolic function.

AIM: To compare patients who have diabetes and ischemic cardiomyopathy (ICM) to those with diabetic cardiomyopathy (DMCMP) regarding LV systolic function, diastolic function, in hospital long term and short-term mortality.

METHODS: Ninety diabetic patients with heart failure and left ventricular ejection fraction (LVEF) ≤ 35%, admitted to Critical Care Medicine department Cairo University were divided into two groups based on coronary angiography results; group I (ICM) n = 48 patients and group II (DMCMP) n = 42 patients.

RESULTS: Group I patients had higher mean age (63 ± 7 years), (p = 0.004), Hypertension (p < 0.001) and dyslipidemia (p = 0.008) were significantly more present in group I compared to group II. No significant differences were found regarding LVEF, global longitudinal strain (GLS), E/A and E/É ratio in both groups. A significant difference in the wall motion score index (WMSI) in group I; (1.4 ± 0.4) versus group II; (1.1 ± 0.2), (p = 0.005) was found. In the study, 6 patients had a cardiogenic shock with no documented in-hospital mortality. At 6 months, statistically, significantly higher mortality rates were found in group I, (p = 0.006), while at one year there was no significant difference in the mortality between the two groups, (p = 0.077). In comparison of the survived and non-survived patients at 6 months and one year in group I (ICM) there was a significant difference in LVEF (40 ± 6% vs 23 ± 6%, p < 0.001), GLS (- 8.1 ± 2.4 vs - 4.6 ± 2.6, p = 0.007), E/A (1.25 ± 0.91 vs 1.8 ± 0.5, p = 0.038), E/É (11.68 ± 7.5 vs 21.3 ± 3.6, p = 0.001) respectively. In group ll (DMCMP) there was no documented mortality at 6 months follow up, however, at one year there was statistically significant difference in the mortality between survived and non-survived patients; the LVEF (35 ± 8% vs 25 ± 2%, p = 0.014), GLS (-7.9 ± 2.9% vs -5 ± 0.1%, p = 0.032), E/A (1.45 ± 0.8 vs 3.3 ± 0, p = 0.006) respectively. The E/É ratio in group ll was not significantly different between the groups (15.73 ± 5.3 vs 15 ± 1, p = 0.873).

CONCLUSION: The combination of cardiomyopathy and diabetes affects LV systolic and diastolic function; however; ischemic cardiomyopathy and diabetic cardiomyopathy had a similar systolic and diastolic function. Ischemic cardiomyopathy is associated with worse prognosis compared to diabetic cardiomyopathy.

Introduction

Diabetes mellitus (DM) is a major risk factor for cardiovascular diseases, including coronary artery disease (CAD), congestive heart failure (CHF) and atrial fibrillation [1]. DM is associated with increased risk of cardiovascular-related deaths. Diabetes can lead to heart failure not only by augmenting coronary artery disease through macroangiopathy but also through structural changes involving the left ventricle (LV) causing systolic and diastolic dysfunction [2].

We aimed to compare diabetic patients, who have ischemic cardiomyopathy (ICM) to those with diabetic cardiomyopathy (DMCMP) in terms of clinical course, left ventricular (LV) systolic function, diastolic function, in-hospital long and short-term mortality.

Methods

Our study included 90 diabetic patients with decompensated heart failure due to cardiomyopathy with LVEF ≤ 35% admitted to Critical Care Medicine department over 16 months (March 2016- July 2017). Excluded from the study were patients with valvular heart disease, patients with diastolic heart failure and those with poor echocardiography window. The study was approved by the ethical committee at the faculty of medicine at Cairo University. Written consent was taken from all patients on admission.

Complete disease history was performed for all patients, analysis of risk factors of coronary artery disease (CAD) and heart failure such as arterial hypertension, dyslipidemia, smoking and family history of CAD; detailed physical examination with special emphasis on Killip classification; coronary angiography to differentiate ischemic from diabetic cardiomyopathy and echocardiographic assessment of LV systolic and diastolic function using ultrasound machine (Philips ultrasound,100-127/220240V~50/60Hz,1010 VA).

Echocardiography included the conventional 2D examination and speckle tracking to assess LV strain. The study was stored in a digital format with patient identity and file number.

The study was analysed by two experienced echocardiographers blinded to the study; the following parameters were measured for evaluation of LV geometry and function: left ventricular end-diastolic dimension (LVEDD), left ventricular end-systolic dimension (LVESD), left ventricular ejection fraction (LVEF) measured using the modified Simpson's method.

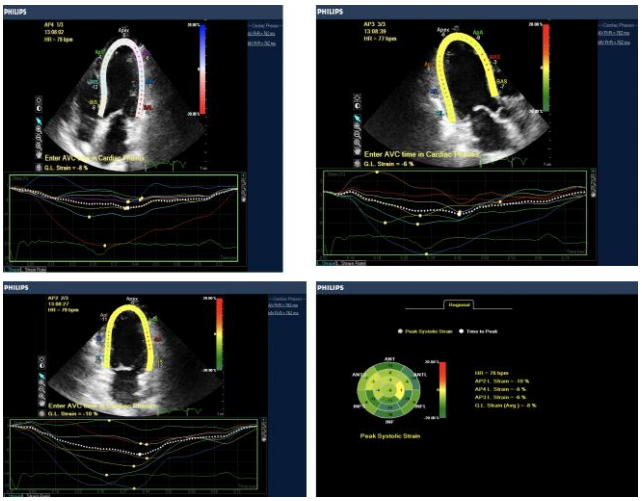

Figure 1: Example of assessment of global longitudinal myocardial strain (GLS) as provided by the EchoPAC software: apical long-axis view, 4-chamber view, and 2-chamber view. In the lower panel, the "bull's eye" plot, using a 17-segment model, provides the value of longitudinal strain for each segment of the left ventricle and the values of longitudinal strain of apical long-axis (GLPSS-LAX), 4-chamber (GLPSS A4C), 2-chamber (GLPSS A2C), and the value of GLS(GLPSS Avg)

Quantification of LV mechanics was done according to the recommendation using 2D speckle tracking echocardiography. A standard 2D ultrasound images were obtained. Three waves were analysed for longitudinal LV strain in the apical 4 chamber, apical 3-chamber and apical 2-chamber views. Cut off values of less than -20% were used as indicators of systolic dysfunction.

The regional wall motion abnormality (RWMA) was expressed by wall motion score (WMSI) which was calculated according to American Society of Echocardiography 17-segments model in which (normal = 1, hypokinetic = 2, akinetic = 3, dyskinetic = 4, aneurysmal = 5) Score was calculated by averaging the sum of the 17 segments. RWMA was considered present if WMSI > 1 [3].

Assessment of diastolic function was done according to the update of the American society of echocardiography imaging and the European association of cardiovascular imaging (2015) [4]. Mitral inflow was assessed by pulsed-wave Doppler from apical four-chamber view during diastole.

A one or two mm sample volume was placed between the tips of mitral flow leaflets during diastole and the following parameters were measured: peak E velocity (m/s), peak A velocity (m/s), E/A ratio, annular É (m/s) by tissue Doppler at the level of mitral annulus and the E/É ratio. The E/É ratio > 15 indicates elevated left ventricular filling pressure (LVFP), whereas E/É < 8 indicates normal left ventricular filling pressure [5].

The study population was divided into two groups based on coronary angiography data: Group I included patients with ischemic cardiomyopathy (n = 48). Group II included patients with diabetic cardiomyopathy (DMCMP) (n = 42).

Ischemia was defined as inadequate blood supply (circulation) to a local area due to blockage of the blood vessels supplying that area. Stenosis of 70% in a main coronary artery (> 2.5 mm) in one angiographic projection, or 50% in two projections, and 50% of the left main coronary artery [6].

Data were coded and entered using the statistical package SPSS (Statistical Package for the Social Sciences) version 24. Data were summarized using mean, standard deviation, median, minimum and maximum in quantitative data Comparisons between quantitative variables were done using the non-parametric Kruskal-Wallis and Mann-Whitney tests *(Chan, 2003a)* [7]. For comparing categorical data, Chi-square ($\chi 2$) test was performed. Exact test was used instead when the expected frequency is less than 5 *(Chan, 2003b)*. P-values less than 0.05 were considered statistically significant [8].

Results

The mean age of the whole study group was 60 ± 10 years, with 18/90 females (20%) and 72/90 males (80%). The mean age in group I was 63 ± 7 years and 55 ± 11 years in group II with a statistically significant difference, (p = 0.004). Both groups had the same gender distribution (9 females in each group), (Table 1).

Table 1: Mean age of study groups

ICM n = 48		DMCMP n = 42	
Age	Mean ± SD	Mean ± SD	*p*-value
	63+7	55 +11	0.004

There was a statistically significant difference between both groups regarding HTN and dyslipidemia, both with a higher incidence in group I, p < 0.001; p = 0.008 respectively, (Figure 2).

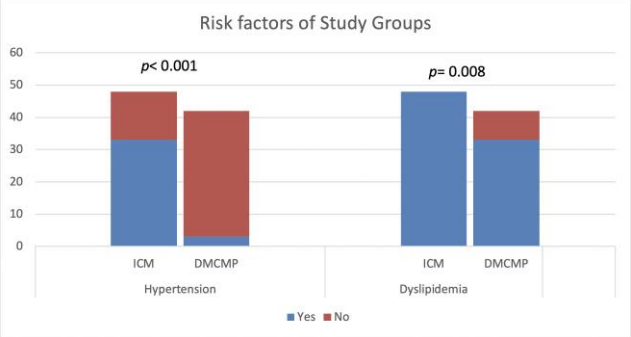

Figure 2: Hypertension and dyslipidemia as risk factors in both groups and p-value

There was no statistically significant difference between both groups regarding the type and duration of DM, wherein group I; 27/48 patients (57%) had non-insulin dependent type two diabetes mellitus (T2DM) and the mean duration of DM was 7.7 ± 2.6 years. In group II; 21/42 patients (50%) were non-insulin dependent T2DM, and the mean duration of DM was 8.3 ± 3.8 years.

All studied patients were classified according to Killip classification; with no statistically significant difference between the two groups, (p = 0.131), (Table 2).

Table 2: Killip classification in both groups

Killip Classification	ICM n = 48 (%)	DMCMP n = 42(%)	*P*-value
Class II	15/48 (32)	18/42 (43)	
Class III	30 /48 (62)	21/42 (50)	
Class IV	3/48 (6)	3/42 (7)	0.131

Out of the studied population, 33 patients (36%) were on inotropic and vasopressor support, namely dobutamine and norepinephrine, out of whom 21 patients were in group 12 patients and I in group II.

Comparison of both systolic and diastolic function of both groups

The left ventricular internal dimensions were not statistically significantly different between both groups; the mean LVEDD was 6.1 ± 0.9 cm in group I and 6.1 ± 1.2 cm in group II with p = 0.926, the mean LVESD in group I was 4.8 ± 1 cm and 5 ± 1 cm in group II with p = 0.682.

There was no statistically significant difference between the two groups regarding global LV systolic function. The mean LVEF was (36 ± 9% in group I versus 35 ± 8% in group II, P = 0.497). The mean GLS was (-7.7 ± 3% in group I vs -7.9 ± 2.9% in group II p = 0.674). The mean WMSI was statistically significantly different in both groups, (1.4 ± 0.4 vs 1.1 ± 0.2, respectively, p = 0.005).

There was no statistically significant difference between both groups as regards to LV diastolic function except for annular É. The mean E/A ratio was (1.5 ± 0.9 in group I vs 1.45 ± 0.8 in group II, p = 0.417) while the mean É was statistically significant (4.6 ± 1.3 m/s in group I vs 6.1 ± 2.7 m/s in group II, p = 0.009), the mean E/É ratio was (15.6 ± 5 in group I vs 15.73 ± 5 in group II, p = 0.278).

In-hospital survival at 6 months and one year of both groups

All patients had survived in-hospital course with no documented mortality, even those admitted with cardiogenic shock. After 6 months, 9 patients died in group I and non in group II with a statistically significant difference, p = 0.006. After one year 3 patients died in group II with no other documented mortality in group I and non-statistically significant difference, p = 0.077. So, the total mortality was 12 patients in the whole study population (Figure 3).

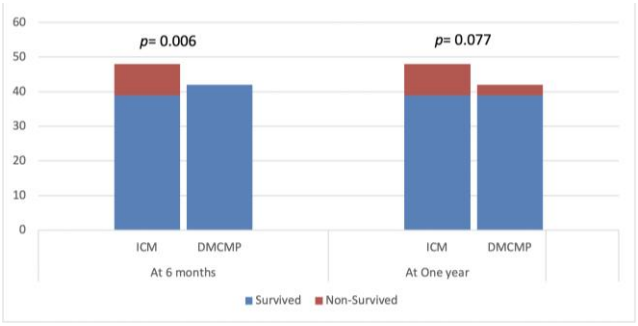

Figure 3: Survival rates at 6 months and one year in both study groups and p-value

The relation of the mean duration of diabetes mellitus to the mortality of patients in both groups was not statistically significant at both 6 months, and one year (p = 0.955 and 0.837 respectively).

Relation of systolic and diastolic function to survival at 6 months and one year in group I

We studied the relation of different echocardiographic values to the mortality after 6 months and one year in group I; there was a statistically significant difference between survived and non-survived patients in terms of LV systolic and diastolic function, with mean LVEF 40 ± 6% vs 23 ± 6%; p < 0.001 respectively, and mean GLS of -8 ± 2.4% vs -4.67 ± 2.6%, p = 0.007 respectively. The mean E/A ratio was 1.25 ± 0.91 vs 1.8 ± 0.5, p=0.038 and the mean E/É was 11.68 ± 7.5 vs 21 ± 3.6, p = 0.001 respectively in the group of survivors versus non-survivors. These findings remained constant after one year as there were no new mortalities recorded in this group (Figure 4).

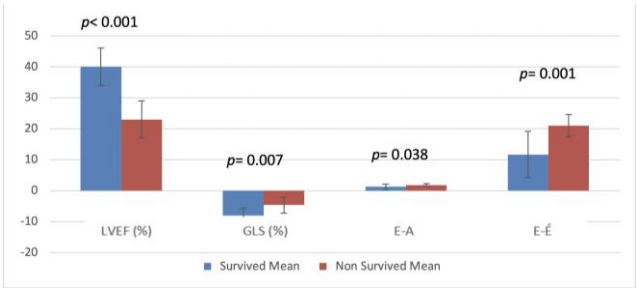

Figure 4: Systolic and diastolic echocardiography values at 6 months and one year in ICM

Relation of systolic and diastolic function to survival at 6 months and one year in group II

We studied the relation of echocardiographic values to the mortality after 6 months and one year in group II; at 6 months there was no mortality in this group, and the mean systolic function for survived patients was; LVEF (35 ± 8%), GLS (-7.9 ± 2.9%) respectively. The mean diastolic LV function for the survived group was: E/A (1.45 ± 0.8), E/É (15.7 ± 5.3) respectively.

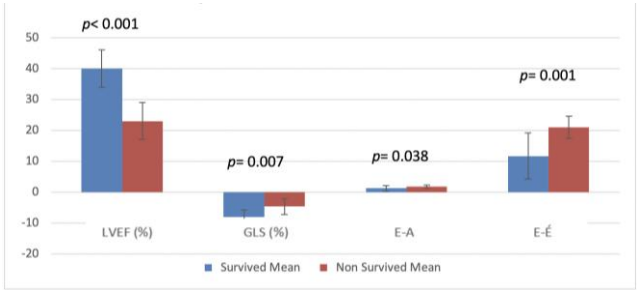

Figure 5: Systolic and diastolic echocardiography values at 6 months and one year in DMCMP

However, there was a statistically significant difference between survived and non-survived patients after one year in LV systolic and diastolic function, where the mean LVEF was 35 ± 8% vs 25 ± 2%, p = 0.014 and the mean GLS was -7.9 ± 2.9% vs -5 ± 0.1%, p = 0.032 respectively for the group of survivors and non-survivors. The mean E/A ratio in survived patients was 1.45 ± 0.8, and in the group of non-survivors 3.3 ± 0, p = 0.006. However, the mean E/É was non-significantly different in both groups of patients (15.73 ± 5.3 vs 15 ± 1, p = 0.873), (Figure 5).

Discussion

Diabetes mellitus (DM) is a chronic metabolic disorder with steadily increasing prevalence all over the world [9]. Diabetic cardiomyopathy (DMCMP) [10] is a cardiac dysfunction which affects approximately 12% of diabetic patients, leading to overt heart failure and death. However, there is no efficient and specific methodology for the diagnosis of diabetic cardiomyopathy, possibly because molecular mechanisms are not fully explained, and it remains asymptomatic for many years [11].

Left ventricular systolic function is routinely quantified by measuring LVEF [12]. Two-dimensional speckle tracking echocardiography in recent years has emerged as a method for assessing LV systolic function. Global longitudinal strain (GLS), obtained by 2-dimensional speckle tracking echocardiography is a measurement that has previously been demonstrated to be of prognostic value, GLS provided incremental prognostic information when added to a model including conventional echocardiographic parameter and clinical predictors [13].

In our study, the mean age in group I was 63 ± 7 versus 55 ± 11 years in group II with a statistically significant p = 0.004. Diastolic echocardiography indices in group I was higher with advanced age compared to group II, indicating the effect of age on diastolic function. These findings in our study were similar to that of Kane et al., (2011) who studied the effect of age on diastolic dysfunction. The study concluded that age-related progression of diastolic dysfunction in the population contributes to the pathophysiologic changes which cause severe heart failure in these patients [14].

In a group, I (57%) had non-insulin dependent T2DM, and the mean duration of DM was 7.7 ± 2.6 years while in group II (50%) were non - insulin-dependent with a mean duration of diabetes of 8.3 ± 3.8 years. We compared our study findings with that of *Zoungas S et* al., (2014), who studied the effect of mean age at diagnosis of diabetes and the duration of the disease which was 7.9 ± 6.4 years. He stated that the long duration of diabetes was associated with the risk of microvascular events and this effect was greater in the younger patients. No interaction was observed between diabetes duration, age and the risk of macrovascular events or death [15].

In our study we measured both LVEF and GLS in both our study groups as a marker of systolic function and we compared our results to Sengeløv et al., (2015) who stated that speckle tracking echocardiography, specifically GLS, is superior to conventional echocardiographic parameters, including left ventricular ejection fraction, in predicting all-cause mortality in patients with heart failure with reduced ejection fraction (HFrEF) [13].

Senglov et al. also investigated the prognostic value of global longitudinal strain (GLS) about the patient with HFrEF and concluded that GLS is an independent predictor of cause mortality and is a superior prognosticator compared to all other echocardiographic parameters in predicting mortality in these patients [13], [16].

The finding goes hand in hand with our results since the mortality rate was higher in patients with low GLS and low LVEF in both our study groups.

Also, Argulian et al., (2016) stated that the GLS is the most reliable method of detecting systolic dysfunction and that cut off value of (-20%) is considered normal while values less than (-20%) are abnormal and indicate systolic dysfunction [16]. We found that most of our patients had a GLS of less than (-15%), which indicated systolic dysfunction.

Radwan et al., in (2016) assessed the GLS in 80 patients who had cardiomyopathy and were divided into two groups, one with CAD and the other without CAD according to angiography. The study showed that the GLS measure is a sensitive and accurate tool in predicting severe CAD. The study used a low cutoff value of GLS -15.6% in which patients with GLS less than -15.6% had significant obstructive CAD stenosis > 70% [17].

In our study, the RWMA was assessed by measuring the (WMSI). There was a statistically significant difference between both study groups.

The wall motion score index (WMSI) in group I with ICM was higher than that in group II and this was explained by the presence of CAD and risk factors such as hypertension, dyslipidemia, and positive family history of CAD which played a role in the occurrence of wall motion abnormalities. In group II with DMCMP, the presence of wall motion abnormality might be explained by atherosclerotic changes which are pronounced in diabetic patients and also the development of micro thrombosis.

The findings in our study go hand in hand with Esmaeilzadeh et al. (2013) who studied the correlation between WMSI with coronary artery lesions. The study stated that a normal LV has a wall motion score index of 1 and the index increases as wall motion abnormalities increase in severity. The study concluded that a WMSI of 1.1-1.9 could predict small infarct size, and an index greater than 2.0 predicts the occurrence of complications and increase mortality [18]. However, a combined study of LVEF, WMSI and GLS proved superiority and accuracy of GLS in predicting long term outcome in ischemic cardiomyopathy [19].

LV diastolic function is assessed by many indices, such as the ratio of peak early to late diastolic filling velocity E/A ratio and tissue doppler mitral early diastolic velocity (E) combined with peak transmittal annular early diastolic velocity (É) in order to obtain a dimensionless index E/É, which provides a fair estimate of LV filling pressure [20], [21].

In group I; the mean É was (4.6 ± 1.3 m/s) while in group II the mean É was (6.1 ± 2.7 m/s) higher than that in group I with a statistically significant difference between the two groups, (p 0.009). However, the E/É ratio in group I was 15.6 ± 5 compared to a ratio of 15.73 ± 5 in group II with no statistically significant difference between the two groups. These findings showed that hypertension and CAD in patients with diabetes added to the risk of developing LV diastolic dysfunction. The E/E' of > 15 in patients with DM is associated with subsequent HF and increased mortality independent of HTN, CAD, or other echocardiographic parameters [22].

In our study, the mean E/A ratio for those who survived at 6 months was (1.25 ± 0.91 vs 1.8 ± 0.5) for non- survived patients in group I, while for group II there was no mortality at 6 months, and the mean E/A was 1.45 ± 0.8. The Strong Heart Study follow-up (2002) showed that, a transmitral E/A ratio < 0.6 (pattern of abnormal relaxation) is associated to a doubled increase of mortality risk and an E/A ratio > 1.5 (pattern pseudonormal/restrictive) is associated to a threefold increase of cardiac mortality [23].

In our study in terms of outcome and complication, both groups had survived the in-hospital course despite the presence of patients with cardiogenic shock. We had nine patients who died in group I after 6 months, and three patients died in group II after one year. Short term outcome goes in hand with Johansson et al., (2016) who found that type two diabetes mellitus (T2DM) was shown to be a predictor of mortality in both ischemic and non-ischemic heart failure, although the presence of ischemic heart disease (IHD) with T2DM appeared to have the worst outcome [24].

Our results also were similar to that of Sarma et al., (2013) who demonstrated in his study that diabetic patients with HF and low LVEF tend to have more co-morbidities and worse long- term outcomes after hospitalization, specifically increased rates of cardiovascular mortality and re-hospitalization after discharge, than those without DM, even after adjusting for baseline risk factors and medications, DM was associated with a (17%) increased risk for cardiovascular mortality and hospitalization for HF over a median follow-up of 9.9 months [25].

Limitation: We excluded a rather big sample from our final study results as the views were not

analysed by speckle tracking software and this was due to poor quality of images. The values for the strain parameters measured in this study were calculated using feature tracking post-processing software. This remains a research application and lacks the clinical validation to enable its adoption into routine clinical practice for the screening of diabetic cardiomyopathy.

In conclusion, the combination of cardiomyopathy and diabetes affects LV systolic and diastolic function; however; ischemic cardiomyopathy and diabetic cardiomyopathy had a similar systolic and diastolic function. Ischemic cardiomyopathy is associated with worse prognosis compared to Diabetic cardiomyopathy. We recommend conducting a larger study to evaluate the impact of DM on heart failure patients over a long period. Further studies are warranted to detect early signs of heart failure in diabetic patients to prevent deterioration of LV function.

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Clinical Science

Comparative Analysis of Approaches and Treatment Results of Patients with Early and Nonearly Rheumatoid Arthritis

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Abstract

The article presents the results of a comparative analysis of different therapy regimens impact on the effectiveness of treatment of patients with early and late rheumatoid arthritis in steady-state. Data on ongoing basis anti-inflammatory therapy of rheumatoid arthritis and the treatment of associated conditions were obtained by continuous copying from case histories of hospital department patients. The observations lasted 12 months. The activity of rheumatoid arthritis before and after the treatment was determined by the DAS 28 (Disease Activity Score) index. The treatment results were evaluated as per the laboratory research and the DAS 28 index, including the counting of painful and swollen joints, erythrocyte sedimentation rate, and health assessment of the patient on a visual analogue scale.

Introduction

Rheumatoid arthritis (RA) is one of the most severe and frequent inflammatory rheumatic diseases, which is associated with progression of joint destruction, decreased functional capacity and quality of life of patients, and the socio-economic hardship is occurring in this context [1].

Rheumatic diseases occur in people of any age, starting with children, but have a clear tendency to a significant accumulation with increasing age of patients. In the Russian Federation, up to 700 thousand new cases of inflammatory and degenerative diseases of the joints and systemic diseases of the connective tissue are diagnosed for the first time every year [2], [3]. The peak of the RA debut falls mainly on the working age. The loss of functional activity due to the development of erosive and destructive changes in the joints leads to disability and incapacitation of patients. Ten years after the onset of the disease, 67% of patients have high (II – III) functional insufficiency of the joints and 44.5% have I and II disability groups [4]. The medical community considers rheumatic diseases as risk factors for the development of severe associated chronic diseases [3], [5]. The accelerated development of cardiovascular and other diseases in RA leads to reduced life expectancy and increased levels of mortality by 50 – 60% as compared to the general population. These are the rheumatic diseases that make the greatest contribution to the reduction of efficiency, deterioration of the general health of the population. All this causes a heavy socio-economic burden of this disease both for the patients, significantly reducing the quality of their life, and for the state health system as a whole due to the required considerable financial expenses for the provision of medical and pharmaceutical care to patients [5], [6], [7].

Literature Review

Currently, the concept of managing patients with RA includes early diagnosis of the disease, timely active treatment commenced with the implementation of a therapeutic window of opportunity, careful monitoring for RA and response to therapy, the maximum suppression of disease activity in the short term in order to achieve and maintain a state of remission that is aimed at preventing the decline of patients' quality of life (program of "Treatment to reach the target") [8], [9], [10].

The primary method of achieving and increasing the duration of the RA remission stages is long-term use of the disease-modifying anti-rheumatic drugs (DMARD) and genetically engineered biologic drugs (GEBD), which reduce the activity of the inflammatory and autoimmune process. A large number of publications of domestic and foreign authors devoted to various approaches to the RA treatment confirm that the application of this approach in the RA treatment has resulted in tremendous progress [11], [12], [13], [14], [15], [16], [17], [18], [19], [20].

However, as noted by rheumatologists, the disease is the most important socio-economic problem because of its high incidence, poor prognosis (at untimely and inadequate treatment), as well as the need for long-term, and sometimes permanent, administration of different combinations of drugs. Moreover, a significant proportion of patients' needs orthopaedic surgery. The modern approaches to RA treatment are associated with significant financial costs. The above explains the significant socio-economic losses associated with RA that were similar to those seen in ischemic heart disease [4], [15], [16].

According to the data of Sh.F. Erdos, D.V. Goryacheva, O.A. Grigoriev et al., as a result of a clinical and economic study, it was revealed that the failure to obtain a social product due to the temporary disability of patients with RA was, on average, EUR 1 million/year in Russia. The support of one disabled person from the moment of disability until death costs the state EUR 20.4 thousand. The total state expenditures for disabled people with RA are EUR 18 billion. At the same time, the state does not receive a profit for EUR 160.5 thousand to EUR 214.0 thousand per each disabled person [21], [22].

The evaluation of the economic impact of two diseases of the musculoskeletal system and connective tissue, namely the RA and ankylosing spondylitis, showed that the value of direct medical costs was equal to EUR 6,454 per year (medical costs – EUR 4,170 per year, nonmedical costs – EUR 2,284 per year). The indirect costs due to disability constitute EUR 6,447 per year [22].

The cost of treatment and examination of the patient for the state is only 1/3 of the costs associated with RA. The remaining costs are determined by a decrease in the quality of life of patients, the termination of their contribution to the creation of the common national product, and a decrease in the labour activity of relatives to provide care for the patient [22], [23], [24], [25].

The financial situation is an important factor that affects the performance associated with quality of life, including the functional activity of the patient [25], [26], [27].

Since RA is a chronic disease, and in fact, patients need expensive drugs for the life term, strategic approach to treatment is required.

Methods

The content analysis of publications of domestic and foreign authors, the methods of mathematical statistics, structural, correlation, the nonparametric analysis were used in the process of work.

Statistica 6.0 (Statsoft, USA) was used for statistical data processing. The results are presented in the form of a median and interquartile interval (Me [25th; 75th percentile]). To compare the frequencies of qualitative traits in groups, the $\chi 2$ criterion was used. When comparing the groups, the Mann-Whitney U test was used, and the correlation analysis was performed using the Spearman's Rank-Order Correlation. Differences were considered significant at $p < 0.05$. RA activity was determined from the DAS 28 value recommended by the European League Against Rheumatism (EULAR) as follows: DAS 28 > 5.1 – first class of activity, DAS 28 [3,2;5,1] – second class of activity, DAS 28 [2,6;3,2] – third class of activity, and DAS 28 < 2.6 – remission [28], [29].

Results

The study included data on the results of treatment in 200 patients with a definite diagnosis of RA (165 women and 35 men) aged 19 to 73 years who had been treated in a special hospital.

Table 1: The structure of the cohort of patients included in the study (n = 200)

Indicator	Value
Gender, female/male, of them:	165/35 or 4.7:1
Women from 19 to 55 years old, n (%)	79 (47.9)
Women over 55 years old, n (%)	86 (52.1)
Men from 26 to 60 years old, n (%)	27 (77.1)
Men over 60 years old, n (%)	8 (22.9)

As can be seen from Table 1, almost half (47.9%) of the women and the majority (77.1%) of the

men suffering from RA were in working age.

One hundred and forty four patients (72.0%) had concomitant diseases, with arterial hypertension most commonly occurring – 60.0%, dyslipidemia – 45.0%, fractures of various localization – 29.5%, coronary heart disease – 21.0%, and also myocardial infarction (1.5%), stroke (1.0%), diabetes mellitus (7.5%), osteoporosis (15.5%), and ulcerative lesions of the upper gastrointestinal tract (14.9%).

Using continuous copying from the case histories, information on the medical prescription of drugs to the population of patients under study was obtained. Methotrexate was administered as the main DMARD; 139 (69.5%) patients received it. Leflunomide (9.0%) and sulfasalazine (1.5%) were also used. The GEBD therapy was used in 21.5% of cases and was represented by TNF-alpha inhibitors (9.0%), rituximab (6.5%), abatacept (4.5%), and tocilizumab (1.0%). Nineteen patients (9.5%) received nonsteroidal anti-inflammatory drugs (diclofenac, nimesulide, and meloxicam), 95 (47.5%) patients – selective COX-2 inhibitors (etoricoxib), and 86 (43%) patients – glucocorticoids (methylprednisolone, prednisone).

Additional therapy was assigned for the treatment of associated diseases. Ninety-eight (81.7%) of 120 patients received antihypertensive drugs; 27 (22.5%) patients – statins; 12 (10%) patients – hypoglycemic therapy; and 27% of the total number of patients received low doses of aspirin.

For a comparative analysis of the approaches and results of treatment of patients with early and nonearly RA, the patients were divided into two groups as follows (Table 2): the first one – with early RA (eRA) with a disease duration of up to two years (n = 60), the second included the patients with nonearly RA (more than two years) (nRA) (n = 140). The patient groups were matched by age, sex, and RA activity.

Table 2: Characteristics of patient groups prior to observation

Indicator	eRA value	nRA value
Gender, female/male (%), of them:	49/11 (82)	116/24 (83)
Women from 19 to 55 years old, n (%)	22 (36.7)	42 (30)
Women over 55 years old, n (%)	27 (45)	74 (52.9)
Men from 26 to 60 years old, n (%)	10 (16.7)	17 (12.1)
Men over 60 years old, n (%)	1 (1.6)	7 (5)
Disease duration, years Me [25th; 75th percentile]	0.7 [0.3; 1.2]	8 [4; 14]
Activity, n (%): I/II/III	28/39/33	31/54/15
DAS 28, Me [25th; 75th percentile]	4.23 [3.1; 5.7]	3.8 [3.1; 4.7]

Methotrexate was prescribed to most patients as basic therapy: in the first group – to 49 patients (81.7%) at a dose of 20 [15; 25] mg/week, in the second group – to 90 (64.3%) patients at a dose of 15 [10; 20] mg/week. All patients treated with methotrexate also received folic acid at a dose of 4.5 [3,3; 10.0] mg/week. Two (3.3%) patients of the first group and 16 (11.4%) patients of the second group received leflunomide; one patient (1.6%) of the first group and two (1.4%) patients of the second group received sulfasalazine. Twelve (20%) patients with eRA and 31 (22%) patients with nRA received GEBD.

The frequency of assigning various GEBD is presented in Table 3. More than half of the patients were additionally administered NSAIDs, of which two (3.3%) patients of the first group and 17 (12%) patients of the second group received nonselective NSAIDs, 32 (53.3%) patients of the first group and 63 (45.0%) patients of the second group received selective NSAIDs.

Table 3: The frequency of use of drugs for the RA treatment in patients with eRA (n = 60) and with nRA (n = 140)

Name of the drug	Assignment frequency, n (%)	
	eRA	nRA
Methotrexate	49 (81.7)	90 (64.3)
Leflunomide	2 (3.3)	16 (11.4)
Sulfasalazine	1 (1.6)	2 (1.4)
GEBD:	12 (20)	31 (22)
Adadimumab	8(13.3)	5 (3.6)
Certolizumab	2 (3.3)	1 (0.7)
Infliximab	-	3 (2.1)
Tocilizumab	-	2 (1.4)
Rituximab	-	13 (9.3)
Abatacept	2 (3.3)	7 (5)
NSAID, of them:	34 (56.6)	80 (57.0)
- nonselective	2 (3.3)	17 (12.0)
- selective	32 (53.3)	63 (45.0)

The patients had been monitored for 12 months. By the end of the study, 26 patients were selected of which three had died, 13 had refused further research, and eight had not taken drugs for various reasons (pregnancy, high cost, and patient reluctance).

The results of the therapy were evaluated after 12 months in 56 patients with eRA and 128 patients with nRA. The characteristics of the patients are presented in Table 4. No differences have been found between weight and disease activity in groups of patients with eRA and nRA after 12 months.

Table 4: Characteristics of patients after 12 months of treatment

Indicator	eRA (n = 56)	nRA (n = 128)
DAS 28, points	3.0 [2.3; 4.4]	3.5 [2.6; 4.5]
Activity, remission/I/II/III, n	13/15/22/6	14/36/61/17

In general, in the eRA group, after 12 months, a decrease in the disease activity was observed as evidenced by the dynamics of the DAS 28 level from 4.23 to 3.0 points ($p = 0.01$), as well as a decrease in the number of patients with a third (III) degree of activity (from 33 to 6 people), and identification of the stage of drug remission in 13 patients ($p < 0.01$). In the nRA group, there was a tendency to a decrease in the level of DAS 28 from 3.8 to 3.5 points ($p = 0.06$).

As a result of analyzing the effect of drug combinations on the DAS 28 index, it was found that only the patients receiving combinations with GEBD had shown a significant decrease in DAS 28. Rituximab in the first group significantly reduced DAS 28 (n = 15, $p = 0.04$) from 4.5 [3.5; 5.3] to 3.8 [3.0; 4.4]. In the second group of patients (n = 14), the reliability of the results decreased ($p = 0.06$).

To verify the data obtained, a parallel study was conducted. It included data on the treatment of 64 patients with RA (54 women and 10 men) aged 55 [48; 60] years, with a prolonged course of the disease (5 [1 – 10] years), with moderate and high clinical disease activity (DAS 28 = 4.5 [3.5 – 5.3]). All patients received GEBD: tumor necrosis factor-α inhibitors (TNF-α) were used in 27/64 (42%) patients, rituximab – in 15/64 (23%) patients, abatacept – in 12/64 (19%) patients, and tocilizumab – in 10/64 (16%) patients. Therapy with DMARDs was carried out in all patients. The patients were monitored for 12 months, and the disease activity was assessed by DAS 28.

As a result of the analysis of the GEBD influence on the DAS 28 index, a significant decrease in the index from 4.5 [3.5 – 5.3] to 3.7 [2.5; 4.6], $p < 0.01$ was revealed. The most significant decrease in the DAS 28 index was observed during therapy with rituximab (4.5 [3.5; 5.3] and 3.8 [3.0; 4.4], $p = 0.04$). The results are consistent with the research of R.M. Balabanova, V.N. Amirjanova, E.L. Nasonova, D.V. Goryacheva [13], [30].

According to the results of the clinical studies, a decrease in the level of C-reactive protein and rheumatoid factor by 73% and 59%, respectively, was observed in the studied group of patients. It was found that the most significant reduction in the average values of these indicators was typical for the patients whom rituximab was administered.

Discussion

As noted above, the indicator of the quality of life is largely determined by the financial situation of the RA patient. For treatment, each patient with RA must be hospitalised once a year for 10 – 21 days (the average cost of hospitalisation is RUB 50,000 (EUR 687.54), and make blood tests once a month (RUB 500/month; RUB 6,000/year); the cost of each injection of the drug is about RUB 1,000. Direct medical expenses of the patient for the treatment will amount to RUB 58,000 – 128,000 (EUR 798 – 1,760) per year, excluding the cost of drugs.

The total direct costs for RA therapy using methotrexate and leflunomide regimens will be RUB 58,714 – 209,265 (EUR 807 – 2,878), respectively. However, these regimens do not have a significant effect on reducing RA activity.

GEBD therapy is 3 – 5 times more expensive due to the high cost of the GEBD (EUR 2,550 – 15,365). These therapy regimens reliably reduce the DAS 28 index, increase the frequency of remissions almost two times, compared to traditional therapy; therefore, improve the quality of life of patients. The frequency of deaths with strategies with the use of GEBD decreases by more than 10% in ten years [26].

In conclusion, the comparative analysis of different approaches to the treatment of RA has revealed a significant decrease of DAS 28 using rituximab (4.5→3.8). At the same time, the total direct cost of the RA therapy with rituximab is significantly lower than that in the treatment of RA using other GEBD. The GEBD treatment is especially important for patients with the nRA, more than half of whom are pensioners. Even the minimum direct cost of the RA therapy is 1.4 times higher than the average old-age retirement pension in Russia. For working people, the total direct costs for the GEBD therapy are 53.4 – 82.0% of the average wage.

It is obvious that the average citizen of Russia cannot afford the GEBD treatment; therefore, the main burden of using biological products must be taken by the state budget, which has significant limitations for carrying out expensive therapies. For the sustainable use of limited budgetary funds in medical organizations of the state healthcare system in Moscow, a commission has been set up to monitor the treatment using GEBD.

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Clinical Science

Prevalence and Associated Factors of Low Back Pain Among Physicians Working at King Salman Armed Forces Hospital, Tabuk, Saudi Arabia

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Abstract

BACKGROUND: Low back pain is a common presenting symptom among workers in primary health care facilities, including physicians.

AIM: This study aimed to identify the magnitude, determinants and sequence of the problem of low back pain among physicians working at the King Salman Armed Forces hospital, Tabuk, Saudi Arabia.

METHODS: A cross-sectional study was carried out among physicians who are working at King Salman Armed Forces Hospital, Tabuk, Saudi Arabia. A previously validated self-administered questionnaire was utilised for data collection including demographic information and data related to low back pain and its characteristics and outcome.

RESULTS: The study included 254 physicians. Their age ranged between 23 and 66 with a mean ± SD of 36.0 ± 9.3 years. Almost two-thirds (66.9%) were males. Most of the physicians (76.4%) ever had LBP whereas 70.5% had LBP in the last 12 months. The only significant factor associated with LBP in the past 12 months was physicians` speciality as all ophthalmologists and majority of emergency physicians and anaesthesia/intensive care physicians (88.9%) compared to only 14.3% of nephrologists and neurologists expressed LBP in the last 12 months. Overall, the association between physicians' speciality and a history of LBP in the last 12 months was statistically significant, p = 0.014. Absence from work because of LBP in the last 3 months was mentioned by 15% of physicians.

CONCLUSION: Low back pain is a very common health problem among physicians working at the King Salman Armed Forces hospital, Saudi Arabia. A considerable proportion of them was absent from work because of LBP.

Introduction

Low back pain (LBP) is one of the most prevalent complaints that require health care. It's the most common type of musculoskeletal disorder [1]. LBP is defined as a pain that exists below the 12th rib, and above the gluteal folds [2], it's a symptom of diseases and not considered itself as a disease [3], [4]. LBP is the most common musculoskeletal condition that is frequently causing disability in both developing and developed countries. It was reported that about 70% to 85% of the population worldwide experience LBP at some time in their life [4].

Physicians are susceptible to twisting, bending, maintaining an awkward posture for a long time and lifting heavy loads which is all considered risk factors for musculoskeletal pains [5], several studies revealed that the prevalence of LBP among medical practitioners is higher than any other musculoskeletal symptoms [6], [7], [8]. LBP represents the main type of occupational injury especially in the healthcare field [9], [10], as it is found to be about twice more than other occupations [9]. It was reported that 18.7% of health care workers with chronic LBP were using pain-relief drugs and or analgesia [10]. Several medical specialities were found to be at high risk of LBP such as obstetrics and gynaecology, orthopaedic, nursing, operation theatre staff, and physical therapist [9].

A study was conducted in China showed that the prevalence of LBP among physicians was 44% [11]. In another study conducted in the U K, 19% of ear, nose, and throat consultants had back pain [12]. In Turkey, the lifetime prevalence of LBP among healthcare workers was determined to be 53% [13]. The lifetime prevalence of LBP among the hospital staff in Tunisia 57.7% and the annual prevalence was

51.1% [14]. The prevalence of LBP among primary healthcare professionals worldwide is 56.8%, 36.8%, 72.5%, 46%, 70.9% in Iran [15], India [16], Malaysia [17], Nigeria [18], and Kuwait [19]. In Saudi Arabia, several studies were performed regarding the prevalence of LBP among healthcare workers in different regions of the Kingdom, in Riyadh, the lifetime prevalence of back pain among all the medical practitioners was found to be 83.9% [20]. Prevalence of LBP among healthcare workers in the eastern region of Saudi Arabia was 79% [21]. While in southwestern Saudi Arabia, the prevalence of LBP among health care workers in the past 12 months was 73.9% [1].

LBP problem is associated with occupationally and personal related consequences such as; frequent absenteeism and disability [1]. The societal burden of injuries to physicians such as LBP will be borne through their productivity reducing, cost of treatment, and absence from work [5]. It was reported that LBP could lead to sick leave and activity limitation for more than 50% of health care workers [1]. Hence, this study to assess the prevalence of low back pain and its associated factors among physicians Working at King Salman Armed Forces Hospital, Tabuk, Saudi Arabia.

Methods

Study design, study setting and subjects

This was a cross-sectional questionnaire-based study conducted in Tabuk city in the north of Saudi Arabia which has a population of 910,030 (2017 census) [22]. It includes one military hospital “King Salman Armed Forces hospital” where the study was specifically conducted. It is a tertiary care hospital with 767 beds, belonging to Ministry of Defense and was opened in 1979 [23]. All physicians, from both genders, all specialities and qualifications working at the King Salman Armed Forces hospital (1440-2019) were eligible for the study (n = 512). Those physicians who have been known to have chronic or recurrent back pain were excluded from the study. Also, those who had a trauma in the back, osteoporosis, infection or neoplasm were excluded.

Data collection

A pre-designed valid questionnaire was used for data collection. It has been used previously in a study conducted in Taif by Keriri HM and proved to be valid and reliable in assessing LBP among health care workers [24]. Permission to use the questionnaire was obtained through personal communication with the author. The questionnaire included information regarding demographic data (e.g., age, sex, marital status, speciality), work-related factors (e.g., hours of work per week, type of work, duration of work in hospital etc.) as well as experience of LBP. The validity of the questionnaire scoring system was assured by three consultants in Family medicine, rheumatology and community medicine (content validity). The questionnaires were distributed by the researcher herself hand to hand to recruited physicians during their rest time. Care was taken not to disturb their clinics. They were collected within one week by the researcher also.

Sample size

The margin of error and confidence level were 5% and 99%, respectively. Likewise, the response distribution of the prevalence of back pain was 79% [21]. The minimum recommended size to meet this criterion was 237 participants. The sample was increased to 260 to compensate for drop out. The sample size was calculated using Raosoft, an open-source calculator.

Questionnaire piloting

A pilot study was done on 15 physicians of various specialities working in the same hospital. The pilot study helped to test the understanding of the participants of the questionnaires, select the relevant variables suitable for the used statistical, determine the time needed to answer the questionnaire, and give an actual situation of the main study. As a feedback, the questionnaire was clear and understandable, and tool on average took 15 minutes to be completed by physicians with LBP and less among those without LBP. Their data were included in the final report since there were non-significant changes.

Sampling technique

Stratified random sample technique with proportional allocation was adopted to select physicians from different departments of the hospital proportional to the total number of physicians in each department. Stratification will be done based on working departments.

Data management and analyses

Data were entered to a personal computer and were analysed using Statistical Package for the Social sciences (SPSS, Chicago Illinois) program version 25. Frequency distributions of responses and cross-tabulations of individual, risk job factors were studied in association with a reported prevalence of LBP. Differences and/or associations were further analysed by the chi-square test (χ^2). Level of significance was determined at $p < 0.05$.

Ethical considerations

Approval of the regional research and ethics committee at King Salman Armed Forces hospital was obtained, and permission from the medical director of King Salman Armed Forces hospital was obtained. Written consents from all participating were obtained. All collected data were kept confidential.

Results

Baseline characteristic of participants

Baseline characteristic of participants: The study included 254 physicians. The mean age of participants was 36.0 ± 9.3 years. The males were 170 (66.9%), and the females were 84 (33.1%), (52.0%) were Saudis (Table 1).

Table 1: Personal characteristics of the participants (n = 254)

	Frequency	Percent
Gender		
Male	170	66.9
Female	84	33.1
Nationality		
Saudi	132	52.0
Non-Saudi	122	48.0
Marital status		
Single	70	27.6
Married	182	71.6
Divorced	2	0.8

Prevalence of LBP

As displayed from Figure 1, most of the physicians (76.4%) ever had LBP, whereas 70.5% had LBP in the last 12 months, as shown in Figure 2.

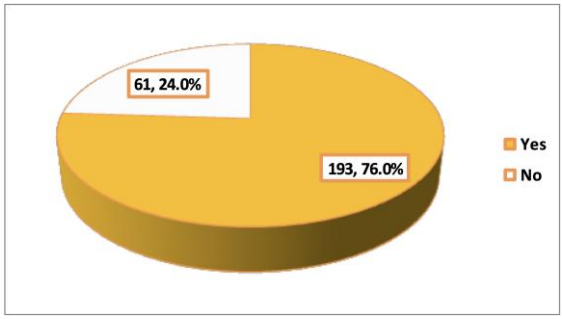

Figure 1: Prevalence of ever back pain among the physicians working at King Salman Armed Forces hospital, Tabuk

Characteristics of the low back pain

In this section, responses of 193 physicians who had LBP ever were described as illustrated in Table 2. The first attack of LBP occurred within the last year among 25.9% of the physicians whereas it occurred for more than five years among 27.5% of them. Only 14% and 3.6% of the physicians had numbness and weakness in their lower limbs, respectively. In the last three months, 39.3% of the physicians had more than three attacks of LBP. Duration of the last LBP problem was less than one week among 56.5% of physicians whereas it lasted more than 5 weeks among 6.7% of them.

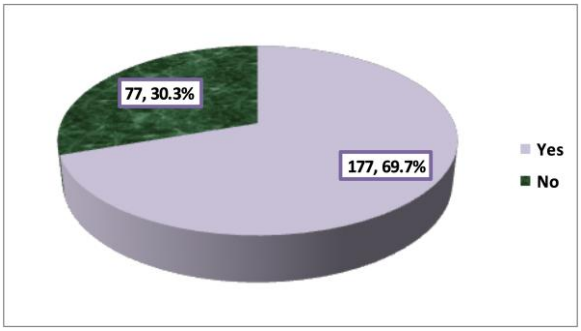

Figure 2: Prevalence of back pain in the last 12 months among the physicians working at King Salman Armed Forces hospital, Tabuk

Absence from work because of LBP in the last 3 months was mentioned by 15% of physicians. Self-rating pain severity with moderate (median was 2 on a scale ranged between 0 and 5). In the last three months, 10.9% of physicians experience no pain, whereas 3.6% experienced continuous LBP (median was 2 on a scale ranged between 0 and 5). Regarding treatment modalities, bed rest (60.6%) and pain medication (49.7%) were the commonest reported by physicians, followed by muscle relaxants (38.3%). Physiotherapy and back support were reported by 15.5% and 14.5% of the physicians, respectively.

Table 2: Characteristics of low back pain among the physicians working at King Salman Armed Forces hospital, Tabuk

	Categories	Frequency (N = 193)	Percentage
Time of the occurrence of the first LBP	Within the last year	50	25.9
	1-5 years	90	46.6
	Since more than 5 years	53	27.5
Having numbness in the lower limb	Yes	27	14.0
	No	166	86.0
Having weakness in the lower limb	Yes	7	3.6
	No	186	96.4
Frequency of LBP in the last 3 months	None	30	15.5
	Once	33	17.1
	Twice	32	16.6
	Three times	21	10.9
	>three times	77	39.9
Duration of the last LBP problem	0-<1week	109	56.5
	1-2 weeks	32	16.6
	3-4 weeks	26	13.5
	4-5 weeks	13	6.7
	>5 weeks	13	6.7
Duration of absence from work because of LBP in the last 3 months	0 days	164	85.0
	1-2 days	17	8.7
	3-7 days	9	4.7
	>7 days	3	1.6
Self-rating of pain severity in the last 3 months	0 (no pain)	12	6.2
	1	25	13.0
	2	62	32.1
	3	70	36.3
	4	18	9.3
	5 (very bad pain)	6	3.1
	Median	2	
Self-rating of experience of pain episodes in the last 3 months	0 (never)	21	10.9
	1	38	19.7
	2	72	37.2
	3	45	23.3
	4	10	5.2
	5 (always)	7	3.6
	Median	2	
Modalities of low back pain treatment*	Bed rest	117	60.6
	Muscle relaxant	74	38.3
	Pain medication	96	49.7
	Physiotherapy	30	15.5
	Back support	28	14.5

* Not mutually exclusive.

Pain severity among physicians with LBP

Only 10.8% of the physicians with LBP either strongly agreed or agreed that they should not do their normal work with their pain while 40.9% strongly agreed that they could do light work for an hour and 43.5% can walk for an hour. Almost one-third of them (33.7%) strongly agreed that they could do ordinary household chores and 40.3% strongly agreed that they could do the weekly shopping. Less than half of them (43.4%) strongly agreed that they could sleep at night (Table 3).

Table 3: Response of the physicians with LBP to questions about pain severity

	Frequency	Percentage
Should I not do my normal work with my present pain?		
0 (strongly disagree)	67	34.8
1	57	29.5
2	27	14.0
3	21	10.9
4	8	4.1
5 (strongly agree)	13	6.7
Median	1	
Can I do light work for an hour?		
0 (strongly disagree)	13	6.7
1	17	8.8
2	23	11.9
3	32	16.6
4	29	15.0
5 (strongly agree)	79	40.9
Median	4	
Can I walk for an hour?		
0 (strongly disagree)	8	4.1
1	21	10.9
2	22	11.4
3	25	13.0
4	33	17.1
5 (strongly agree)	84	43.5
Median	4	
Can I do ordinary household chores?		
0 (strongly disagree)	11	5.7
1	22	11.4
2	29	15.0
3	34	17.6
4	32	16.6
5 (strongly agree)	65	33.7
Median	4	
Can I do the weekly shopping?		
0 (strongly disagree)	9	4.7
1	14	7.3
2	31	16.1
3	28	14.5
4	33	17.1
5 (strongly agree)	78	40.3
Median	4	
Can I sleep at night?		
0 (strongly disagree)	10	5.2
1	14	7.3
2	26	13.5
3	22	11.4
4	37	19.2
5 (strongly agree)	84	43.4
Median	4	

Pain intensity among physicians with LBP

Table 4 demonstrates that 14.5% of the physicians strongly disagreed that they can tolerate the pain they have without using pain medications and 15% of them strongly agreed that they could manage pain without taking pain medication. Almost one-fifth of them (19.2%) strongly agreed that pain medications provide them complete relief from pain. Only 1.6% of them strongly agreed that pain medication has no effect on relief from pain. Twenty-four physicians with LBP (12.4%) strongly agreed that an increase in pain is an indication that they should stop what they are doing until the pain decreases.

Table 4: Response of the physicians with LBP to questions about pain intensity

	Frequency	Percentage
Can I tolerate the pain I have without having to use pain medication?		
0 (strongly disagree)	28	14.5
1	29	15.0
2	36	18.7
3	20	10.4
4	33	17.
5 (strongly agree)	47	24.3
Median	3	
The pain is bad, but I can manage without having to take pain medication?		
0 (strongly disagree)	39	20.2
1	44	22.8
2	31	16.1
3	19	9.8
4	31	16.1
5 (strongly agree)	29	15.0
Median	2	
Pain medication provides me with complete relief from pain?		
0 (strongly disagree)	36	18.7
1	33	17.1
2	41	21.2
3	17	8.8
4	29	15.0
5 (strongly agree)	37	19.2
Median	2	
Pain medication provides me with moderate relief from pain?		
0 (strongly disagree)	46	23.8
1	44	22.8
2	31	16.1
3	33	17.1
4	27	14.0
5 (strongly agree)	12	6.2
Median	2	
Pain medication provides me with little relief from pain?		
0 (strongly disagree)	70	36.3
1	44	22.8
2	40	20.7
3	13	6.7
4	15	7.8
5 (strongly agree)	11	5.7
Median	1	
Does pain medication have no effect on relief from pain?		
0 (strongly disagree)	125	64.8
1	32	16.6
2	19	9.8
3	13	6.7
4	1	0.5
5 (strongly agree)	3	1.6
Median	0	
An increase in pain is an indication that I should stop what I'm doing until the pain decreases?		
0 (strongly disagree)	57	29.5
1	27	14.0
2	36	18.7
3	29	15.0
4	20	10.4
5 (strongly agree)	24	12.4
Median	2	

Personal factors

As shown in Table 5, none of the studied personal factors (age, gender, nationality, marital status, smoking history and body mass index) was significantly associated with LBP in the last 12 months.

Table 5: Personal factors associated with LBP among physicians in the last 12 months

	Low back pain		p-value
	No N=77 N (%)	Yes N=177 N (%)	
Gender			
Male (n=170	55 (32.4)	115 (67.6)	
Female (n=84)	22 (26.2)	62 (73.8)	0.315*
Nationality			
Saudi (n=132)	42 (31.8)	90 (68.2)	
Non-Saudi (n=122)	35 (28.7)	87 (71.3)	0.588*
Marital status			
Single (n=70)	21 (30.0)	49 (70.0)	
Married (n=182)	55 (30.2)	127 (69.8)	
Divorced (n=2)	1 (50.0)	1 (50.0)	0.831*
Age (years)			
Mean±SD	35.3±7.9	36.3±9.9	0.451**
Smoking			
Non-smoker (n=178)	59 (33.1)	119 (66.9)	
Current smoker (n=56)	14 (25.0)	42 (75.0)	
Ex-smoker (n=20)	4 (20.0)	16 (80.0)	0.296*
Body mass index			
Underweight (n=4)	1 (25.0)	3 (75.0)	
Normal (n=76)	23 (30.3)	53 (69.7)	
Overweight (n=93)	30 (32.3)	63 (67.7)	
Obese (n=81)	23 (28.4)	58 (71.6)	0.948*

*Chi-square test; **Student`s t-test.

Work-related factors

All of ophthalmologists and majority of emergency physicians and anaesthesia / intensive care physicians (88.9%) compared to only 14.3% of nephrologists and neurologists expressed LBP in the last 12 months. Overall, the association between physicians' speciality and a history of LBP in the last 12 months was statistically significant, p = 0.014. While, the experience of physicians, their number of working days per week and working hours per day was not statistically significant to LBP (Table 6).

Table 6: Work-related factors associated with LBP among physicians in the last 12 months

	Low back pain		
	No N = 77 N (%)	Yes N = 177 N (%)	p-value*
Experience as physician (years)			
≤ 5 (n = 124)	37 (29.8)	78 (70.2)	
6-10 (n = 44)	19 (43.2)	25 (56.8)	
11-15 (n = 33)	9 (27.3)	24 (72.7)	
> 15 (n = 53)	12 (22.6)	41 (77.4)	0.166
Working days/week			
≤5 (n = 177)	52 (29.4)	125 (70.6)	
>5 (n = 77)	25 (32.5)	52 (67.5)	0.623
Working hours/day			
< 8 (n = 12)	4 (33.3)	8 (66.7)	
8 (n = 148)	41 (27.7)	107 (72.3)	
> 8 (n=94)	32 (34.0)	62 (66.0)	0.730
Specialty			
Family medicine (n = 54)	11 (20.4)	43 (79.6)	
Internal medicine (n = 17)	5 (29.4)	12 (70.7)	
Pediatrics (n = 25)	5 (20.0)	20 (80.0)	
General surgery (n = 25)	9 (36.0)	16 (64.0)	
Obstetrics / Gynecology (n = 25)	9 (36.0)	16 (64.0)	
Dentistry (n = 10)	4 (40.0)	6 (60.0)	
Emergency Medicine (n = 9)	1 (11.1)	8 (88.9)	
Psychiatry (n = 9)	4 (44.4)	5 (55.6)	
Orthopedics (n = 15)	6 (40.0)	9 (60.0)	
Ophthalmology (n = 4)	0 (0.0)	4 (100)	
Neurology (n = 7)	6 (85.7)	1 (14.3)	
Preventive medicine (n = 7)	2 (28.6)	5 (71.4)	
Radiology (n = 6)	2 (33.3)	4 (66.7)	
Nephrology (n = 7)	6 (85.7)	1 (14.3)	
Oncology (n = 7)	2 (28.6)	5 (71.4)	
ENT (n = 5)	1 (20.0)	4 (80.0)	0.014
Anaesthesia/Intensive care (n=9)	1 (11.1)	8 (88.9)	
Others (n = 13)	3 (23.1)	10 (76.9)	

* Chi-square test.

Discussion

Most studies investigating LBP among health care professionals were carried out among nurses with few epidemiological studies have estimated the prevalence and identified associated risk factors of LBP among physicians. This study attempted to estimate the prevalence of LBP and identify its determinants among physicians working at King Salman Armed Forces hospital, Saudi Arabia. Most of the physicians in the current study (76.4%) ever had LBP, whereas 70.5% had LBP in the last 12 months. Comparable figures have been reported in another two Saudi studies carried out recently in the Eastern Region and Riyadh. In the Eastern Region, the prevalence of lifetime LBP was 67.7% among physicians whereas the rate of visiting clinics for LBP was 32.6% [21]. In Riyadh, the lifetime prevalence of LBP was 87.7% [20]. Most of the physicians with LBP (94%) had a minimal disability, 6% had moderate disability, and none of them had a severe disability. Different rates have been reported in international studies. In Turkey, LBP lifetime prevalence was 53.9% among physicians [13]. In Iran, LBP prevalence was 15.1% among physicians [25]. In another study carried out in Iran, the overall prevalence of LBP among resident physicians was 56.8% [15]. In Malaysia, the cumulative prevalence of LBP was 72.5% while the 12-months prevalence was 56.9% [17]. In Kuwait, the 12-month LBP prevalence among physicians was 13.7% [19]. The difference in the prevalence rate between various studies including the present one could be attributed to the difference in demographic and work-related characteristics of the participated nurses in these studies as well as different tools utilised in identifying LBP.

It has been documented that females are more prone to LBP than males as a result of the anatomical, physiological and structural difference between them; also the fact that mechanical disadvantage, sprain and strain, are more common in women than men [26]. However, in the current survey, there was no difference between male and female physicians regarding the prevalence of LBP. This finding agrees with what has been reported by others in Riyadh (KSA) [20], Malaysia [17] and Kuwait [26]. However, in numerous studies [13], [15], [16], [21], [27], females were more likely to have LBP compared to males.

In the present study, the higher rate of LBP was observed among ophthalmologists, emergency physicians and those specialists in anaesthesia and intensive care. This higher prevalence may be due to the greater workload places such as standing for a long time and a lot of movement among those physicians comparing to physicians in other specialities, particularly nephrology and neurology. By what has been reported in a systematic review study, smoking was not a significant predictor for LBP in the present study [22]. In another study conducted in Turkey, smoking was a statistically significant risk factor for LBP [27]. In this study, physicians` body mass index was not a predictor for LBP. In other studies, carried out among healthcare workers (nurses), overweight and obesity were associated with LBP [23], [28]. The exact mechanism underlying the association between obesity and LBP is not fully identified.

Despite the high prevalence of LBP observed in this study, the aetiology and nature of LBP are not yet well understood. Many studies have reported a strong association between musculoskeletal disorders and work-related factors [16] and work pressure [29] among health care workers, including physicians. In the present study, LBP prevalence was not associated with work-related factors, except the physicians` speciality. However, others [27], [30], [31] reported an association between long hours of work and increased risk of back pain among healthcare workers.

In the present study, about 68.4% of the participants who are complaining of pain reported that the score of pain was either 2 or 3 out of a scale of 5. However, about 12.4% reported a score of 4 or 5. Results of another Greek study showed that the pain severity ranged from moderate to intolerable in 38% of the sufferers [32]. Comparison between the two studies is difficult due to using different scales. However, severe pain, in general, affects, for sure the work productivity.

In this study, among physicians who had LBP, 10.8% reported that they could not do their normal work with their pain while 40.9% can do light work for an hour. In another Saudi study carried out in Riyadh [20], a minimal disability was observed among the majority of physicians with LBP, which means that this pain did not prevent them from performing activities of daily living. Therefore, determination of the severity of pain is very essential for those with LBP.

In the present study, 15% of physicians who had LBP reported an absence from work in the last three months because of LBP. Similarly, in a study carried out by AlMalki et al. in Riyadh [20], 13% of those who had back pain had taken days off from work. Also, in Malaysia, 7.3% of physicians reported an absence from work because of LBP [17]. LBP has been identified as one of the main causes of loss of hours and days among the healthcare workers [20]. This finding is very important as it showed that LBP could have consequences on the productivity of the affected physicians, which consequently affect the quality of care delivered to their patients.

In the current study, bed rest and pain medications were the commonest reported treatment modalities for LBP. This finding agrees with what has been reported in Riyadh [20], as most affected physicians did not seek medical help and only used simple analgesics and heat / cold fomentations as mostly LBP is a mechanical procedure. This study includes some important limitations. First of all, results may not be generalised to include physicians in other healthcare settings. Future research might include physicians working in other governmental and private health care sectors and can provide additional information. Moreover, since the design in this study was a cross-sectional, the results should be interpreted with great caution because they express only association and not causation between the risk factors and prevalence of LBP. Lastly, using a self-reported questionnaire as a study tool is subjected to recall bias.

In conclusion, this study revealed that LBP is a very common health problem among physicians working at the King Salman Armed Forces hospital, Saudi Arabia as it affected more than 70% of them in the last 12 months. This problem may negatively impact their health and economy. The possible risk factor of LBP was physicians` speciality as ophthalmologists, emergency physicians and those specialists in anaesthesia or intensive care were more likely to have LBP. This research should be extended to other Saudi hospitals to obtain a broader assessment of the LBP problem among Saudi physicians.

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Clinical Science

Heavy Metals Can either Aid or Oppose the Protective Function of the Placental Barrier

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Abstract

BACKGROUND: In developing countries, toxic heavy metals are a threatening catastrophe to human health, particularly in the vulnerable group of pregnant mothers and their fetuses. Fortunately, the placenta can be a protective barrier to the fetuses.

AIM: To explore the relationship between serum lead, cadmium and arsenic levels in pregnant mothers and their newborns, to address the placental barrier in this situation.

METHODS: A cross-sectional study was conducted on 100 pregnant mothers at the time of labour and their newborns. Serum cadmium, lead, and arsenic levels were measured using the Inductively Coupled Plasma Mass Spectrometry.

RESULTS: All the studied heavy metals concentrations showed a significant elevation in the maternal blood relative to the cord blood. There was a significant association between the maternal lead and both fetal lead and arsenic. Meanwhile, a negative but insignificant correlation was recorded between the maternal cadmium and each of the fetal cadmium, lead, and arsenic.

CONCLUSION: The study findings indicated a weak relation between maternal and fetal blood heavy metals, except for the influence of maternal lead, so it can be assumed that the placental barriers are partially protective against those toxic pollutants, putting into consideration the influence of their different natures.

Introduction

Environmental contamination with heavy metals is considered as a public health problem in Egypt. Their progressive contamination of the soil and water are increasing to an alarming rate [1]. Besides, the growing industrial development and disorganised urbanization, contribute to the raised levels of heavy metals in the urban environment of our country [2].

Prenatal exposure to environmental contaminant may occur through the placenta and the umbilical cord. It has been widely evidenced that the protection provided by the placental barrier is not complete, as several harmful agents can pass through it, like some drugs and toxic agents [3].

Nowadays, toxic heavy metals are considered as major sources of the progressively growing problem of environmental pollution. Cadmium, lead, and arsenic are all toxic heavy metals present in the surrounding environment almost always together as co-occurrences [4]. Collectively, they reach our bodies through the air, food, and water, with a special referral to cadmium, whose main entry source, is smoke [5].

Several studies have investigated the passage of these contaminants from the mother to the fetus, and their influence on the health of pregnant mothers and their fetuses where they can affect growth and development [6], [7].

They are all neurotoxic besides their other hazards, mostly due to the oxidative stress [8]. Fortunately, on the other side, they can induce the synthesis of low molecular weight proteins, rich in cysteine, named metallothioneins (MTs) at the placenta. These, in turn, are protective to the fetus against the different stressful conditions exemplified

by heavy metals, oxidative damages and inflammation. They can regulate cell growth differentiation repair and apoptosis. Moreover, they are a pillar for immune regulation and are protective against immune-mediated apoptosis [9].

The purpose of this study was to identify the relation between serum lead, cadmium and arsenic levels in pregnant mothers and their newborn's cord blood, to justify the function of the placental barrier in this condition.

Material and Methods

A cross-sectional study that was conducted in the period from September 2016 to June 2017, 100 pregnant mothers and their newborns were recruited at the time of labour [10]. They were chosen randomly from those attending AL-Galaa Teaching Hospital as a research project, funded the by National Research Centre 10th research plan, entitled "immunological profile in cord blood and growth assessment of the newborn about maternal exposure to environmental contaminant". (Grant No. 11010140), which was approved by the Medical Ethical Committee of the National Research Centre (Registration No.16-295).

All mothers gained comprehensive and clear knowledge about the aim of our work, and written consents were signed before enrollment.

The mother's ages ranged between 18 and 40 years. Neonates were of both sexes. Pregnant mothers with a history of chronic diseases or major illnesses during pregnancy were excluded. Neonates with any apparent congenital abnormalities, genetic, metabolic or neurological problems were also excluded.

The following data were collected

- Sociodemographic data about mothers included age, social status, economic responsibility, water source availability, sanitary disposal, smoke exposure and education.

- Food frequency questionnaire (FFQ) for dietary assessment of the different foodstuff.

- Gestational age, type of labour, history of delivery problems and chronic diseases.

- Maternal anthropometric measurements of weight in kilograms (kgs) height in centimetres (cm).

- Neonatal Apgar scoring, at one and five minutes, was measured to assess neonatal condition at birth.

- Neonatal anthropometric measurements of weight (kgs), height (cm), head circumference and mid-upper arm circumference (MAC) in cm were all taken.

Blood sample collection

Five ml of blood were collected from mothers, whether in normal or section delivery at the time of labour and put in 3 free EDTA tubes. Another 5 ml of blood were collected from the cord blood during delivery before placental separation and put in free EDTA tubes.

These blood samples were for measuring the cadmium, lead, and arsenic levels in mothers and umbilical cord of fetus using inductively coupled plasma mass spectrometry, as shown in a previous study [10].

Statistical analysis

The analysis was performed using SPSS version 21 (SSPS Inc., Pennsylvania, USA). Mean ± SD, median and interquartile ranges were used to present quantitative data. While frequencies and percentages used for qualitative data. Mann Whitney U test was used for comparison between groups. Pearson's correlation analysis was carried out to evaluate the association between variables. $P < 0.05$ value was considered as significant.

Results

A total of 100 mothers with their infant (46 males and 54 females) were analyzed for the current study; their mean age was 26.25 ± 5.44 years. The study population had no history of occupational exposure to toxic elements, and they were all living in an urban environment. None of the mothers was active smokers, while about 80% were passive smokers. Table 1 shows the demographic characteristics of our target population

Table 1: General characteristics of the study population

Categorical variables	Category	N (%)
Maternal Age	below 20 years	8 (8%)
	20-30 years	63(63%)
	More than 30 years	29 (29%)
Type of delivery	Normal delivery	55 (55%)
	Cesarean section	45 (45%)
Newborn's gender	Male	46 (46%)
	Female	54 (54%)
Vegetables intake	1 / week	27 (27%)
	3 / week	55 (55%)
	> 3 / week	17 (17%)
Fruits intake	1 / week	44 (44%)
	3 / week	38 (38%)
	> 3 / week	17 (17%)
Animal proteins intake	1 / week	59 (59%)
	3 / week	35 (35%)
	> 3 / week	5 (5%)
Continuous variables	Mean	SD
Gestational age	37.04	2.1
Newborn Weight	2.9	0.64

As shown in Table 2, all the studied heavy metals concentrations displayed significant

enhancement in the maternal blood serum versus to the cord blood serum.

Table 2: Heavy metals concentrations in maternal and cord blood sera

	Maternal					Fetal						
	Mean	SD	Percentile 25th	Percentile 50th Median	Percentile 75th	Mean	SD	Percentile 25th	Percentile 50th Median	Percentile 75th	Z	P
Lead (µg/ml)	23.56	36.1	0.30	12.84	19.7	13.15	22.2	0.05	5.5	13.2	-2.84	0.005*
Arsenic (µg/L)	60.86	527.2	1.08	2.30	7.7	3.43	7.5	0.499	0.98	2.72	-4.66	0.000*
Cadmium (ng/ml)	20.59	155.9	0.35	1.35	7.1	1.95	3.2	0.15	0.60	1.6	-3.14	0.002*

* Significant at p < 0.05 level.

The correlations between maternal and fetal heavy metals are shown in Table 3; a significant positive association was recorded between the maternal lead and both fetal levels of lead and arsenic. There was also an insignificant negative association between maternal cadmium concentrations and the cord blood serum levels of cadmium, lead and arsenic

Table 3: Correlation between maternal and neonatal heavy metals

		Fetal Pb	Fetal As	Fetal Cd
Maternal Cd	r	-0.007	-0.001	-0.018
	P - Value	0.944	0.992	0.859
Maternal Pb	r	0.718*	0.593*	-0.010
	P - Value	0.000	0.000	0.919
Maternal As	r	-0.047	0.022	-0.042
	P - Value	0.642	0.827	0.679

* Significant at p < 0.05 level.

Discussion

The protective function of the placental barrier against some toxic heavy metals was assessed in the present study, by evaluating the relationship between levels of arsenic, cadmium and lead in the maternal and cord blood, we observed statistically significantly higher concentrations of all mentioned heavy metals in maternal blood serum than in cord blood serum.

In comparison to other recent studies, concentrations of the above-mentioned heavy metals were assessed in Malaysia in maternal and cord blood; they observed significantly lower levels of lead and cadmium but not arsenic in cord blood than in maternal blood [11]. Our findings were also in partial agreement with those of Zhuo et al., [12] who reported higher concentrations of lead and cadmium in a maternal blood clot in comparison to an umbilical cord blood clot, but again arsenic levels showed insignificant differences. These investigators also stated that the barrier function of the placenta works most effectively with cadmium. Simultaneously, our findings indicate that the placental barriers act as a partial defence against those agents especially arsenic and cadmium.

On the opposite side, the finding of this research indicated that maternal blood cadmium levels correlated negatively, but insignificantly with the umbilical cord blood levels of cadmium, lead, and arsenic. The oxidative stress which cadmium in poses should mal-affect the placental barrier, which in turn should allow more levels of toxic metals to pass to the fetus. However, the observed negative correlation in this study could be ascribed to the capability of the maternal cadmium, even at relatively low doses, to act as a powerful driver of metallothioneins synthesis at the placental barrier [13], [14]. MTs are proteins of low molecular weight and abundant in cysteine; they can hinder the passage cadmium from the mothers to their fetuses in several ways. It is worth mentioning that MTs, however, do not completely stop the movement of Cd from mothers to their fetuses [14], but Cd had been considered a more potent inducer of MTs in comparison to other heavy metals [15].

Several recent types of the research reported results consistent with ours where they found out that the placenta works as a strong barrier against Cd passage to the fetuses whose umbilical cord blood Cd levels were significantly lower than those of mothers' blood (P < 0.001) [11], [16].

On the other hand, this study found a highly significant positive correlation between the maternal lead levels and the fetal lead and arsenic levels. In accordance, previous work demonstrated that lead and arsenic have positive correlations with each other [17], [18]. Moreover, both metals showed a synergistic action [19].

Also, a recent meta-analysis mentioned a positive association in most of the included studies, between placental lead level and that of cord blood [20]. The easy transfer of lead across the placental barriers by a passive diffusion process was documented previously [3]. Also, lead was declared to be detrimental even at low levels of exposure [21]. It is crucial to emphasise that lead was documented to induce oxidative stress through various mechanisms, causing structural injury and perturbation of various vital functions at the cellular level [22], [23].

Meanwhile, both lead and arsenic impair nitric oxide production, which is a known endothelial relaxing agent, leading to increased reactive oxygen species (ROS) generation [24], causing deterioration to the vascular endothelium, and this will end in vasoconstrictions [25]. Accordingly, we assume that they impair blood and nutritional supplies to the foetus via hampering the placental barrier proper function, and hence there will be no proper detoxification.

In conclusion, based on the present findings, it seemed that arsenic and lead might have a synergistic effect, causing derangement of the barrier function of the placenta. However, this function is partially effective regarding the transfer of arsenic and cadmium but less effective against lead. Importantly, cadmium showed a weak protective action mostly attributed to MTs action, but that needs to be clarified in further future research.

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Clinical Science

Clinical Significance of Minimal Residual Disease at the End of Remission Induction Therapy in Childhood Acute Lymphoblastic Leukemia

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Abstract

BACKGROUND: Detection of minimal residual disease (MRD) in the early phase of therapy is the most powerful predictor of relapse risk in children with acute lymphoblastic leukaemia (ALL).

AIM: We aimed to determine the significance of MRD at the end of remission induction therapy in the prediction of treatment outcome in children with ALL.

METHODS: Sixty-four consecutive patients aged 1-14 years with newly diagnosed ALL were enrolled in this study from January 2010 to October 2017. All patients were treated according to the ALL IC BFM 2002 protocol. MRD was detected at the end of remission induction therapy (day 33) by multiparameter 6-colour flow cytometry performed on bone marrow specimens with a sensitivity of 0.01%.

RESULTS: Overall, 42.2% of patients had detectable MRD on day 33 of therapy. MRD measurements were not significantly related to presenting characteristics but were associated with a poorer blast clearance on day 8 and 15 of remission induction therapy. Patients with negative MRD status on day 33 had a 5-year event-free survival of 94.6% compared with 76.1% for those with positive MRD status (P = 0.044).

CONCLUSION: MRD levels at the end of remission induction therapy measured by multiparameter flow cytometry have clinical significance in childhood ALL. High levels of MRD are strongly related to poor treatment outcome.

Introduction

Minimal residual disease (MRD) is defined as the presence of sub-microscopic levels of leukaemic cells. [1] Detection of MRD during remission induction and consolidation therapy is the most sensitive method to evaluate treatment response and one of the strongest predictors of outcome in childhood acute lymphoblastic leukaemia (ALL). Many studies have demonstrated the prognostic significance of measuring MRD in childhood ALL, suggesting that MRD positivity at serial time points during the treatment is highly predictive of relapse, and it is associated with poor treatment outcome [2], [3], [4], [5], [6], [7], [8], [9].

Current contemporary protocols incorporate MRD monitoring as the main stratification criterion for risk-adapted treatment. Recent studies have shown that personalised treatment based on MRD can improve the clinical outcome of children with ALL [16], [17], [18], [19], [20], [21]. Because of the strong correlation between MRD levels and risk of relapse, this concept includes treatment intensification for children with higher MRD levels and treatment de-intensification for patients with early MRD clearance. The techniques for MRD assessment allow an average detection of one leukemic cell among 10^4 to 10^5 normal cells, which represents a 100-fold increase in sensitivity compared to conventional bone marrow

cytomorphology. The most widely used methods for MRD assessment are multiparameter flow cytometric (FCM) analysis of aberrant immunophenotypes and polymerase chain reaction (PCR) amplification of different fusion genes transcript or the antigen receptor rearrangements for immunoglobulin (Ig) or the T-cell receptors (TCR). [10], [11], [12], [13], [14], [15]. Both methods are highly sensitive and specific, but expensive, complex, require qualified staff and, because of that, their use is restricted in countries with limited resources.

The purpose of our study was to determine the prognostic significance of MRD detected by flow cytometry at the end of remission induction therapy in children with ALL.

Material and Methods

Patients and treatment

From January 2010 to October 2017, 74 consecutive patients aged 1 to 14 years with newly diagnosed ALL were treated at the Department of Hematology and Oncology of the University Clinic for children's diseases in Skopje, Macedonia. Among the 74 patients, 64 patients in whom flow cytometric MRD assessment was done on day 33 of remission induction were enrolled in this study. Data on demographic characteristics, diagnostic immunophenotyping, molecular risk factors, early treatment response, flow cytometric MRD assay and treatment outcomes were retrospectively collected from the hospital electronic system and paper-based – medical records. The study was approved by the Ethics Committee of the Medical Faculty in Skopje.

Diagnosis of ALL was based on standard morphologic, cytochemical, immunophenotype and genetic studies. Patients were treated based on the intermediate-risk arm of ALL-IC BFM 2002 protocol consisted of induction (protocol I), consolidation (protocol M), delayed intensification (protocol II) and maintenance therapy with a total duration of 2 years. Induction chemotherapy consisted of glucocorticoids, vincristine, daunorubicin and L-asparaginase with intrathecal methotrexate. In protocol M all patients received four courses of high dose ($5gr/m^2$) methotrexate. Eight children in our cohort (2 children with BCR-ABL positive ALL and 6 with high positive levels of MRD at the end of induction therapy) were allocated into a high-risk group, and they were treated according to the high-risk arm of ALL – IC BFM 2002 protocol. One of the 2 patients with BCR-ABL positive ALL was treated with intensive chemotherapy alone, whereas the second patient was treated with chemotherapy plus imatinib. Informed consent had been obtained for all patients from their guardians before initiation of chemotherapy following The Declaration of Helsinki.

In Macedonia flow cytometry, which is performed at the Clinic of Hematology, is used in diagnosis for acute leukaemia, but its applicability in MRD assessment is limited. MRD analysis at the end of induction therapy (day 33) was performed in the reference flow cytometric laboratory of the General Hospital George Papanikolaou in Thessaloniki, Republic of Greece by multiparameter 6 color flow cytometry using bone marrow mononucleated cells which were sent immediately after bone marrow aspiration (sternum puncture) collected in ethylenediamine tetra-acetic acid tube. Leukaemia-associated immunophenotypes were investigated with various combinations of monoclonal antibodies conjugated to the following fluorochromes: fluorescein isothiocyanate (FITC), phycoerythrin (PE), allophycocyanin (APC), phycoerythrin-cyanin 5.1 (PC5), phycoerythrin-cyanine 7 (PE Cy7) and *allophycocyanin-cyanine 7* (APC-Cy7), (Table 1 and 2).

Table 1: Monoclonal antibody combinations used for MRD detection in precursor B ALL

FITC	PE	APC	PC5	PECy7	APC-Cy7
CD58	CD38	CD10	CD45	CD34	CD19
CD24	CD38	CD10	CD45	CD34	CD19
CD38	CD22	CD10	CD45	CD34	CD19
CD81	CD20	CD10	CD45	CD34	CD19
CD38	CD200	CD10	CD45	CD34	CD19

Cell staining was performed on FACSCanto II flow cytometer, using the FACSDiva software (BD Biosciences) for analysis. This detection method allows the identification of one leukemic cell among 10 000 or more normal bone marrow cells. MRD positivity was defined as ≥ 0.01% of mononuclear cells expressing leukaemia-specific immunophenotypes [3].

Table 2: Monoclonal antibody combinations used for MRD detection in T cell ALL

FITC	PE	APC	PC5	PECy7	APC-Cy7
CD7	CD1a	CD3	CD5	CD34	CD45
CD38	CD1a	CD3	CD5	CD34	CD45
CD99	CD1a	CD3	CD5	CD34	CD45

Statistical analysis

October 31, 2018, was chosen as the reference date for the collection of data. The mean observation time was 45.7 months (range 1-100 months). Associations between MRD, presenting features, and early treatment response was analysed with the χ^2 test or Fisher exact test. Event-free survival (EFS) was calculated from the date of diagnosis to date of first event or date of last follow up if no event occurred. The event was resistance to therapy, relapse or death from any cause. EFS and survival curves were estimated according to Kaplan-Meier and groups were compared by log-rank test. The significance level of 0.05 was used in all statistical test. All statistical analyses were performed using SPSS (Statistical Package for the Social Science) version 23.0.

Results

Patients' characteristics

Presenting clinical features of the 64 patients were summarised in Table 3. The median age of patients was 5.6 years (range 1-14 years). There was a slight predominance of males (57.8%). The median WBC count at presentation was 32.07 x 10^9/L (range 0.89-194.3 x 10^9/L). Precursor B cell ALL was diagnosed in 52 (81.2%) patients and T cell ALL in 12 (18.8%). CNS involvement at diagnoses was confirmed in 3 (4.7%) patients. The majority of patients were considered as standard risk based on NCI criteria. BCR-ABL was documented in 2 out of 45 patients.

Relation among MRD levels, presenting features, and early treatment response

Of the 64 patients, 37 (57.8%) had a bone marrow that was negative for MRD (< 0.01%), whereas 27 (42.2%) were MRD positive (≥ 0.01%) at the end of remission induction therapy. Among patients with MRD positive status, the levels of MRD were 0.01-≤ 0.1% in 11 (40.8%) children, 0.1-≤ 1% in 12 (44.4%) patients and >1% in 4 (14.8%) patients. Regarding MRD distribution by immunophenotype, we observed a higher rate of MRD positive findings among patients with precursor B cell ALL compared to patients with T cell ALL (44.2% versus 33.3% respectively), but it should be noted that quantitative MRD levels were higher in T cell ALL.

Table 3: MRD distribution according to patients' clinicobiological features

Characteristics	Total	MRD level on day 33		*P*
		< 0.01%	≥ 0.01%	
	64 (%)	37 (%)	27 (%)	
Gender				0.523
male	37 (57.8)	21 (56.8)	16 (59.3)	
female	27 (42.2)	16 (43.2)	11 (40.7)	
Age				0.194
1 to < 10	56 (87.5)	34 (91.9)	22 (81.5)	
10 -14	8 (12.5)	3 (8.1)	5 (18.5)	
WBC count (x 10^9/L)				0.615
≤ 50 x10^9/L	52 (81.2)	30 (81.1)	22 (81.5)	
> 50 x 10^9/L	12 (18.8)	7 (18.9)	5 (18.5)	
Immunophenotype				0.362
Precursor B-ALL	52 (81.2)	29 (78.4)	23 (85.2)	
T cell-ALL	12 (18.8)	8 (21.6)	4 (14.8)	
CNS involvement				
present	3 (4.7)	2 (5.4)	1 (3.7)	
absent	61 (95.3)	35 (94.6)	26 (96.3)	
NCI risk group				0.329
standard	48 (75.0)	29 (78.4)	19 (70.4)	
high	16 (25.0)	8 (21.6)	8 (29.6)	
BCR-ABL				
positive	2 (3.1)	0 (0)	2 (7.4)	0.151
negative	43 (67.2)	24 (64.9)	19 (70.4)	
unknown	19 (29.7)	13 (35.1)	6 (22.2)	
Prednisone response				0.019
PPR	7 (10.9)	1 (2.7)	6 (22.2)	
PGR	57 (89.1)	36 (97.3)	21 (77.8)	
BM - 15 day				0.003
M1	45 (70.3)	32 (86.5)	13 (48.2)	
M2	14 (21.9)	4 (10.8)	10 (37.0)	
M3	5 (7.8)	1 (2.7)	4 (14.8)	

Abbreviations: WBC = white blood cells; CNS = central nervous system; NCI = National Cancer Institute; NCI standard risk group = age 1 to <10 years, and WBC < 50 x 10^9/L; NCI high risk group= age ≥ 10 years or WBC > 50 x 10^9/L; PPR= absolute blast count in the peripheral blood ≥ 1 x 10^9/L; PGR = absolute blast count in the peripheral blood less than 1 x 10^9/L after 7 days of prednisone and one dose of intrathecal methotrexate on day 1; M1 BM (bone marrow) = < 5%, M2 = 5 to 25% and M3 = ≥ 25% leukemic blasts in BM.

Table 3 shows the relation between levels of MRD on day 33 and the clinicobiological features of the disease**.** In our analysis, the presenting features including gender, age, WBC count at diagnoses, CNS involvement, immunophenotype, NCI risk status and molecular risk factors did not differ significantly between patients with negative and positive MRD status at the end of induction therapy. Two cases with BCR-ABL positive ALL, which is prognostically unfavourable had ≥ 0.1% leukemic cells at the end of remission induction, but this failed to reach statistical significance due to small sample size (*P* = 0.151). These findings indicate that presenting prognostic features could not identify patients who will have undetectable MRD at the end of remission induction therapy. We also tested whether earlier treatment response determined by prednisone response and bone marrow morphology on day 15 would predict the presence of MRD after completion of induction therapy. In our series, MRD levels differed significantly between prednisone poor and good responders. Patients with prednisone poor response (PPR) were more likely to be MRD positive at day 33 than patients with prednisone good response (PGR) (*P* = 0.019). Bone marrow morphology on day 15 also had a significant impact of MRD status on day 33. Patients with M2 and M3 bone marrow morphology on day 15 were more likely to be MRD positive at the end of induction therapy than patients with M1 (*P* = 0.003). In contrast to presenting clinical features, our findings indicate that the earlier clearance of leukemic blasts in peripheral blood and bone marrow could predict patients who will attain negative MRD status at the end of remission induction therapy.

When comparing 2 groups of patients based on end-induction MRD status, 27 MRD positive patients on day 33 had a significantly lower 5-year EFS than 37 MRD negative patients (76.1% versus 94.6; respectively, *P* = 0.044; Figure 1). The main event in our series that contributed to the EFS was the occurrence of relapse. Relapses were recorded in 8 (12.5%) out of a total of 64 patients. Among patients with positive MRD findings, the relapse rate was 22.2% compared to 5.4% among MRD negative patients (Table 4). Of note, none of the MRD negative patients with precursor B cell ALL experienced relapse during the follow-up study period. Thus, relatively rapid elimination of MRD in patients with this subtype of leukaemia identifies cases with an excellent prognosis.

Table 4: Description of events contributing to EFS comparing MRD negative and positive patients

	MRD negative patients N = 37	MRD positive patients N = 27
Events	N (%)	N (%)
Isolated BM relapse	2 (5.4)	5 (18.5)
Combined relapse (BM+CNS)	0	1 (3.7)
Death in remission	0	2 (7.4)
Total deaths	2 (5.4)	7 (25.9)

Discussion

The prognostic importance of MRD in childhood ALL is well established in numerous clinical studies [2], [3], [4], [5], [6], [7], [8], [9], [10], [11], [12], [13], [14], [15]. Modern treatment protocols for childhood ALL recommended MRD monitoring at multiple time points to evaluate the effectiveness of chemotherapy in the elimination of leukemic cells. Complex and sophisticated methods such as PCR-based techniques or multiparametric flow cytometry have been developed and evaluated to detect MRD. MRD measurement during the early phase of treatment (on day 8 and day 15/19) and at the end of induction therapy (day 29, 33 or 42) is considered the main predictor of treatment outcome and essential tool for risk stratification, aimed at both treatment intensification and reduction [8], [9], [10], [11], [12], [13], [14], [15], [23], [24], [25].

Patients with less than 0.01% leukemic cells at the end of remission induction are likely to have an excellent treatment outcome, whereas patients with high levels (i.e., ≥ 1%) of MRD at the end of the induction phase have a significantly higher risk of relapse and should be considered for alternative treatments. [3,4,8] Recent studies have demonstrated that intensification of therapy for patients with high levels of MRD can improve their outcome [12], [15].

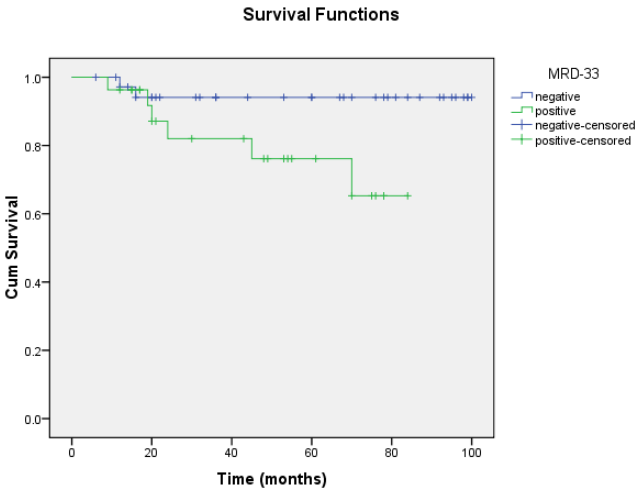

Figure 1: Kaplan-Meier estimate of 5-year Event-free survival based on MRD status on day 33 (negative versus positive) EFS = 94.6% versus 76.1% respectively, P = 0.044

In this study, MRD measurement was performed at the end of induction therapy, as this time point seemed most relevant for the treatment decision. Our date defines a cohort of 37 patients with negative MRD status at the end of induction therapy who have a superior EFS compared to those with positive MRD and this finding is consistent with numerous clinical studies [2], [3], [5], [6], [7], [8], [10], [12], [13], [14], [15], [23]. Researchers of Children's Oncology Group (COG) studied the prognostic impact of MRD measured by flow cytometry in the peripheral blood at day 8, end-induction (day 29) and end-consolidation bone marrows in 2143 children with precursor B-cell ALL. The presence of MRD in day-8 blood and day-29 bone marrow was associated with a lower EFS in all risk groups; even patients with 0.01% to 0.1% day-29 MRD had poor outcome compared to MRD negative patients at the end of remission induction (5-year EFS 59% vs 88%) [8]. In the AIEOP – BFM ALL 2000 study, 3184 patients with precursor B cell ALL were stratified by MRD measured on days 33 and 78 based on immunoglobulin and TCR gene rearrangements into three groups with a significantly different outcome. Patients defined as standard risk (42%) showed a 5-year EFS estimated at 92.3%, while intermediate (52%) and high-risk patients (6%) showed a 5-year EFS of 77.6% and 50.1%, respectively [10] In the study by Basso et al. measurement of MRD by flow cytometry on day 15 bone marrow was the most powerful early predictor of relapse. Standard risk patients had a significantly lower 5-year cumulative incidence of relapse compared to patients from the intermediate and high-risk group (7.5% vs 17.5 vs 47.2%, respectively) [9].

In our cohort, certain clinicobiological features of ALL didn't show relation to the speed of leukemic blasts reduction. Investigators of Total Therapy studies XIIIA and XIIIB at St. Jude Children's Research Center have shown that the patient`s age and the presence or absence of adverse genetic abnormalities were directly related to the speed and extent of initial cytoreduction [3]. And other study groups have observed slow clearance of MRD in NCI high-risk children, or with leukemic blasts expressing BCR-ABL [2], [26]. In our cohort, the BCR-ABL fusion transcript was confirmed in two patients, and both of them were MRD positive at high levels at the end of induction therapy. Despite the lack of statistical significance, probably due to the small sample size, it can be said that our results are in agreement with the previous studies [2], [3], [26]. MRD clearance is depended by the biology of the leukemic blasts, but other factors as well including specific host germline pharmacogenetics polymorphisms can affect in vivo treatment response and regulate treatment efficacy in each patient [27].

Prednisone response and bone marrow morphologic evaluation on day 15 are an integral part of the BFM protocol`s stratification scheme. Prediction of treatment outcome by conventional morphological assessment of treatment response is still of great importance, especially to countries with limited resources for MRD monitoring [28]. Our study confirmed a significant association between rapid early clearance of leukemic cells from peripheral blood and bone marrow and attaining MRD negative status at the end of induction therapy. This is in agreement with the previous study of Fronkova et al., who observed that in patients treated according to ALL IC BFM 2002 protocol, MRD negativity at day 33

was associated with good prednisone response and non – M3 morphology at day 15 [29]. Researching whether it is possible to avoid MRD testing in some subgroups of ALL patients, Fronkova et al., found that morphological criteria in ALL-IC are able to identify most MRD high-risk patients, but fail to define the MRD low-risk group, for who is possible treatment reduction to avoid long-term toxicities, which is the challenge of modern leukaemia treatment [29].

In conclusion, measurement of blast clearance allowing the quantitative definition of MRD is a mandatory tool for favourable treatment outcome of childhood ALL. Our study confirmed the excellent outcome for childhood ALL in patients with negative MRD monitoring by flow cytometry at the end of induction therapy. MRD assessment is still not available in our treatment centre and considering its prognostic importance; there is an urgent clinical need to introduce in routine practice.

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Clinical Science

Long-Term Results of Multiple Anterior Cervical Discectomy with Cage Fusion Technique: Results of Multiple Centre Study

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Abbreviations: ACDF = Anterior cervical discectomy and fusion; MRI = Magnetic resonance imaging; C.T = Computed tomography; VAS = Visual analogue scale

Abstract

BACKGROUND: Cervical herniation is commonly treated by anterior cervical discectomy and fusion (ACDF) if conservative management has failed in relief of the patient's symptoms. Disc fusion is needed after ACDF as anterior longitudinal ligament will be absent after doing the operation, especially if multiple levels are needed. The occurrence of complications as cage subsidence and adjacent segment failure related to the length of follow up as they are increasing in percentage is directly proportional to the length of follow up.

AIM: Analysis of the results for patients who underwent 3 levels of ACDF with cage fusion for short term and long term follow up in multiple centres as the visual analogue score for neck pain & brachialgia.

METHODS: This retrospective cohort series of 68 patients selected out of 136 patients suffering from 3 levels of degenerative cervical disc disease who were unresponsive to adequate conservative therapy. All cases were treated at one of the neurosurgery departments of 3 different hospitals (Naser institute for research and treatment hospital, Haram hospital for research and treatment and Misr university for science and technology) by the same surgical team in the period from February 2012 to February 2017.

RESULTS: We found in this study;68 patients fulfilling the inclusion criteria, of the 29 patients underwent 3 levels of ACDF starting from C3-4 (42.65%) and 39 patients who underwent 3 levels of ACDF starting from C4-5 (57.35%). Clinical assessment for VAS pain score for both neck pain and radiculopathy were done before the surgery and immediately post-operative and during each time follow up visit and we found statistically significant immediate postoperative improvement. ($P < 0.05$)

CONCLUSION: Stand-alone three levels of an anterior cervical discectomy with cage fusion technique improved the clinical outcomes on long term follow up.

Introduction

A cervical herniation is commonly treated by anterior cervical discectomy and fusion (ACDF) if conservative management has failed to relieve the patient's symptoms. The usage of cervical intervertebral disc replacement with cage achieves immediate load-bearing support, restoration of disc height, intervertebral foraminal decompression and facilitates interbody fusion [1]. Cage subsidence is considered a major long-term complication after insertion of a cervical intervertebral cage [1], [2]. Another important complication is the occurrence of adjacent segment failure after fusion of intervertebral disc whether it is single fusion or multiple levels [3], [4], [5], [6].

Fusion is needed after ACDF as anterior longitudinal ligament will be absent postoperatively, especially if multiple levels are operated upon [7]. For that reason, some authors prefer to do fixation with fusion while others have shown that fusion alone would give the same results as fixation with fusion [7], [8].

The occurrence of complications such as cage subsidence and adjacent segment failure is related to the length of follow up as their increase in percentage is directly proportional to the length of the follow up [5], [9], [10], [11], [12].

Neck pain and brachialgia are the main symptoms that result from cervical disc subsidence and adjacent segment failure due to narrowing of the intervertebral foramen in some cases [14].

We reviewed and analysed data from patients who underwent 3 levels ACDF cage fusion for short term and long term follows up in multiple centres regarding the visual analogue scale (VAS) for neck pain and brachialgia [15].

Material and Methods

This retrospective cohort series of 68 patients selected from a total of 136 patients suffering from three levels of degenerative disc disease who were unresponsive to adequate conservative therapy. They were operated at neurosurgery department of 3 different hospitals (Naser institute for research and treatment (MOH), Haram hospital for research and treatment (MOH) and Misr university for science and technology, by the same surgical team in the period from 2012 to 2017. Patients were selected from the data saved in each hospital with these inclusion criteria: 1) degenerative disc herniation with or without osteophytes, 2) brachialgia and neck pain were the main complains of these patients, 3) all patients underwent 3 levels ACDF with no other procedure in the cervical spine. Any patient with recurrence or history of previous cervical posterior laminectomy were excluded from the study. The sheets of all patients included in the study included proper history is taken from the patients and complete general and neurological examination (Table 1).

Table 1: Frequency of preoperative clinical symptoms. (No. = numbers of cases, % = percentage)

Symptom	No.	%
Brachialgia	68	100
Neck pain	64	94.1
Numbness	64	94.1
Motor weakness	39	57.3
Sphincter disorder	12	17

The selected patients (68) in the three hospitals underwent three levels ACDF with the insertion of intervertebral disc cage of the PEEK type and using a synthetic graft to enhance fusion (Figure 1).

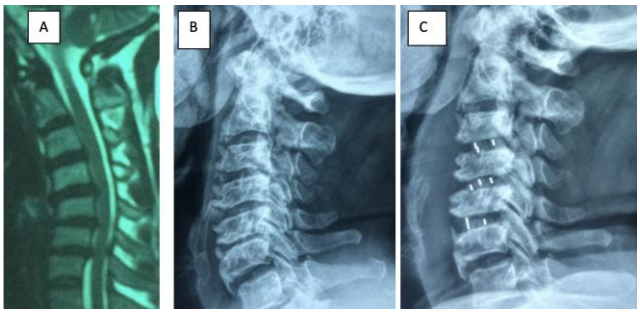

Figure 1: A) Preoperative MRI showing C3-4, C4-5, and C5-6 disc prolapse; B) Preoperative X-ray of the cervical spine lateral view of the 3 levels showing osteophyte disc complex; C) Postoperative X-ray lateral view of the cervical spine showing good positioning of 3 cages of the C3-4, C4-5, and C5-6

The mean age was 57.1 years (range from 46 to 71 years). 30 of our 68 cases were females, while the other 38 cases were males. Follow up using visual analog scale (VAS) was done for at least 3 years postoperative at 3 months, 6 months, 1 year, 2 years and three years postoperative for both neck pain and shoulder pain (radicular pain) out of 10 points with words that convey “no pain” at one end and “worse pain” at the opposite end (Table 2).

Table 2: Incidence of complications

Complication	No. of cases	Percentage %
Transient dysphagia	11	16.17
Transient hoarseness	6	8.82
Infection	1	1.47
Hematoma	0	0
Dural tear	2	2.94
Spinal cord injury	4	5.88
Cage subsidence	20	29.41
Adjacent segment	8	11.76
pseudarthrosis	11	16.17

Statistical analysis

Statistical analysis was done using IBM SPSS statistics version 20. Data were presented as mean ± standard deviation, frequencies and range. All the numerical data were analysed by analysis of variance (ANOVA). Non-numerical data were analysed using chi-square. A probability value (P-value) less than 0.05 was considered statistically significant.

Results

We found in this study, 68 patients fulfilling the inclusion criteria. 29 patients underwent 3 levels ACDF starting from C3-4 (42.65%) and 39 patients underwent 3 levels ACDF starting from C4-5 (57.35%). Preoperative complaints of the patients obtained from their sheets are listed in Table 1. Postoperative assessment of the patients was done immediately postoperative, and the follow up was done after 3 months, 6 months, 1 year, 2 years and 3 years with overall complications are shown in (Table 3), also at the end of 3 years there is no pseudarthrosis.

Table 3: Frequency of clinical improvement

Symptom	No. of cases improved	Percentage %
Brachialgia	59	86.76
Neck pain	53	82.81
Motor weakness	31	79.48
numbness	54	84.45
Sphincter disorder	7	58.33

Clinical assessment for VAS pain score for both neck pain and radiculopathy were done before the surgery and immediately post-operative and during each, for follow up visit, and we found statistically significant improvement immediately postoperative after ($P < 0.05$). Assessment of VAS for both neck pain and brachialgia done at the 3 months and six months follow-ups were statistically

insignificant compared to the immediate post-operative assessment (P < 0.05), while in the follow up at 1, 2, 3 years the VAS score for neck pain and brachialgia became worse in comparison of the immediate post-operative. Patients VAS score for both neck pain and brachialgia both pre-operative and post-operative, immediate,3 months, 6 months, 1, 2, 3 years is given in Table 2. Motor weakness improved gradually after surgery up to six months postoperative, and in our study 31 out of 39 patients improved (79.48%), numbness improved in 54 patients out of 64 (84.43%) and sphincter disorder improved in 7 patients out of 12 patients (58.33%) (Table 4).

Table 4: Assessment of clinical outcome for pain in the neck and brachialgia using VAS score for pain

Values are mean ± standard deviation							
Type of pain	Preop.	1 month	3 months	6 months	1year	2 years	3 years
Neck pain	7.1 ± 1.0	2.2 ± 1.2	2.3 ± 1.2	2.3 ± 1.2	2.9 ± 1.3	3.4 ± 1.3	3.6 ± 1.4
Brachialgia	7.5 ± 1.1	2.2 ± 1.1	2.1 ± 1.1	2.1 ± 1.1	2.6 ± 1.1	3.9 ± 1.1	3.5 ± 1.2

Cage subsidence was reported at the end of the study in 20 cases (29.41%), and in four cases we needed to do revision surgeries with plate fixation as the patients do not improve conservatively (Figure 2).

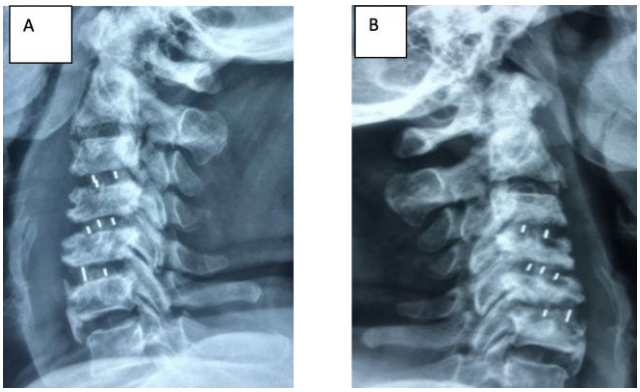

Figure 2: A) Three months postoperative C3-4, C4-5, and C5-6 cervical invertebral cages with good placement of cages; B) One year postoperative follow up of the same patient showing cage subsidence of the three cages

Adjacent segment failure has been noted in 8 cases,11.76% (Figure 3).

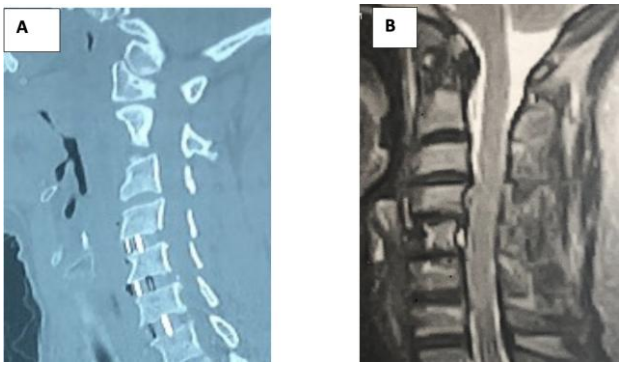

Figure 3: A) Postoperative C.T scan of the cervical spine of a patient complaining of axial pain after doing ACDF with the insertion of C4-5, C5-6, and C6-7 cages; B) MRI cervical spine of the same patient showing adjacent segment failure at the level of C3-4

Fusion of the intervertebral disc was assessed radiologically through detection of the presence of trabecular bone across the interfaces without any lucencies between the cage and the endplate of the vertebra and in our study at 6 months the rate of pseudarthrosis was 16.17% (11/68 patients) while at the end of study at 3 years it was 100% (Table 5) and (Figure 4).

Table 5: Incidence of fusion rate. Values are in percentage

Follow up period	No. of cases	Percentage %
1 year	53	77.9
2years	64	94.1
3 years	68	100

A case ACDF with the insertion of C3-4, C4-5, and C5-6 showing good fusion is shown in Fig. 4.

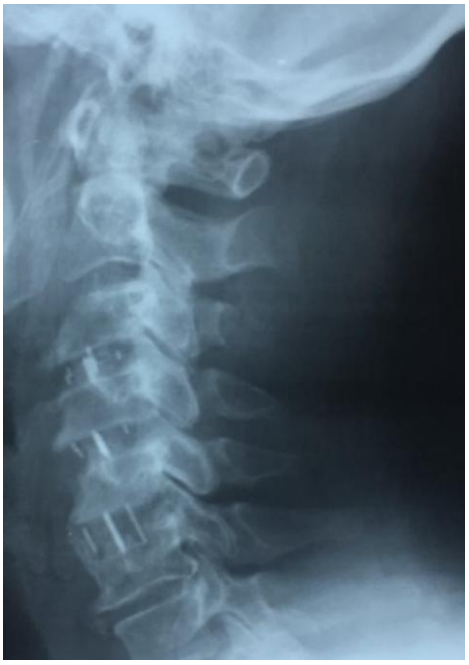

Figure 4: A case ACDF with the insertion of C3-4, C4-5, and C5-6 showing good fusion

Discussion

Anterior cervical discectomy (ACDF) and placement of intervertebral cage were commonly performed for patients with single-level and double-level pathologies as stand-alone without fixation to degenerative cervical disc disease. Many authors also recommend the same procedure for three levels of cervical disc disease [16], [17]. The idea for putting the intervertebral cervical cage with synthetic bone material is to restore the disc height, enhance bone fusion and lastly working as weight shearing device [18], [20]. Some authors noted that stand-alone cage fusion in three cervical levels without plate fixation has more complications than cage fusion with fixation [20].

In our retrospective study, cervical neck pain

showed statistically significant relief (P < 0.05) throughout follow up till 3 years using the VAS score for cervical neck pain. Song Kj et al. reported improvement of the clinical outcome after 3 level discectomy and cage fusion [19]. In this retrospective study, we noticed that brachialgia significantly improved according to the VAS score from 7.5 ± 1.1 to 2.2 ± 1.1 with (P < 0.01). Zajonz D et al. reported his work on 17 patients with a stand-alone cage on 33 cervical cages with the postoperative improvement of brachialgia in spite of cage subsidence [23]. Transient dysphagia occurred in 11 patients (16.17%), and transient hoarseness of voice in 6 patients (8.82%), the cause of dysphagia in this study is not well known, and it may be explained by long-time of retraction of the oesophagus or manipulations on its wall during surgery. Also, hoarseness of voice is usually transient and disappeared after 3 months, and it is due to unilateral affection of recurrent laryngeal nerve. De La Garza-Ramos R and his colleagues reported a high incidence of dysphagia and transient hoarseness of voice in three and four levels stand-alone cage fusion [2], [21].

In our study we noticed mild increase of VAS score for neck pain (from 2.3 ± 1.2 at 3 months to 3.6 ± 1.4 at 3 years) and for brachialgia (from 2.1 ± 1.1 at 3 months to 3.5 ± 1.2) and this might be due to new osteophyte formation, mild instability, disc subsidence and loss of cervical lordotic curvature. Liu Hong et al., reported in their series of 25 patients the same results about the improvement of clinical symptoms with three levels stand-alone cervical cages with the use of allograft [4], [22]. Cage subsidence occurred when there is a decrease of the disc space ≥ 3 mm, from the original postoperative X-ray. In our retrospective series study, the rate of subsidence was 20 cases (29.41%), and only four of them required plating at six months, and the rest of patients improved on conservative management. The causes of cage subsidence may be due to over distraction, aggressive removal of the endplate and improper large size of the cage placed. Reducing the rate of subsidence could be achieved by avoiding these causes. Zajonz D et al., in their retrospective cohort study on 33 cervical segments that were treated by ACDF with stand-alone cage fusion in 17 patients and noted the occurrence of cage subsidence was observed in half of their cases with no effect on the clinical results [23].

Adjacent segment failure is defined as degeneration of the adjacent disc superior to the fusion levels or inferior to them [21]. In our study 8 patients (11.76%) developed adjacent segment disease during the follow-up time. Clinal improvement was achieved with conservative management and required no further intervention. Song Kj et al. noted the same observation when they operated on 21 patients with a degenerative cervical spinal disease requiring three levels ACDF using PEEK cages and plate fixation [19].

In our study, the rate of bone fusion was 77.9% at the end of the first year, 94.1% by the end of the 2nd year and 100% at 3rd year. The criteria of bone fusion are the presence of bone formation between the cage and vertebral endplate, lack of motion during dynamic cervical X-ray and confirmed by doing a C.T scan of the fused levels. The results at the end of the third year reached 100% in our study due to using plate fixation for the four cases who developed cage subsidence. Pereira EAC et al. observed these results in their study on patients requiring three and four-level discectomy and stand-alone cage fusion [6], [23].

In conclusion, stand-alone three levels anterior cervical discectomy with cage fusion technique improved the clinical outcomes on long term follow up, disc subsidence and adjacent segment disease did not affect the clinical results.

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Availability of Data and Materials

They are available from the corresponding author on responsible request. E-mail of the corresponding author (hamdi.nabawy@gmail.com)

Authors' contributions

HN performed study design, wrote the manuscript, the main surgeon in all cases, did the statistical analysis and revision of the work. ML helped in the study design, helped HN in the cases done in Misr University, and revised the paper. MW helped in the surgeries, collecting data and references and helped in the statistical analysis.

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Clinical Science

Functional Outcomes of Surgical Management for Spinal Epidural Masses in an Egyptian Tertiary Hospital

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Abstract

BACKGROUND: The spinal epidural space, covering the dural sac, is located along the posterior longitudinal ligament anteriorly, the ligamentum flavum and the periosteum of laminae posteriorly, and the pedicles of the spinal column by the intervertebral foramina containing their neural elements laterally. It could be affected variably by different types of diseases, either as primary lesions or as an extension from a disease process in the nearby tissues and organs.

AIM: We aimed to present clinically and surgically patients with spinal epidural masses operated in the Neurosurgery Department of Cairo University Hospitals, Cairo, Egypt, along a time interval of one year.

METHODS: In this prospective cohort study, we analysed motor deficits, sensory deficits, and bowel and bladder dysfunction. We have performed decompressive laminectomy on 19 patients with spinal epidural masses together with mass excision as long as the tumour was accessible, with or without fixation.

RESULTS: All patients were radiologically assessed by MRI over the affected side of the spine. D10 was the commonest site in our study to be affected in 10 cases of our participants (23%), followed by D5, D7, and D12 each of them was affected in 6 cases (14%), in another word spinal segments by order of frequency to be affected were dorsal followed by lumbar spine. All patients included in this study (100%) showed an obvious improvement as regard pain and tenderness.

CONCLUSION: Surgical interventions have improved the quality of life for our patients with spinal epidural masses.

Introduction

Spinal Epidural Space is a space between the dura and the ligamentum flavum and periosteum of the vertebral bodies, pedicles and laminae. Spinal epidural space pathologies, both intrinsic and extrinsic to the epidural space have been described in scattered articles in literature [1].

In this prospective cohort study, we will report a one-year surgical experience in managing 44 patients with different types of epidural spinal masses, presented at the Department of Neurosurgery in Kasralainy Medical School Teaching Hospitals, Cairo, Egypt, regarding motor deficits, sensory deficits, bowel and bladder dysfunction.

Methods

Patients

In this study, we clinically and surgically evaluated 44 patients with spinal epidural masses. All patients were operated in the Neurosurgery Department of Cairo University Hospitals, Cairo, Egypt, along a time interval of one year. We followed very strict criteria in selecting our candidates from the patients who were suffering spinal epidural masses which resulted in neurological deficits.

Inclusion criteria were patients indicated for surgical management for unknown primary tissue diagnosis, spinal instability or deficits, Radio-resistant tumours, recurrence after maximal XRT

and rapid neurologic deterioration. Exclusion criteria included patients with very radiosensitive tumours, total paralysis, expected survival < 3-4 months, multiple lesions at different spinal levels and the inability to tolerate the surgery.

Data Collection

All candidates provided the necessary data fulfilling our study protocol and were investigated clinically prior surgical interventions than were maintained on a scheduled post-operative follow-up visits.

A) History Taking: Age, gender, occupation, time of initial complaint till the time of presentation, history of any type of cancer that the patient suffered from, history of co-morbidities, e.g. diabetes mellitus, smoking, history of previous operations and history of tuberculosis or anti-tuberculosis treatment.

B) Clinical Examination: All patients were clinically evaluated and examined with special care to the following signs and symptoms: low back pain, motor deficit, sensory deficit, sphincteric deficit, upper motor neuron (UMN) manifestations in case of cervical, thoracic, or upper lumbar lesions and lower motor neuron (LMN) manifestations in the lower lumbar manifestations.

C) Radiological Investigations: Cervical, thoracic, lumbar and sacral spine X-ray: anteroposterior, lateral and stress views (flexion-extension) were done with the emphasis on the signs of instability (in stress views).

1) Computed Tomography of the spine: For assessing the bony spines and the ability of instrumental fixation.

2) Cervical, thoracic and lumbosacral MRI (with contrast): To detect possible pathological abnormalities and for feasible interventions.

D) Management: All patients were indicated for surgery; we emphasised on certain points for the study design:

1) Any history of the previous operation

2) Type of the selected operation:

- Cervical spine: Posterior laminectomy, lateral mass fixation, anterior corpectomy or anterior plate placement.

- Thoracic spine: Usually, circumferential fixation was done.

- Lumbosacral spine: Decompressive laminectomy and transpedicular fixation, Intra-operative cause of neural compression;

- Intra-operative findings of Abscess, pott's disease, neoplastic causes (to correlate with pre-operative imaging), need for fusion (confirmed intra-operative instability) and intra-operative or postoperative complications.

E) Preoperative Clinical Evaluation Measures: All candidates were clinically examined preoperatively and postoperatively for comparative evaluation between the clinical condition preoperative and postoperative.

The Frankel Grade, according to the Congress of Neurological Surgeons, serves as a classification guide for spinal injuries. When a spinal cord injury occurs, patients were often told that they have an injury at a given spinal cord level and were given a qualifier indicating the severity of the injury, such as "complete" or "incomplete."

Frankel Grading system:

1. Complete neurological injury. No detected motor nor sensory functions below the level of the lesion.

2. The preserved sensation only. No detected motor function below the level of lesion with some preserved sensory function below the level of the lesion.

3. Preserved motor, nonfunctional. Some preserved voluntary motor functions below the level of the lesion but too weak to serve any useful purpose, sensation may or may not be preserved.

4. Preserved motor, functional. Functionally preserved useful voluntary motor function below the level of injury.

5. Normal motor function. Normal motor and sensory functions below the level of the lesion and abnormal reflexes may persist.

Results

History Taking

a) Sex: There were 36 males and 8 females (4.5:1), which shows male predominance by 81.8% *(Figure 1A)*.

b) Age: Participants in this study ranged from 15 to 69 years old; the mean age was 48.3 years while the majority of patients, 22 cases. i.e. 50%, were between 50 and 60 years old (the sixth decade) *(Figure 1B)*.

c) Smoking: 32 cases (72.7%) in this study were smokers, while 12 ones (27.3%) were non-smokers.

d) Trauma: Only 4 cases of our participants (9%) gave us a history of preceding trauma in about 4 days, 6 days, 1 week and ten days before the onset of symptoms. There was no history of

trauma in the other 40 cases (91%).

e) Diabetes Mellitus: There was a history of diabetes in 14 cases (31.8%). it was controlled in 6 cases (13.6%) and uncontrolled in 8 cases (18.2%) *(Figure 1C)*.

No history of diabetes was given in 30 cases (68.2%).

f) Hypertension: There was a history of hypertension in 20 cases (45.4%), controlled in 8 cases (18.1%) and uncontrolled in 12 cases (27.2%). No history of hypertension in the other 24 cases (54.4%).

g) Malignancy:

- 40 cases of our patients were proved to be metastatic tumours (90%) classified as follows:

- 4 cases proved to be multiple myeloma (9%).

- 4 cases proved to be renal cell carcinoma (9%).

- 8 cases with bronchogenic carcinoma (18%).

- 14 cases with hepatocellular carcinoma in origin (32%).

- 2 cases proved to be malignant lymphoma (4.5%).

- 2 cases were affected by breast carcinoma (4.5%).

- 2 cases with undifferentiated metastatic carcinoma (4.5%).

- 2 cases proved to be spindle cell sarcoma (4.5%).

- 2 cases proved to be chondrosarcoma (4.5%) and;

- In 8 cases of our patients, spinal epidural metastasis was the first presentation of malignancy (18%) *(Table 1), (Figure 1D).*

Table 1: Summary of Patients Data

Gender	Male	36	82%
	Female	8	18%
Trauma	Yes	4	9%
	No	91	91%
Diabetes Mellitus	Yes	14	31.8%
	No	30	68.2%
Smoking	Yes	32	72.7%
	No	12	28.3%
Hypertension	Yes	20	45.5%
	No	24	54.5%
Malignancy	Yes	40	91%
	No	4	9%

h) Systemic Infection: 4 cases of our patients (9%) gave us history of respiratory tract tuberculosis and they also gave us history of receiving medical treatment of tuberculosis for about nine months.

i) History of initial complaints and presentations:

- All our cases (100%) gave us history of back pain varying in both sites, based on the site of the lesion, and severity, i.e how severe is the degree of spine affection.

- 40 cases were suffering from motor dysfunction (91%).

- 24 cases were suffering from sensory dysfunction (55%) and;

- 28 cases of our patients were suffering from sphincteric affection (64%) *(Figure 1E)*.

j) Clinical Evaluation:

- In this study, we found that back pain was the presented symptom in all our cases (100%). Spine tenderness was also found in all cases (100%) with variable sites according to the site of the lesion. Limb weakness was found in 40 cases (91%), from which 4 cases (9%) were suffering from both upper and lower limb weakness and 36 cases (82%) were suffering from lower limb weakness with variable degrees from Grade 0 to Grade 4 motor power. Grade 0 was found in 8 cases (18%), Grade 1 was also found in 8 cases (18%), Grade 2 in 6 cases (14%), Grade 3 in 12 cases (27%) and Grade 4 was found in 6 cases (14%). Sensory dysfunctions detected in 24 cases (55%) of our participants.

- Sphincteric affection recorded in 28 cases (64%) of our participants. 24 of these patients were suffering from urine and stool incontinence (55%) and 2 cases were affected with urinary retention (4.5%).

- 30 cases of our patients were suffering from hyperreflexia (68%) while 14 cases were suffering from hyporeflexia (32%) *(Figure 1F)*.

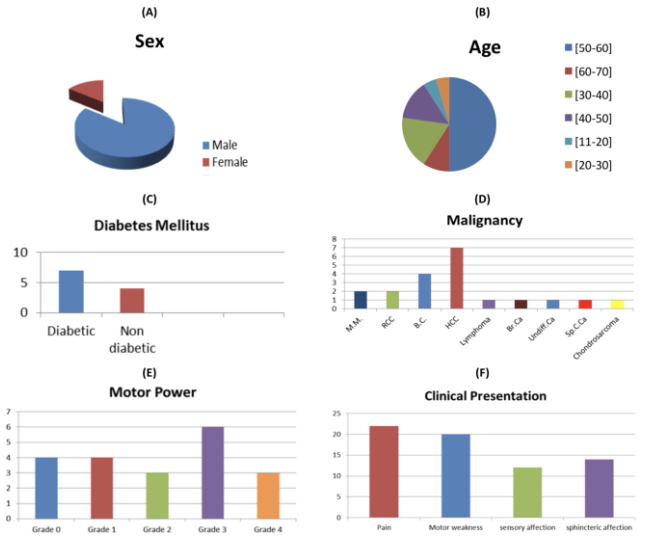

Figure 1: A), B), C), D), E), and F) Summary of Patients Data

Radiological Evaluation

- All patients in this study were radiologically assessed by MRI over the affected sites of the spine.

- D10 was the commonest site to be affected in our study (23%), followed by D5, D7, and then D12 which were affected in 6 of our cases (14%). The dorsal spine was the most commonly affected spinal portion in our study, 26 cases (59.1%), followed by lumbar spine in 12 cases (27.3%), cervical spine in 4 cases (9.1%), and then by sacrum in 2 cases (4.5%) *(Table 2)*.

Table 2: Patients Radiological Findings

Type of Investigation	Findings	No. of Patients	%
Site of lesion	Lumbar	26	59.1%
	Dorsal	12	27.3%
	Cervical	4	9.1%
	Sacral	2	4.5%
Signs of instability			
Slippage at the same or another level:	Yes	20	45%
	No	30	68.1%
MRI Spine	Neoplasm	40	91%
	Infection	4	9%

- MRI revealed spinal metastasis in 40 participants with intraspinal extension and cord compression, while 4 cases (9%) were affected by pott's disease.

- MRI of the spine also showed that none of our patients has any previous spine surgeries.

- Preoperative MRI, CT scans and Plain X-ray have shown the evidence of spinal instability in 20 cases (45.5%), but intraoperative scans have revealed that 26 cases were unstable and were fixed *(Figure 2)*.

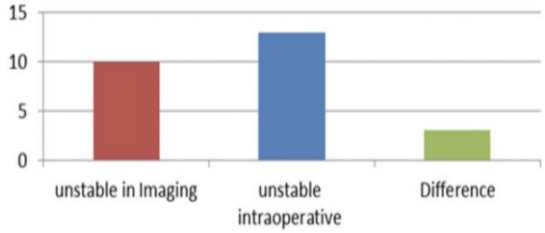

Figure 2: Difference between Radiological assessment and Intraoperative assessment of Instability

- Follow up CT scans and / or plain X-rays were done to all our cases in this study *(Table 2)*.

Surgical Intervention

a) Preoperative:

The time interval between the first presentation and the operation ranges from 5 days to 150 days, mean of 26.22 days:

- 38 cases (86%) were operated by a posterior approach.

- 4 cases (9%) were operated through lateral thoracotomy.

- 2 cases of our participants (4.5%), were operated by an anterior approach.

b) Intraoperative findings:

- 40 cases of our patients (91%) proved to be neoplastic in origin and 4 cases (9%) proved to be inflammatory (Abscess).

- 26 cases in this study (59.1%) show evidence of instability and their need for fixation with decompression of the spinal cord together with tumor excision.

- 24 cases (54.5%) were operated by decompressive laminectomy and transpedicular fixation, 4 cases of them (9%) were unilaterally fixed on the left side only.

- 10 cases (23%) were operated by decompressive laminectomy together with total mass excision.

- 4 cases (9%) were operated by decompressive laminectomy only where there were no need for fixation.

- 2 cases of our patients (4.5%) was operated by anterior corpectomy and pyra mesh insertion in a case of dorsal spine metastasis with no need for posterior fixation.

- 2 cases (4.5%) was operated by anterior corpectomy with total mass excision and lateral plate insertion with screws.

- 2 cases (4.5%) were operated by anterior corpectomy with total mass excision and expandable cage insertion.

c) Postoperative Outcomes:

All candidates in this study were postoperatively evaluated according to Frankel Grading system (A, B, C, D, and E) with the following outcomes:

- 22 cases (50%) showed improvement of their condition from Frankel grade C to Frankel grade D.

- 2 cases (4.5%) showed improvement of their conditions from Frankel grade A to Frankel grade C.

- 4 cases (9%) remained the same postoperatively as preoperatively with grade C.

- 6 cases (13.6%) remained the same postoperatively as preoperatively with grade D.

- 6 cases (13.6%) remained same postoperatively as preoperatively with grade A.

- 4 cases of our patients (9%) remained same postoperatively as preoperatively with grade E *(Figure 3)*.

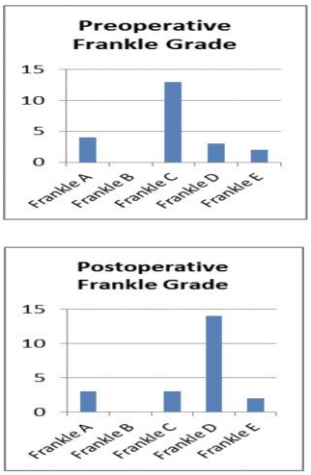

Figure 3: Comparison Between Preoperative & Postoperative Frankel Grade

Table 3: Summary of Neurosurgical Operations, Details And Outcomes

Neurosurgery; Details and Outcomes	No. of Patients	%
Intraoperative Findings:		
Instability	30	68.1%
Canal stenosis	8	18.2%
Operation:		
Decompressive laminectomy only	4	9%
Decompressive laminectomy with mass excision	10	22.7%
Decompressive Laminectomy with Transpedicular Fixation With: Unilateral mass excision	24	54.5%
Bilateral mass excision	20	45.5%
Anterior corpectomy with mass excision and pyra mesh insertion	2	4.5%
Anterior corpectomy with mass excision and lateral fixation with plate and screws	2	4.5%
Anterior corpectomy with mass excision and expandable cage insertion	2	4.5%
Complication:		
Dural tear directly repaired with sutures	2	4.5%
Wound infection	6	13.6%
CSF leak	2	4.5%

- All included participants in this study (100%) showed an obvious improvement regarding pain and tenderness.

- Regarding motor power, 24 cases out of the 40 cases presented with the deterioration of their motor power (55%) showed improvement of their motor power. However, 16 cases out of the 40 (36%), did not show improvement with no further deterioration of these cases.

- Regarding sensations, 18 cases (41%) out of the 24 presented with sensory dysfunction, showed marked sensory improvement. However, 6 cases

- (14%) out of the 24 cases showed no improvement in their sensory dysfunction, but there was no further deterioration.

- Regarding sphincteric affection, only 6 cases (14%) out of the 28 cases with sphincteric affection showed improvement, while the rest 22 cases (50%) showed no improvement at all.

d) Complications:

- 6 cases of our patients (13.6%) suffered from superficial wound infection for which they received intravenous systemic and local antibiotics for 14 days with total remission.

- 6 case (13.6%) in this study was complicated by a dural tear from which one of them, the tear was accessible and directly repaired with sutures while for two other cases, the tears were inaccessible, so they were covered by muscle and gel foam. Both two cases were complicated by postoperative CSF leakage which stopped in one case spontaneously with medications and re-stitching the wounds while the other case stopped after insertion of a continuous lumbar drain for 5 days with total stoppage of CSF leak and complete wound healing.

Case Study

Case 1

A 58-male patient, presenting with low back pain with tenderness, bilateral lower limb weakness, motor power grade 4 for about two weeks, intact sensations and intact sphincters. He gave us a history of bronchogenic carcinoma with brain metastasis two months before his

recent complaint, which was surgically removed. MRI dorsal spine showed D12 metastatic lesion which was operated by surgical decompression of the spine and unilateral transpedicular fixation of D10, 11, 12, L1, 2.

Postoperatively, the patient experienced marked improvement of back pain with the same motor power as preoperative *(Figure 4)*.

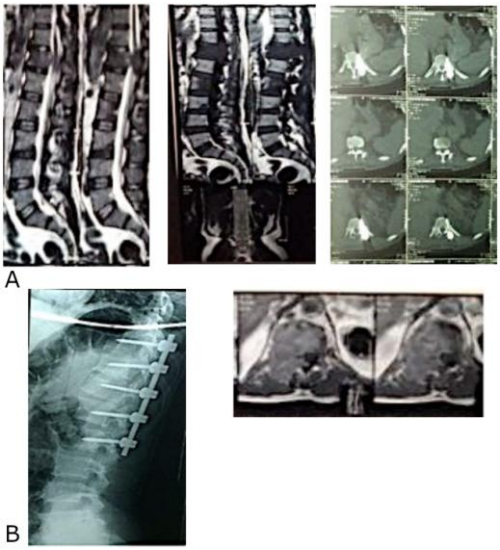

Figure 4: A) Preoperative Sagittal T1WI, T2WI and Axial T1WI; B) Postoperative Plain X-ray and Axial CT scan after fixation

Case 2

A 49-male patient, presenting with severe back pain and tenderness with bilateral lower limb weakness grade 3 weeks, sensory level at xiphisternum, and urinary incontinence for two weeks' duration. MRI dorsal spine showed D8 metastatic lesion, which was operated through lateral thoracotomy by anterior decompression with the insertion of pyra mesh. Postoperatively, the patient showed improvement of sensory function and motor power as it became of grade 4 with regaining his urinary continence. Pathology revealed that the patient was suffering from plasma myeloma *(Figure 5)*.

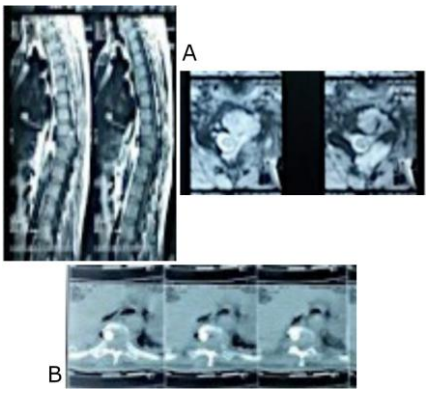

Figure 5: A) Preoperative Sagittal T1WI and Axial T2WI; B) Postoperative Axial CT scan after pyra mesh insertion

Case 3

A 29-male patient, presenting with back pain with tenderness, bilateral lower limb weakness of grade 2, the sensory level at the umbilicus, and urinary incontinence of 10 days' duration. MRI spine revealed D9 metastatic lesion, operated by lateral thoracotomy fixation with plate and screws together with decompressive laminectomy. Postoperatively, the back pain and tenderness were markedly relieved; motor power became of grade 4, sensory dysfunction improved, without improvement of sphincteric affection. Pathology was a high-grade spindle cell sarcoma (malignant peripheral nerve sheath) *(Figure 6)*.

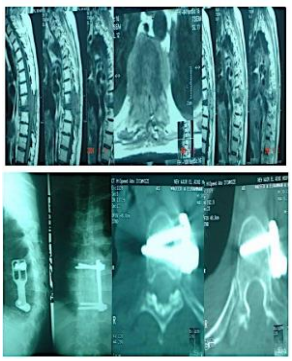

Figure 6: Preoperative Sagittal T1WI, Axial T1WI, and Sagittal T1WI with contrast Postoperative Plain X-ray Ap and lateral views and Axial CT scan post Fixation

Case 4

A 37 diabetic male patient presenting with back pain and tenderness, bilateral lower limb weakness of grade 3, intact sensations and urinary incontinence for five months. MRI dorsolumbar spine showed L1 metastatic lesion, operated by decompressive laminectomy and fixation of D12 and L2. Postoperatively, the patient showed no improvement with the same motor power and urinary incontinence. Pathology revealed renal cell carcinoma which firstly presented with spinal metastasis *(Figure 7)*.

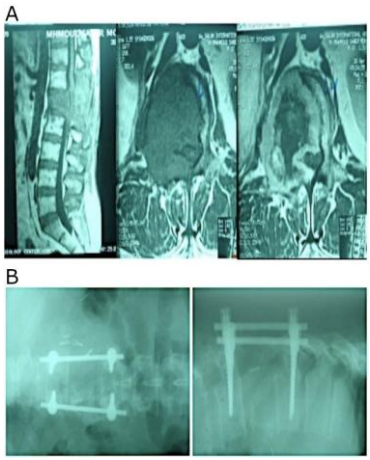

Figure 7: A) Preoperative Sagittal T1WI, Axial T1WI and Axial T1WI with contrast; B) Postoperative Plain X-ray A-P and Lateral Views

Case 5

A 53-male patient, with a history of chronic renal failure, presented with back pain and tenderness, bilateral lower limb weakness grade 0, hypothesis till the level of the umbilicus, and urinary and stool incontinence for about 12 days.

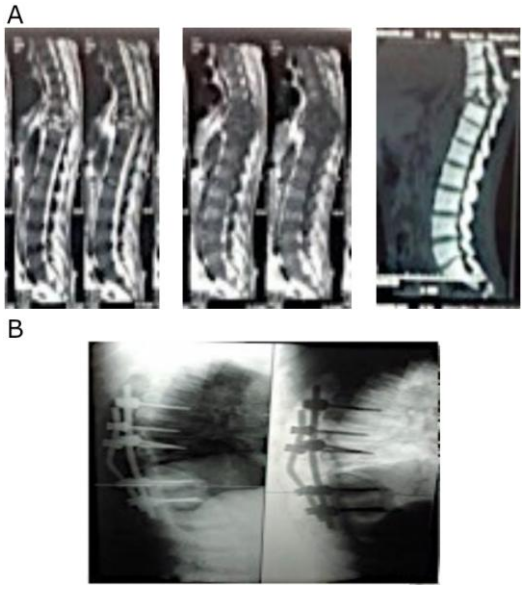

Figure 8: A) Preoperative Sagittal T1WI, Axial T1WI and Sagittal T1WI with contrast; B) Postoperative Plain X-ray Lateral and Axial views

MRI Spine showed D10 metastatic spinal lesion, operated by decompressive laminectomy and transpedicular fixation of D8, 9, 11 and D12. Postoperatively, the motor power showed a minimal degree of improvement as it became of grade 1 and sensations slightly improved. However, there was no improvement of sphincteric affection. This case was complicated by dural tear and CSF leak which was repaired intraoperatively. The CSF leakage persisted for 2 days and treated conservatively under intravenous antibiotic coverage. Pathology revealed renal cell carcinoma *(Figure 8)*.

Discussion

Patients with spinal epidural masses were complicated with spinal cord compression presented with back pain, sphincteric affection, motor and sensory dysfunctions.

Sensory affection in 24 cases (55%) of our participants. Near the findings obtained by Bach et al., [2], were a retrospective study over 398 patients from 1979 to 1985 in which 83% of patients suffered from pain, 50% suffered from sphincteric affection, and around 70% suffered from motor dysfunction and only 10% was of normal sensations.

In this study, all patients were radiologically assessed by MRI over the affected side of the spine. D10 was the commonest site in our study to be affected in 10 cases of our participants (23%), followed by D5, D7, and D12 each of them was affected in 6 cases (14%), in another word spinal segments by order of frequency to be affected were dorsal followed by lumbar spine, these results were in agreement of the results of Jacobs WB et al., [3], [4], where a retrospective study over 282 patients was done from 1990 to 2001 for patients operated for neurological deficits due to thoracic and lumbar deficits and the patients with dorsal spine affection were 78% of the patients. 20 cases of our participant show signs of spinal instability and the need of spinal fixation (45.5%), but intraoperatively 26 cases needed Spinal fixation (59.1%) and 4 cases as operated through anterior approach by anterior corpectomy and replacement with pyra mesh in one case and with expandable cage in the other one (9%), 14 cases (31.8%) needed no fixation. MRI also revealed Spinal metastasis in 40 cases with intraspinal extension and cord compression, and 4 cases (9%) were with pott's disease.

In the current study, we have performed decompressive laminectomy on 19 patients with spinal epidural masses together with mass excision as long as the tumour was accessible, with or without fixation. Fusion with instrumentation is indicated when postoperative segmental instability is inevitable after decompression or pre-existing instability is present or in the treatment of spondylodiscitis. Fixation with posterolateral fusion and instrumentation using rods and transpedicular screws was used in 12 patients (54.5%), 10 cases were detected preoperatively (45.5%) and 2 cases (9%) with inevitable instability due to aggressive bilateral medial facetectomy. By this technique we were able to stabilize the anterior and middle spinal columns as stated by *Denis (Denis F et al., 1984), these results were also concluded by Klimo P, et al.,* [5]*, in which they concluded that posterior approaches have traditionally been the most common surgical procedure used for MSECC, with superior results for decompressive laminectomy combined with instrumentation of the spine.*

All patients included in this study (100%) showed an obvious improvement as regard pain and tenderness. This results are in agreement with that reported by Sundaresan et al., [6], [7], [8], [9], [10], [11], [12], a retrospective study over 110 patients and the results revealed 82% improvement regarding pain relief and ambulatory status. Also in Jansson et al., about 70% of their patients showed improvement of at least one Frankel grade compared to that in Weigel et al., [13], [14], in which a retrospective study was done over 76 patients, where there were 58% Frankel improvement in the ability to walk 93%, and pain relief was noted in 89% of patients. Moreover, Wang et al., [15], a retrospective study over 140 patients in which there was the improvement of pain and improvement or stabilisation of their motor condition in 96% of their patients, and 75% of their patients regained the ability to walk again. Finally, in North et al., [16], [17], only 85% of patients were ambulatory preoperatively, and after surgery, about 97% were ambulatory.

The time interval between clinical presentation and surgical intervention revealed that the shorter the duration between them, the better were the results. Meanwhile, all cases reported improvement of pain and some cases reported improvement of their neurological deficit with the surgical interventions. These results in agreement with Michael et al., [18] as their results concluded that rapid diagnosis and emergency surgical treatment maximise neurological recovery. However, patients with complete neurological lesions or long-standing compression can improve substantially with surgery.

Table 4 summarises the suggested strategies to be followed with similar cases to our patients based on the clinical outcomes of the present study and the review of literature of the surgically treated patients with spinal epidural masses.

Table 4: Suggesting a strategy for management of spinal epidural masses

Tumour Localization	Approach	Tumour	Reconstruction	Fusion	Option
Cervical posterior	posterior	Complete resection	-	Lateral mass screws	
Cervical anterior	anterior	Complete resection	Cage or bone	Anterior plating	Lateral mass screws
Cervicothoracic junction posterior	posterior	Complete resection	-	Lateral mass +pedicle screws	
Cervicothoracic junction anterior (good prognosis)	combined	Complete resection	Cage	Lateral mass +pedicle screws +anterior plating	Pedicle screws
Thoracic posterior	posterior	Complete resection	-	-	
Thoracic Anterior (good prognosis)	combined	Complete resection	Cage	Pedicle screws	Anterior plating
Thoracolumbar posterior	posterior	Complete resection	-	Pedicle screws	
Thoracolumbar anterior (good prognosis)	combined	Complete resection	cage	Pedicle screws	Anterior plating
Lumbar posterior	posterior	Complete resection	-	-	Pedicle screws
Lumbar anterior (good prognosis)	Combined	Complete resection	Cage	Pedicle screws	Anterior plating
Sacrum	Posterior	Complete or subtotal resection	-	-	Posterior fusion

Spinal Epidural Masses are a heterogeneous group of patients with variable pathologies which can cause pain and irreversible loss of neurological functions. In most cases, this syndrome is caused by compression of the thecal sac and the spinal cord by these extradural masses.

The functional outcomes of surgical management for 44 patients with spinal epidural masses obtained from this study could be concluded as follows:

Spinal epidural masses were more common in males due to the higher incidence of malignancy and more exposure to environmental stresses.

The most common symptom was back pain and tenderness, and the most affected signs were motor weakness and sphincteric affection.

The time interval between the initial complaint until diagnosis and treatment correlated well with the outcomes. The earlier the diagnosis and treatment, the better the prognosis.

The sensitivity of MRI with contrast as verified by operative findings was found to be 100%, and it proved to be the gold standard of treatment and the diagnostic tool recommended for more delineation, accuracy and specification.

The most presenting pathology was metastatic spinal cord compression. Most of the cases were operated by neural tissue decompression and tumour excision together with fixation.

The most common complications of these surgeries encountered in our study was superficial wound infection and dural tear complicated with CSF leak.

We found that all our patients experienced variables degrees of pain improvement, and most of our patients experienced improvement of motor weakness with variable improvement in sensory dysfunction.

Surgical intervention for spinal epidural masses has improved the quality of life for our patients.

It is widely accepted that the results of surgery regarding spinal masses, especially those with instrumentation, have superior results over the traditional surgery.

In conclusion, surgical intervention for spinal epidural masses can be very useful and successful as regard pain, motor power, sensations, and sphincteric affection. Patients have experienced a better quality of life and regained their ambulatory power which gave them the ability to resume activities in life.

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Pre-Surgery Planning of Lower Limbs Major Joints Arthroplasty

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Keywords: Arthroplasty; Hip joint Limb shortening; Computer tomography; Load; Hip-spine syndrome; Contracture; Axial load; Functional study

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Abstract

BACKGROUND: Knee and hip joints endoprosthetics are the main surgical method of arthrosis treatment. The epidemiological incidence rate of the disease is growing steadily every year, affecting younger and younger people. Despite the proven tactics of joint endoprosthetics, an important issue is quality planning of surgery.

AIM: The aim of this research is to develop a device and a method that would contribute to solving the existing challenges of pre-surgery planning of hip endoprosthetics in patients with related pathologies, which have caused compensatory deformation, and making long vertebrarium-pelvis-lower limbs scout images with the patient lying on his back with an axial load in a computer tomography.

METHODS: Analog X-ray photographs of the pelvis made on film, digital DICOM images, and special planning programs are used for planning. However, according to numerous studies, the disease of the hip joint is not an independently isolated pathology. In most cases, this pathology is accompanied by changes in the lumbar spine. Often, patients prepared for endoprosthetics have a congenital deformity of tarsus or hip segment, which, during the knee, joint endoprosthetics surgery causes difficulties with the installation of an intramedullary guide.

RESULTS: The results after total knee arthroplasty according to the method modified at the Department showed a reduction of the WOMAC index slightly more than twice down to 37.26 ± 7.92. The number of revision surgeries after endoprosthetics decreased from 5 (5.7%) to 1 (1.1%) for the hip joint, and from 7 (4.3%) to 2 (1.3%) for the knee joint, respectively.

CONCLUSION: To form a proper guide entry point, it is necessary to assess the segment at the stage of surgery planning and examination of patients, which can be done using the proposed method. To remove the complications during the pre-surgery planning of hip joint endoprosthetics in patients with related pathologies, a device and methods have been developed for obtaining long topograms of the vertebrarium-pelvis-lower limbs complex with the patient lying on his back with the axial load in computer tomography.

Introduction

Depending on the duration of the disease, compensatory deformations in every patient are expressed to varying degrees due to limb shortening on the side of the affected joint [1]. All these changes together affect the vertebrarium-pelvis-lower limbs anatomic complex [2], [3], [4]. Especially in case of marked changes after joint endoprosthetics with the observation of all rules of components installation, patients are troubled by pain both in the early and the late post-surgery periods, which is not associated with the prosthesis [2], [5], [6], [7]. Many papers have been published that are focused on studying this problem, and various authors propose methods of components installation concerning the characteristics of each patient [8], [9], [10], [11], [12]. It should be noted that there are various methods of visualisation and assessing the adjacent hip joint structures [13], [14] (Figure 1).

An example is X-ray imaging of the entire lower extremity and the pelvis. However, this examination is often performed in the lying position on the back with the use of several films (Figure 2) [15], and, in case of analog X-ray photography, without the possibility of assessing the vertebrarium-pelvis-lower limbs anatomical complex with the baseload [16], [17], [18] (Figure 3).

The aim of this research is to develop a device and a method that would contribute to solving the existing challenges of pre-surgery planning of hip endoprosthetics in patients with related pathologies, which have caused compensatory deformation, and making long vertebrarium-pelvis-lower limbs scout

images with the patient lying on his back with an axial load in a computer tomography.

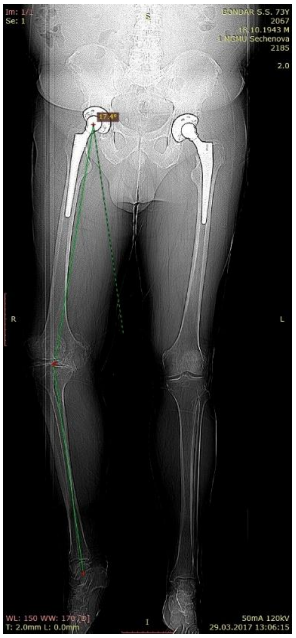

Figure 1. Long digital X-ray image

Material and Methods

To test the method, the authors conducted 247 studies along with the routine planning procedures to assess the efficiency of the method. The study was performed both in the standard conditions and with the use of the device developed at the Department of Traumatology, Orthopedics and Disaster Surgery of the Sechenov University (Figure 4).

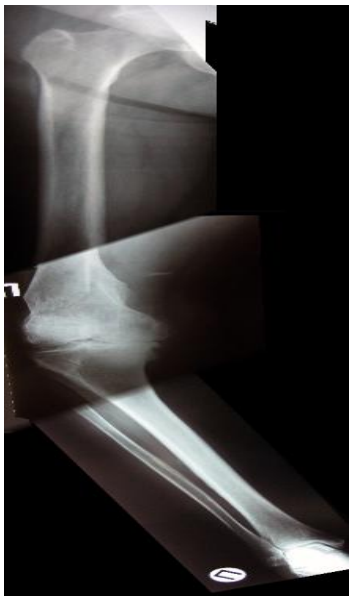

Figure 2: A patient with posttraumatic arthrosis of the knee joint. Planning by the method of bonding films

The device is a vest that is put on the patient. The vest is connected to a rigid platform under the patient's feet with rods. The rods are fitted with force meters connected to the monitor that displays the pressure on the platform.

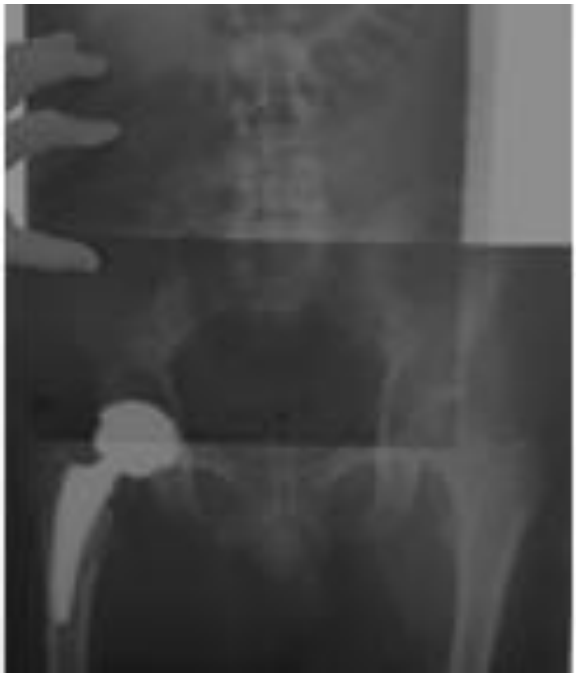

Figure 3: The use of several films for a rough estimation of the vertebrarium-pelvis-lower limbs anatomical complex

Thus, the device allows creating an axial load on the vertebrarium, hip joints, knee joints, ankle joints and feet with the patient lying on his back. The monitor continuously records the force applied to force meters and displays the axial load in kilograms.

Figure 4: The use of the device developed at the Department of Traumatology, Orthopedics and Disaster Surgery

A CT scanner Toshiba Aquilion ONE 640 was used for the study. The analysis was performed by standard X-ray imaging of the pelvis and knee joints followed by CT scanning of the same patients with the use of the method developed at the Department of Traumatology, Orthopedics and Disaster Surgery of the Sechenov University.

The results of the research with the use of the new method were assessed with the help of building axes and angles before (Figure 5) and after surgery (Figure 6).

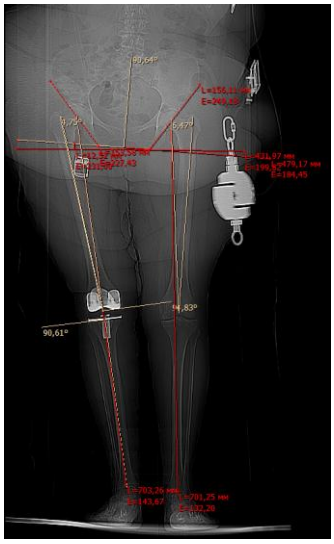

Figure 5: Planning endoprosthetics of the knee joint

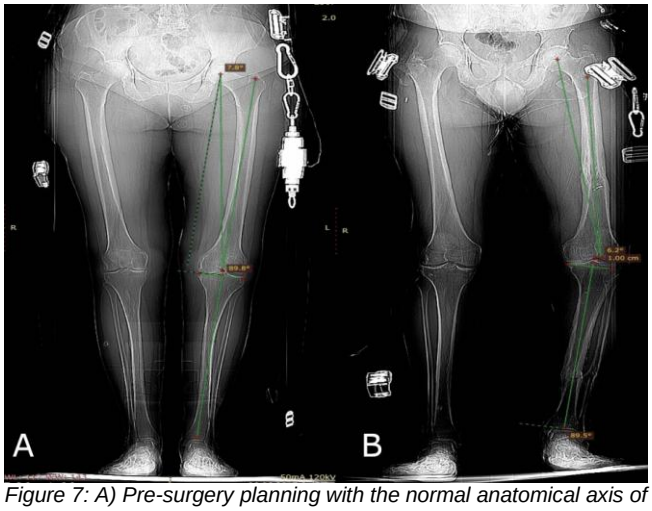

Figure 7: A) Pre-surgery planning with the normal anatomical axis of the lower limb; B) Pre-surgery planning with varus deformity of the lower limb

The data were statistically processed with the use of Statistica 8.0 software suite. The quantitative variables were described using standard methods of variation statistics, for which the arithmetic mean was (M), and the standard deviation was (δ). The mean values were presented as M + δ. Qualitative variables were characterised as absolute and relative frequency ratios. The differences were considered veracious with $p < 0.05$. For assessing the results, the following methods of statistical analysis were used: Student's t-test; nonparametric tests for variable samples that were incompatible with the law of normal distribution (Mann–Whitney U test, Wilcoxon test).

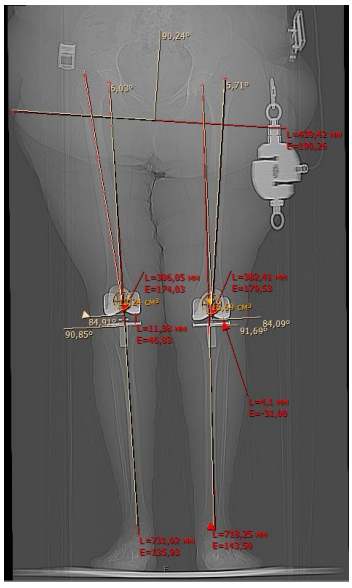

Figure 6: After knee joint endoprosthetics

All studies were approved by the appropriate Ethics Committee and were therefore performed by the ethical standards laid down in the 1964 Helsinki Declaration. All persons gave their informed consent before being involved in the research.

To determine the tactics of knee joint treatment, the authors assessed the anatomical axis of the limb, the mechanical axis of the limb, the Q-angle, and evaluated positioning of the components in the post-surgery period. During pre-surgery planning, the method made it possible to identify predominant lesion of the medial sections of the knee joint under axial load, which changed the tactics of the planned surgery – making unicondylar endoprosthetics instead of total knee arthroplasty. In planning the hip joint endoprosthetics with concomitant pathology of the vertebrarium, the authors managed to estimate the degree of pelvic bones displacement, the degree of scoliotic vertebral deformity, lower limb shortening (Figure 7A and 7B). This allowed more precise pre-surgery planning and monitoring the positioning of endoprosthesis components after surgery.

In the framework of pre-surgery planning before total hip arthroplasty, the authors evaluated the anatomical and mechanical axes of the limbs, and the degree of pelvic bones displacement during a CT examination with the help of devices that simulated the axial load, and without it. An important role for proper pre-surgery planning and for determining further tactics of treatment, in the opinion of the authors, was also played by studying the lumbar spine for identifying the vertebral column deformities, pathologies of the sacroiliac joint, and the degenerative-dystrophic changes in the spinal motion segment.

To monitor the efficiency of the method, CT scanning was performed by standard methods with pre-surgery planning, after which an examination with the use of the new method was performed with subsequent planning for further analysis and identifying the differences in the tactics of the surgical treatment.

The study was performed with 247 patients, of which 87 (35.3%) had hip joint endoprosthetics, and 160 (64.7%) had knee joint endoprosthetics. From the

second group, 37 (23.1% of patients with knee endoprosthetics) had total endoprostheses installed in both knee joints.

Out of 247 patients, in 54 (21.9%) post-traumatic arthrosis was diagnosed, 163 patients (66%) had degenerative arthrosis, and the remaining 30 patients (12.1%) had dysplastic arthrosis.

As a result, information was obtained about changes in the plan of surgical intervention with fairly pronounced differences during the pre-surgery planning in the position of the components of the endoprosthesis after using the standard and the modified methods of examination, respectively. In planning hip joint endoprosthetics, surgeons most often changed the angles of acetabular components; the second frequent pattern was the difference in selecting the length of the prosthesis head. In planning knee joint endoprosthetics, surgeons most often changed the positioning of the tibial component and calculated the entry point for the intramedullary guide (Figure 8A and 8B).

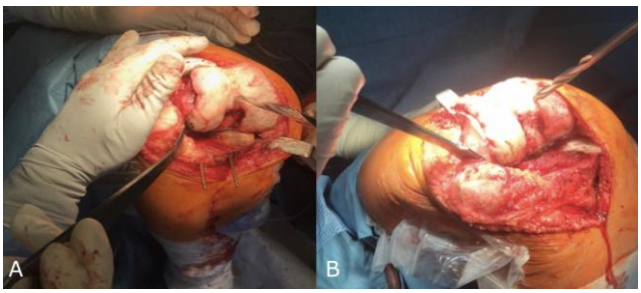

Figure 8: A) Determination of the entry point for the femoral intramedullary guide with the normal axis; B) Determination of the entry point of the femoral intramedullary guide with varus deformity of the lower limb

Results

For comparison of long-term results of prosthetics with the use of the new method, the authors chose for reference 247 case reports of patients with similar models of pathology, which had undergone endoprosthetics with the use of the standard planning methods. The data of assessment scales, the amount of movement and survival of the prosthesis were obtained (Table 1; Figure 9). The WOMAC (Western Ontario McMaster Universities OA Index) estimation scale, which allows assessing the intensity of the pain syndrome in the osteoarthritis of the hip or knee joints in course of five activities in the standing position – walking, climbing stairs, at rest, and at night [15], [19], [20], was mainly used. The clinical characteristic of the patients was the following: most patients were female: 156 patients (63.2%), the average age was 63.16 ± 12.41. The duration of the disease ranged from 1 to 12 years; the average duration of aggravation was 6.0 ± 1.5 weeks. In most patients, radiographic osteoarthritis stage III was found (by Kellgren – Lawrence) – in 180 patients (72.9%), stage II – in 25 patients (10.1%), and stage IV – in the remaining 42 patients (17 %). In most patients (92%), a limited range of movement was noted, mostly in the degree of affected knee joints bending.

Table 1: The data of assessment scales, the amount of movement and survival of the prosthesis

	WOMAC		The number of revision interventions	
	Standard method	Modified method	Standard method	Modified method
Total hip arthroplasty (THA)	32.81 ± 9.63	31.32 ± 8.13	5	1
Total knee arthroplasty (TKA)	84.14 ± 8.74	37.26 ± 7.92	7	2

Pain syndrome intensity during movement ranged from 0 to 93 (82.43 ± 4.76) by the visual analogue scale. The functional state of the joints by the WOMAC index before surgery was 68.26 ± 7.51 mm. After a surgical intervention with the use of the standard method for the hip joint, the WOMAC index was 32.81 ± 9.63, and after total hip arthroplasty according to the modified method with pre-surgery planning with the use of the developed device, the index reached 31.32 ± 8.13. For the knee joint, the differences between the two groups were much more significant. WOMAC for the standard methodology was 84.14 ± 8.74. The results after total knee arthroplasty according to the method modified at the Department showed a reduction of the WOMAC index slightly more than twice down to 37.26 ± 7.92. The number of revision surgeries after endoprosthetics decreased from 5 (5.7%) to 1 (1.1%) for the hip joint, and from 7 (4.3%) to 2 (1.3%) for the knee joint, respectively.

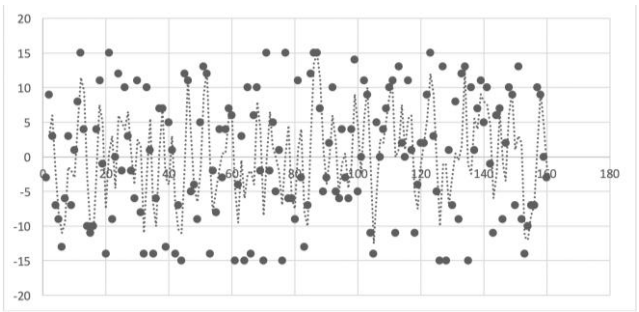

Figure 9: Adjustment of the acetabular component inclination in surgical treatment planning (before and after making scout images with axial load)

Discussion

In many countries, the importance of pre-surgery planning is acknowledged, and they try improving it to achieve the most optimal result. In Japan, during pre-surgery planning of hip joint endoprosthetics, they are guided by the pelvis

inclination angle [21], [22]. German surgeons during pre-surgery planning use a 3D modelling application, magnetic resonance imaging, and fluoroscopy without axial load [23], [24]. In the United States, the importance of using digital templates in surgical practice is noted [25], [26]. In the studies in German authors, digital calibrated X-ray photographs are made, while in North America, they use individual tools prepared before the surgery [5], [27]. The work of specialists from Delft, the Netherlands is quite interesting; they note the correlation between the patient's shoe size and the size of implants used for primary knee joint endoprosthetics [28], [29].

The obtained results of our study show attractiveness of the pre-surgery planning method developed at the Department of Traumatology, Orthopedics and Disaster Surgery of the First MSMU n.a. I. M. Sechenov (Sechenov University). Modern literature describes many various techniques of improving pre-surgery planning of endoprosthetics of large joints of the lower extremities [11], [12].

The authors' method involves the use of a standard horizontal tomographic scanner, which makes it unique in terms of the scope of application, unlike the methods that involve the presence of a vertical tomographic scanner. The examination in the tomographic scanner takes a short time, usually less than one minute; there is a possibility of making additional slices to facilitate surgical planning. In medical literature, the use of the above device in combination with a horizontal computer tomograph, has not been described. In Russia, this method of pre-surgery planning for the patients with combined pathology of the hip and knee joints and lumbar spine has been granted a patent for invention No. 2651056 dated April 18, 2018 [30]. As a result, this method has a distinct advantage in minimising the duration of examination and radiation exposure. With that, the informative value of the data obtained exceeds the standard methods of patient examination.

In conclusion, the use of the new method of pre-surgery planning for knee and hip joint endoprosthetics with concomitant spinal pathology allows to reduce the duration and improve the quality of pre-surgery planning to avoid repeated CT studies with low informative value of scout images, to reduce the time of surgery on the average by 17 minutes, and to improve treatment results and survival rate of the endoprostheses. For the examination, one can use a tomographic scanner with any number of spirals from various manufacturers. There is no need to use special planning software. The use of this method will significantly improve the quality of arthroplasty and reduce the time and costs for preparing a surgery.

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Clinical Science

A Randomized Control Trial Comparing Transparent Film Dressings and Conventional Occlusive Dressings for Elective Surgical Procedures

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Abstract

BACKGROUND: Surgical site infection is one of the major health-care-associated problems causing substantial morbidity and mortality and constituting a financial burden on hospitals as well. The wound management is one of the crucial evidence-based strategies in the reduction of surgical site infection rates

AIM: To study the impact of standardisation of transparent semipermeable dressing procedure on the rate of surgical site infection in comparison with conventional dressing in clean and clean-contaminated surgeries.

METHODS: The study included 100 patients who were admitted to surgical wards in Cairo university hospitals, for clean and clean-contaminated operations, in the period from February 2017 to August 2017. Immunocompromised and uncontrolled diabetic patients were excluded. Patients were randomly allocated into two groups; in the first group, patients wounds were covered using transparent semipermeable dressing, while the second group patients' wounds were covered using conventional occlusive gauze dressing. Patients were followed up for criteria of infection every other day during the first week then at two weeks, three weeks and four weeks.

RESULTS: In clean and clean-contaminated operations, the transparent dressing group showed a significantly lesser rate of surgical site infection at (2%), compared with the conventional occlusive gauze dressing group with a surgical site infection rate of (14%) (p-value of 0.02).

CONCLUSION: The transparent semipermeable dressing is effective in reducing surgical site infection rate in clean and clean-contaminated operations.

Introduction

Surgical site infections (SSIs) are those occurring in a surgically created wound within 30 days. SSIs are the commonest hospital-acquired infections**.** They represent a significant burden on the health care system worldwide with significant patient comorbidity and mortality. The human and financial costs of treating surgical site infections (SSIs) are rising. It is estimated that approximately half of SSIs are deemed preventable using evidence-based strategies [1], [2].

The Centers for Disease Control and Prevention (CDC) classified SSI to superficial; within the skin and subcutaneous fat, deep; musculo-facial layers or organ space; in an organ or cavity, if breached during surgery [3].

Different classifications of risk factors have been proposed to be associated with SSIs. They can be classified to Preoperative, intraoperative and postoperative risk factors. SSI risk factors can also be divided into modifiable, e.g. cigarette smoking and non-modifiable as extreme of age and severity of illness [4].

In developing countries, the distribution of Hospital-acquired infections is different from more developed, with fewer bloodstream infections since fewer devices are used and a higher proportion of SSIs; which can be redeemed preventable through

evidence-based measures [5].

SSI (defined using Centers for Disease Control and Prevention's National Healthcare Safety Network criteria) is defined as infection that occurs within 30 days after any operative procedure (where day 1 = the procedure date) and patient has at least one of the following: purulent drainage, organisms identified from an aseptically-obtained specimen from the incision by a culture or non-culture based microbiologic testing method [1]. The incision that is deliberately opened by a surgeon, and the patient has at least one of the following signs or symptoms: pain or tenderness; localised swelling; erythema; or heat and diagnosis of incisional SSI by the surgeon or attending physician [1].

In 11 Egyptian hospitals, 510 SSIs were identified following 4,246 surgeries with overall SSI rate of 12% [6]. The incidence of SSI at Cairo university hospitals was 9.2%. A significant increase was associated with a prolonged preoperative hospital stay, prolonged surgery, contaminated wounds and presence of the drain. The most common organism was Staphylococcus aureus (24.3%) then Klebsiella pneumonia (18.5%) [7].

Good wound care will minimise the inflammatory response, speed healing and minimize scarring. A dressing is a sterile pad or compresses applied to a wound to promote healing and/or prevent further harm. Most of the procedures result in wounds in which the edges are brought together to heal using stitches, staples, clips or glue to allow healing by primary intention. Afterwards, wounds are often covered with a dressing that acts as a barrier between them and the outside environment. One advantage of this may be to protect the wound from micro-organisms, and thus infection. Many different dressing types are available for use on surgical wounds [8].

The clean wound is an uninfected operative wound in which no inflammation is encountered, and the respiratory, alimentary, genital, or uninfected urinary tracts are not entered, and clean wounds are primarily closed. While Clean-Contaminated wounds are operative wounds in which the respiratory, alimentary, genital, or urinary tracts are entered under controlled conditions and without unusual contamination [1].

Low adherence dressings and wound contact materials are usually cotton pads that are placed directly in contact with the wound. They are either non-medicated (e.g. paraffin gauze dressing), or medicated (e.g. containing povidone-iodine or chlorhexidine) [9].

Transparent film dressings are semi-permeable membrane dressings; that is waterproof yet permeable to oxygen and water vapour which help in preventing bacterial contamination. They may be used as a primary or secondary dressing. They also maintain a humid wound environment, facilitate cell migration and encourage necrotic tissue autolysis by trapping moisture on the surface of the wound [10], [11].

The main aim is to study the impact of standardisation of transparent semipermeable dressing procedure on the rate of surgical site infection in comparison with a conventional occlusive gauze dressing.

Methodology

The study included 100 patients who were admitted to surgical wards in Cairo university hospitals, during the period between February 2017 to August 2017.

This study was revised and approved by the research ethics committee, Faculty of Medicine, Cairo University. The study was designed as a randomised controlled trial in which patients were allocated to two different groups, according to the chronological order of their presentation, to A and B; group A patients received the transparent dressings for their surgical wounds and group B patients had the conventional occlusive gauze dressings.

Patients were then assessed for eligibility according to the inclusion and exclusion criteria listed below. Patients were informed of the nature of the study, consented to participate.

Inclusion criteria

- Patients who presented to the plastic and general surgery department in the study period.

- Patients with clean surgeries.

- Patients with clean-contaminated surgeries.

Exclusion criteria

- Extremes of age; children under the age of 10 and adults beyond the age of 60.

- Patients with uncontrolled diabetes.

- Immuno-compromised patients.

- Any patient with a history of impaired healing.

- Patients on medication that may impede wound healing or render them susceptible to infection (eg. Steroids)

- Patient with contaminated or infected surgery.

- Patients presenting in the trauma department.

- Drop-outs from follow up.

Group A: Included fifty patients' undergone different clean and clean-contaminated surgeries, and received postoperative semipermeable transparent wound dressing since day one and throughout their postoperative course.

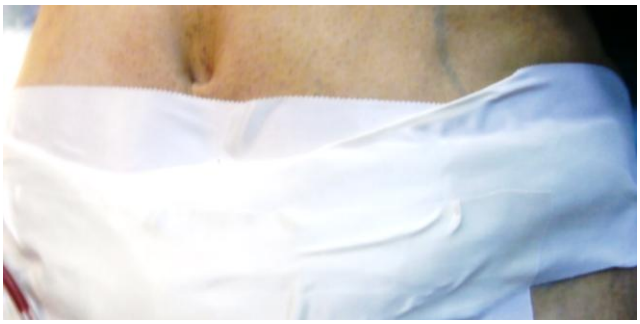

Figure 1: Gauze dressing covering abdominal incision postoperatively

Group B: Included fifty patients' undergone different clean and clean-contaminated surgeries, and received conventional occlusive gauze dressings since day one and throughout their postoperative course.

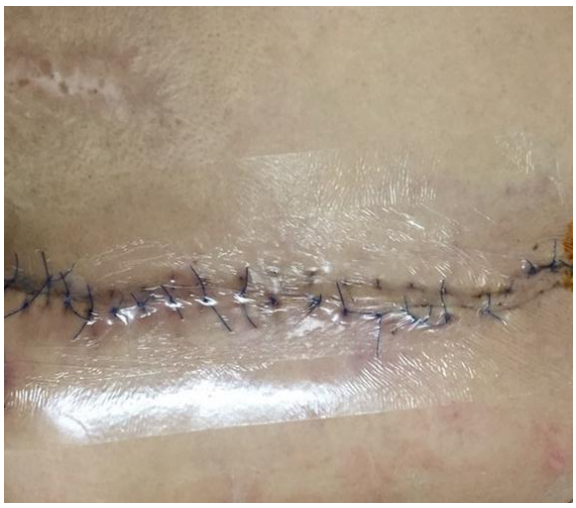

Figure 2: Transparent dressing covering incision postoperatively

The following data were collected from patients upon enrollment in the study: - Full medical history analysis including age, sex, cigarette smoking and medical comorbidities, regular medications that may impede healing and drug allergies; - Operative details including the use of any foreign material and previous surgical history; - Full general examination including body weight, vital signs, and skin conditions preceding the surgical operation; - Preoperative investigations including complete blood picture, fasting blood glucose and HbA1c; and - Preoperative prophylaxis was done according to hospital policy; ampicillin-sulbactam was given within minutes to one hour before incision. One dose was sufficient, yet additional doses were given for operating procedures longer than three hours.

Follow up and criteria of infection

Wounds were evaluated postoperatively every other day in the first week then weekly till the end of the month.

Group A: transparent wound dressings were applied intraoperatively. Afterwards, they were evaluated for adherence, underlying exudate, and transparency and stigmata of infection, and were only changed when a leak was detected or lost adherence. Under aseptic conditions, films were removed, and the wounds were cleaned with normal saline, and povidone-iodine was used as a disinfectant and allowed to dry, and a new film was reapplied.

Group B: basic gauze dressing was used intraoperatively, with every other day, dressing changes starting from day 2 postoperatively till the third week. The dressings were removed, the wounds were inspected and cleansed with saline, povidone-iodine was applied, and the wound was covered with gauze followed by adhesive plaster.

The equation to calculate rates of SSIs:

$$\frac{\text{No. of SSIs in a specific group X 100}}{\text{No. of operations in the same group}}$$

Microbiological analysis for patients with suspected wound infection: Using a sterile technique, a sterile cotton-wool swab was used to collect a sample from the infected site.

Sample processing: All samples were cultured on blood MacConkey agar incubated aerobically at 37°C for 24-48 hrs. Direct Gram-stained films were prepared from each wound swab and examined microscopically. Identification of isolated microorganisms according to standards using: Gram stain, colony morphology, Biochemical reactions for gram-positive isolates (catalase, coagulase, mannitol, DNase). Also, novobiocin disc was used for further identification of Staphylococci. Biochemical reactions for gram-negative isolates (TSI, LIA, MIO, citrate, urease, and oxidase). Antimicrobial susceptibility was done by disk diffusion method.

Statistical Method

Data were analysed using SPSS win statistical package version 20 (SPSS Inc., Chicago, IL). Numerical data were expressed as mean and standard deviation or median and range as appropriate. Qualitative data were expressed as frequency and percentage. Chi-square test (Fisher's exact test) was used to examine the relation between

qualitative variables. For quantitative data, a comparison between the two groups was made using either student t-test or Mann-Whitney test (non-parametric t-test) as appropriate. A p-value < 0.05 was considered significant.

Results

The age of the patients included in group A ranged from 15 – 55 years, with a mean of 34 ± 10.02. The age of the patients included in group B ranged from 18 – 57 years with a mean of 34.46 ± 9.157. Group A included 40 females (80%) and 10 males (20%), while group B included 41 females (82%) and 9 males (18%) as shown in Table 1.

Table 1: Patients' baseline demographics and wound characteristics

	Group A Transparent dressing	Group B Conventional dressing
Age:		
Y Mean (SD*)	34 (10.022)	34.46 (9.157)
Sex in (percent %):		
Male	20%	18%
Female	80%	82%
Risk factors (per cent %):		
Obesity	42%	34%
Smokers	4%	6%
Type of wounds:		
Clean	44	44
Clean-contaminated	6	6

* Denotes standard deviation.

Our findings confirm that the rate of SSI is lower when transparent dressings were used on surgical incisions when compared to the conventional occlusive gauze dressing, as shown in Table 2 and 3, (p-value 0.02).

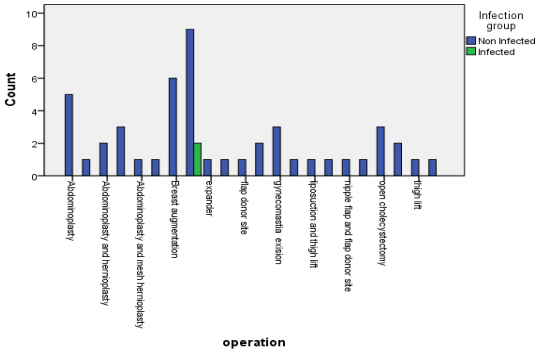

Figure 3: Types of operations in a transparent dressing group

To study obesity as a risk factor of SSI, we found that of the 100 patients, 38 % were obese, and 62% were non-obese. Of the 38 patients suffering from Obesity, 6 had SSI which is 15.7%, while those are not suffering from Obesity only 2 out of 62 patients suffered from SSI with 3.2% only. (p-value < 0.02) which is statistically significant.

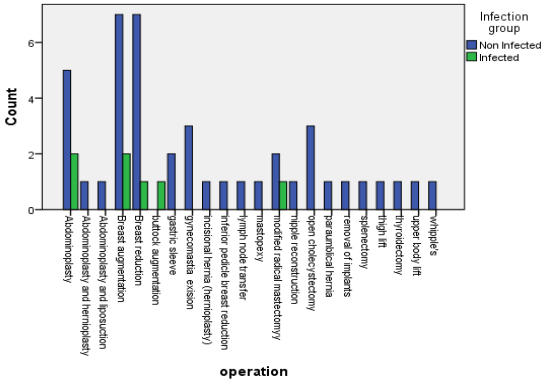

Figure 4: Types of operations in a conventional dressing group

Age of the patients included in our study ranged from 15 – 57 years, with a mean of 34 ± 9.579. Mean age in the infected group was 37.2 ± 9.7 in comparison to 33.9 ± 9.5 in the non-infected group.

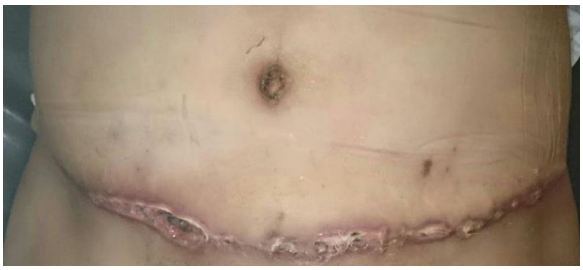

Figure 5: showing infected wound postoperatively after abdominoplasty operation

The incidence of smoking as a risk factor among both groups was 5%. Such an incidence was not high enough to reliably assess the impact of smoking as a risk factor of SSI.

Table 2: Comparison between Transparent dressing and Conventional dressing

		Dressing type				
		Transparent dressing		Conventional dressing		P value
		Number	%	Number	%	
Age (Mean ± SD*)		34 ± 10		34.5 ± 9.2		0.81
Sex	Male	10	52.6%	9	47.4%	0.79
	Total	50	50.0%	50	50.0%	
Obesity	Yes	21	55.2%	17	44.7 %	0.5
	Total	50	50.0%	50	50.0%	
Smoking	Yes	2	40.0%	3	60.0%	0.65
	Total	50	50.0%	50	50.0%	
Type of wound	Clean	44	50.0%	44	50.0%	1
	clean contaminated	6	50.0%	6	50.0%	
	Total	50	50.0%	50	50.0%	
Infection group	Infected	1	12.5%	7	87.5%	0.02
	Total	50	50.0%	50	50.0%	

* Denotes standard deviation.

Follow up

The transparent dressing stayed in place for a mean of 6 days (range 5 – 7), and the gauze dressing

was removed after a mean of 1.5 days (range 1 – 3). The transparent dressing, therefore, stayed in place, a mean of four and a half days longer than the gauze dressing.

We gained the impression that the patient's comfort and well-being were better in the transparent group as it made bathing possible and allowed earlier postoperative mobilisation.

Table 3: Comparison between infected and non-infected groups

		Non-Infected		Infected		P-value
		Number	%	number	%	
Age (Mean ± SD*)		33.9 ± 9.5		37.2 ± 9.5		0.33
Sex	Male	19	20.6%	0	0.0%	0.15
	Total	92	92.0%	8	8.0%	
Obesity	Yes	32	34.7%	6	15.7%	0.02
	Total	92	92%	8	8.0%	
Smoking	Yes	5	100.0%	0	0.0%	0.49
	Total	92	92.0%	8	8.0%	
Total leucocytic count	High	7	87.5%	1	12.5%	0.62
	Total	92	92.0%	8	8.0%	
Type of wound	Clean	80	90.9%	8	9.0%	
	clean contaminated	12	100.0%	0	0.0%	
	Total	92	92.0%	8	8.0%	0.27
Dressing type	Transparent dressing	49	98.0%	1	2.0%	
	Conventional dressing	43	86.0%	7	14.0%	0.02
	Total	92	92.0%	8	8.0%	

* Denotes standard deviation.

The patients with transparent dressing felt more satisfied as the dressing was conformable, and the frequency of dressing change was less than the gauze dressing.

Inspection of the wound through the transparent dressing was transparent in 24 patients (48%), slightly opaque in 11 patients (22%) but the sutures could be seen, and 15 (30%) were opaque that the sutures couldn't be seen and the dressing was replaced to visualise the sutures.

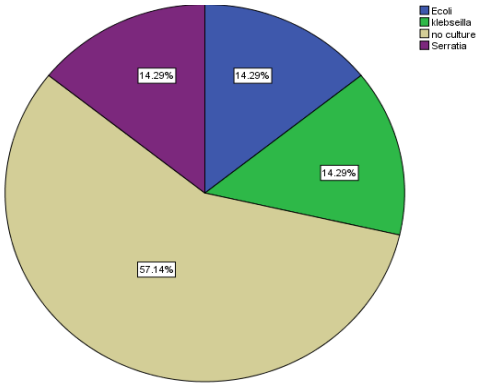

Figure 6: Frequency of various pathogens causing surgical site infections in the conventional group

The TLC was elevated among 7 patients 14% in the conventional dressing group and 1 patient 2% in the transparent dressing group (p-value < 0.03)

Table 4: Frequency of pathogens causing surgical site infections in patients with occlusive transparent dressing group

	Frequency	Per cent	Per cent
negative culture	1	2.0	100.0
Total	1	2.0	100.0

Discussion

In this study, our goal was to evaluate the impact of a transparent semipermeable film dressing versus the conventional occlusive gauze dressing on rates of SSIs in the period of February 2017 to August 2017.

The age of the patients included in the study ranged from 15 – 57 years, with a mean of 34 ± 9.579. The mean age in the infected group was 37.2 ± 9.7 compared to non-infected group 33.9 ± 9.5 in the non-infected group (p-value 0.33). However, the mean age was higher in the study conducted by C. Holm et al., 1998 which was 61.3, (range 25 – 90) years, and also in the study conducted by Shinohara et al., 2008 the mean age was 63.5 years (range 31 – 91 years) [12], [13]. This may be due to the conduction of our study in a developing country where the mean age of the general population is lower than that where C. Holm and Shinohara conducted their study. Also, this could be attributed to the fact that we excluded patients above the age of 60 years old.

Our study included 81 females and 19 males, which differs from the studies conducted by C. Holm et al., 1998 and by Shinohara et al., 2008 which included equal numbers of males and females. While the study conducted by Ubbink et al., 2008 included 92 males and 50 females in the transparent dressing group and 93 males and 50 females in the conventional dressing group [12], [13], [14]. That difference, in our opinion, had no effect on the results of our study. This larger number of female patients involved in this study might be since females are more prone to go for plastic procedures. Also, on a national level, the female's super number the males which make our finding sensible.

In our study, the type of wound dressing used had an influence on the rate of SSI; this influence was found to be statistically significant. One patient (2%) of a total of 50 patients who had used transparent semipermeable wound dressing suffered from SSI, compared to seven patients (14%) of a total of 50 patients suffered from SSI in the other group that used conventional occlusive gauze dressing. This shows the superiority of transparent semipermeable wound dressing usage following elective clean and clean-contaminated surgical procedures in reducing the rate of SSI (p-value 0.02).

Collated data from 50 controlled trials on a variety of wounds yielded infection rates of 5.37% and 3.25% rates (p < 0.001) between conventional gauze and transparent dressings, respectively [15].

In a study conducted by Maki and Ringer 1987, Cutaneous colonisation using transparent dressing was lower in level and comparable with a gauze dressing and other dressings (range, 100.58 to 100.70 colony-forming units) [16].

There is also evidence that moist wound healing results in better cosmesis, decreased pain, and improvement in the granulation tissue of the wound bed [17].

This differs from a study conducted by Sastry et al., 2015. They implemented the use of sterile gauze or a transparent semipermeable dressing to cover the wound of cardiac implantable electronic devices, with no inclination to the use of any of the fore-mentioned types [18].

This agrees with another study conducted by Cosker 2005 showing that there was no statistically significant difference in the number of SSIs in the basic wound contact-dressed group (5 / 100; 5%), compared with the transparent film-dressing group (9 / 200; 5%) [19].

The overall SSI incidence rate in the current study was 8 %. Nearly similar findings were concluded from a study conducted at Tanta University Hospital in Egypt by Afifi and Baghagho, 2010 who detected an overall SSI incidence rate of 8.3% [20]. Meanwhile lower SSI rates could be detected in France, Italy and Germany, SSI rates of 3.3%, 3.3% and 1.2% were detected in three studies conducted by Rioux et al., 2006, Moro et al., 2005 and Hirschmann et al., 2005 [21], [22], [23]. This could be attributed to the conduction of our study in a developing country which usually shows higher rates of SSIs than more developed countries.

In the current study, obesity was statistically significantly associated with an increased risk of SSI (P-value = 0.02). This was like a study of Egyptian orthopaedic patients by Abdel-Halim et al., 2010 who reported in their study that obesity was a significant risk factor for SSI (P < 0.001) [24]. To study obesity as a risk factor of SSI, we found that of the 100 patients, 38% were obese, and 62% were non-obese. Incidence of infection in the obese group was 15.7% and in the non-obese group was 3.2% (p-value < 0.02) which is statistically significant. Our study detected that the incidence of obesity among the transparent dressing group included 21 patients, 42%, while among the conventional dressing group included 17 patients, 34%.

Our study as well as Shinohara et al., 2008 gained the expression that Patients seemed more comfortable with the transparent dressings, which allowed them to move about freely and to take a shower when necessary and early postoperative mobilization was also facilitated, and studies suggested that film dressings might be less painful for patients than basic wound contact dressings [13].

AS for the SSI microbiology in our study, the organisms isolated from the infected wounds from the transparent dressing group: negative culture 2%. Organisms isolated from the infected wounds gauze dressing group: negative culture 8%, E-coli 2%, Klebsiella 2%, Serratia 2%. This differs from results of a study conducted in Japan by Shinohara published in 2008 showing the isolation of Bacteroides fragilis (3/63) 4.8% in transparent dressing group and isolation of Bacteroides fragilis and Enterococcus faecalis (4/71) 5.6% in gauze dressing group (p = 0.567) [13]. Staph aureus was the main causative organism of SSI (44.4%), all S. aureus isolates were MRSA, followed by Klebsiella pneumoniae 22/90 (24.44%) and Acinetobacter 15/90 (16.67%). The implant was highly associated with SSI cases 80 / 90 (89%) according to Helal et al., 2015 [25].

One of the limitations encountered during our study was negative cultures despite the presence of SSI that was diagnosed by the surgeons and according to the CDC criteria for SSI. The appearance of postoperative SSI in the absence of culturable bacterial pathogens is a common dilemma for surgeons. The potential causes of culture-negative SSI include prior antimicrobial therapy, the presence of fastidious or slow-growing microorganisms or infection caused by ordinary bacteria that may be dismissed as “contaminants” and performing aerobic cultures only [26]. Other limitations in our study included a lower number of clean-contaminated surgeries in our study in comparison to clean surgeries.

In conclusion, our study showed that the transparent semipermeable dressing is effective in reducing surgical site infection rate in clean and clean-contaminated operations as well as, reducing its burdens as additional hospital stay and additional costs associated with the occurrence of infection.

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Clinical Science

Comparison between Results of Microdiscectomy and Open Discectomy in Management of High-Level Lumbar Disc Prolapse

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Abstract

AIM: This work aims to compare between results of microdiscectomy and open discectomy in management of high-level lumbar disc prolapse.

METHODS: This is a controlled randomised study, where patients having upper lumbar disc herniations were evaluated preoperatively both clinically and radiologically, randomisation was planned to perform open discectomy in odd number patients and to perform microdiscectomy in even number patients, patients were evaluated and followed up for deficits and outcomes.

RESULTS: We operated ten patients in this study, five cases were operated upon with microdiscectomy, and five cases were operated upon with open discectomy, the median age of presentation in this study was 44 years, there were five males and five females, postoperative pain improvement was better in microdiscectomy. Hospital stay, blood loss, bone loss and postoperative complications were less in microdiscectomy.

CONCLUSION: Microdiscectomy allows good surgical visualisation and is less traumatic to the involved tissues. The results of this study indicated that microsurgery reduces hospitalisation time, improves the overall surgery-related outcome. The main differences between the two procedures were the length of the incision and blood loss. We found that lumbar microdiscectomy allows patients earlier return to work and normal life with less reliance on postoperative narcotic analgesic agents.

Introduction

This is a prospective study of 10 cases of high-level lumbar disc prolapse which were surgically managed in the period between May 2014 and April 2015 in the neurosurgery department at Cairo university hospitals.

The rationale of this work was to compare the results of microdiscectomy and open discectomy in management of high-level lumbar disc prolapse.

This is a controlled randomised study where randomisation was planned to perform open discectomy in odd number patients while microdiscectomy was performed in even number patients.

Inclusion criteria: 1) Single level high lumbar disc prolapse (L1-2 or L2-3) and 2) Patients indicated for surgery with intractable low back pain associated with radiculopathy.

Exclusion criteria: 1) Multiple levels high disc prolapses; 2) Recurrent cases (previous disc surgery); 3) Presence of another pathology; 4) Morbid obese patients, and 5) Patients with osteoporosis.

The following methods were applied for the studied cases:

History Taking

Personal history including name, age and sex, symptomatology including back pain, lower limb pain and claudication pain.

Pain analysed according to site, character, severity and distribution.

Patients were assessed for presence or absence of motor deficit, sensory deficit and cauda equina.

Examination

The patients were examined for 1. Vital signs (pulse, arterial blood pressure, temperature and respiratory rate); 2. Assessment of the pain. (Site, character, referral, severity, exaggerating and relieving factors); 3. Assessment of the presence of motor weakness, sphincteric manifestations and other neurological examination; and 4. Back examination and deformity and associated medical conditions.

Table 1: Visual analogue pain scale (Flynn D et al, 2004)

0	2	4	6	8	10
No hurt	Hurts a little bit	Hurts a little more	Hurts even more	Hurts whole lot	Hurts as much as you can imagine

Investigations

Routine laboratory investigations: During preoperative preparation of the patients, all cases were subjected to complete blood picture, blood glucose, liver and kidney functions, bleeding profiles and serum electrolytes, ESR, CRP.

Table 2: Master Table 1

Case	Age	Sex	Site of disc prolapse	Neurological deficit	Back pain	Femoralgia
1	56	M	L2-3	Motor &sensory deficit	+	+
2	34	M	L2-3	Sensory hypoesthesia	+	+
3	46	F	L1-2	Sensory deficit	-	+
4	44	M	L1-2	-	+	+
5	44	F	L1-2	Sensory hypoesthesia	+	+
6	24	F	L2-3	-	-	+
7	38	M	L1-2	Sensory hypoesthesia and urinary affection	+	+
8	37	M	L2-3	-	+	+
9	42	F	L2-3	Motor and sensory deficit	+	+
10	33	F	L2-3	-	-	+

Radiological investigations

1) Plain X-ray lumbosacral spine: - Anteroposterior view; - Lateral view; - Both oblique views to detect fracture pars; and - Dynamic flexion and extension views for determination of stability.

2) Magnetic resonance imaging lumbosacral spine. It was performed in all cases to define: - Cause and degree of neurological compression; - Bone marrow changes: Presence of Modic Type 1 changes suggests instability (hypointense on Tl-weighted imaging and hyperintense on T2-weighted imaging and were shown to represent bone marrow oedema and inflammation); - Any abnormality of the pars interarticularis, pedicles, or facet joints; and - Nerve structures, including those exiting neural foramina, and the spinal canal should be evaluated for stenosis.

Operative management: five cases were operated upon with microdiscectomy and five cases were operated upon with open discectomy.

Postoperative Management: - Postoperative antibiotics (cephalosporins, penicillin) were continued for two days postoperatively; - Narcotic analgesics were used in the first twenty-four hours; - Oral diet was started in the second day; and - Patients were ambulant in the first postoperative day.

Follow-Up

A. Clinical follow-up: immediately after surgery and on an outpatient basis.

B. Radiological follow-up: immediate postoperative and after six months.

Included: Plain radiography anteroposterior & lateral radiograph

Table 3: Master Table 2

Case no	Total laminectomy	Hemi laminectomy	Total facetectomy	Medial facetectomy	Blood loss during surgery	Time of surgery	Hospital stay
1	+	-	+	-	450 cc	110 minutes	2 days
2	-	+	-	+	250cc	133 minutes	2 days
3	+	-	+	-	500 cc	115 minutes	3 days
4	-	+	-	+	200cc	122 minutes	2 days
5	+	-	+	-	700 cc	120 minutes	2 days
6	-	+	-	-	150 cc	127 minutes	One day
7	+	-	+	-	500 cc	115 minutes	4 days
8	-	+	-	+	200 cc	130 minutes	2 days
9	+	-	+	-	400 cc	130 minutes	2 days
10	-	+	-	-	150 cc	125 minutes	One day

Clinical Evaluation:

- Patients are evaluated according to the presence or absence of neurological deficit, and sphincteric affection and - Patients are evaluated according to pain improvement using a visual analogue scale for pain immediately after surgery and after six months.

Table 4: Master Table 3

No.	The clinical indication of surgery		Dural tear	Clinical outcome	
	Intractable pain (LBP and LL pain)	Motor deficit		Post-operative pain (VAS)	Neurological deficit due to surgery
1	+	-	-	2	-
2	+	-	-	2	-
3	+	+	-	4	-
4	+	-	-	2	-
5	+	-	+	4	-
6	+	-	-	2	-
7	+	-	+	2	-
8	+	-	-	2	-
9	+	+	-	4	-
10	+	-	-	2	

Results

The data collected from 10 cases of high-level lumbar disc prolapse were analysed prospectively. In our study, 50% of the cases were females, while 50% of the cases were males.

The mean age for patients that had upper lumbar disc surgery was (44.92) years old, the mean height for them was (162.6) cm, the mean weight was (82.8) kg, the mean BMI was 31.42.

Table 5: The average age, weight, height and duration of symptoms

	Minimum	Maximum	Mean
Age in years	24	56	44.92
Height in cm	150.00	175.00	162.60
Weight in Kg	65.00	95.00	82.80
BMI	27.06	42.22	31.42
Duration in Months	0.20	96.00	13.03

Five cases of high-level lumbar disc prolapse were operated upon with open discectomy; the other five cases were operated upon with microdiscectomy.

Table 6: Radiology of the herniated disc

	Calcified disc	Non calcified disc	Central	Para central	Diffuse	Focal
L1-2	3	1	2	2	1	3
L2-3	3	3	2	4	2	4

Incidence of L1-2 disc prolapse was 40% while the incidence of L2-3 was 60%.

Incidence of left-sided disc prolapse was 50%, right-sided disc prolapse was 10%, central disc prolapse was 40%.

Femoralgia was the most common indication for surgery 100% followed with low back pain 70%, sensory deficit 60%, motor deficit 20% and urinary affection 10%.

Table 7: Surgical procedure

	L1-2	L2-3
Open discectomy	3	2
Microdiscectomy	1	4

Total laminectomy was done in 50% of cases & hemilaminectomy was done in 50% of cases & unilateral total facetectomy was done in 50% of cases and medial facetectomy was done in 40% of cases.

Table 8: Differences between microdiscectomy and open discectomy

	Microdiscectomy	Open discectomy
Average blood loss	190 cc	510 cc
Average hospital stays	1,8 day	2,9 day
Average time of surgery	127 minutes	105 minutes
Average pain improvement (VAS)	2	2,3
Postoperative complications	0	Two cases of dural tear

Case No 1: Open Discectomy

History: Male patient 56 years old.

Complaint: severe right femoralgia for 4 months according to the pain scale (8).

Examination: weakness GIV in right knee extension and hypoesthesia in right L2, 3, 4 roots.

Diagnosis: L2-3 disc prolapse.

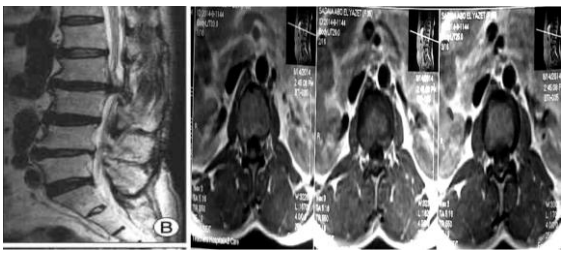

Figure 1: Preoperative MRI lumbar spine T2 axial and sagittal showing L2-3 disc prolapse

Operation: total laminectomy, facetectomy and L2-3 discectomy.

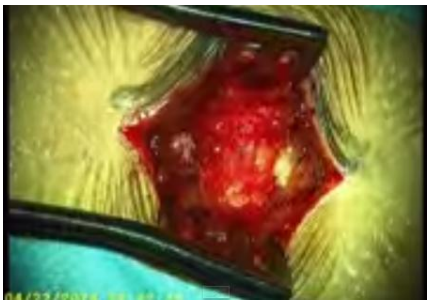

Figure 2: Intraoperative image showing skin incision during open discectomy

Postoperative: pain improved according to analogue pain scale [4].

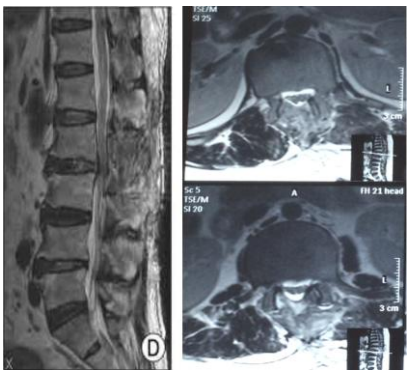

Figure 3: Postoperative MRI T2 axial and sagittal lumbar spine showing L2 total laminectomy and L2-3 discectomy

Case No. 2: Microdiscectomy

History: male patient 34 years old.

Complaint: Low back pain and severe pain in the lateral aspect of thigh for one month according to analogue pain scale hurts a whole lot [8].

Diagnosis: L2-3 disc prolapse.

Examination: FMP, hypoesthesia in the lateral aspect of the thigh and positive femoral stretch test.

Operation: hemilaminectomy, medial facetectomy and L2-3 microdiscectomy.

Postoperative: pain improved according to the scale hurts a little bit [2].

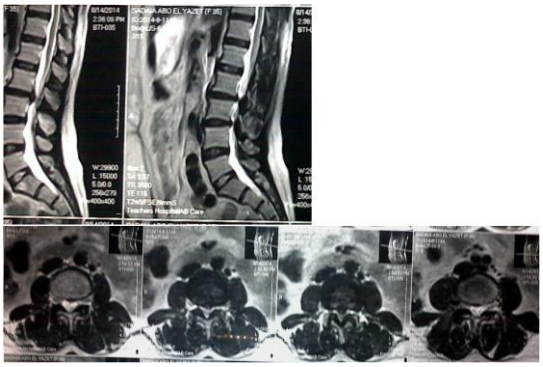

Figure 4: Preoperative MRI lumbar spine T2 axial and sagittal showing L2-3 disc prolapse

Discussion

Unique characteristics of upper lumbar disc herniation include ill-defined polyradiculopathies that cannot be clearly categorised into typical muscle group weakness or reflex deficits [1]. These polyradiculopathies may be associated with a narrower upper lumbar spinal canal compared with the lower spinal canal, resulting in compromise of multiple roots by a single disc herniation. Clinical symptoms are quite variable, localised sensory change or pain was rarely demonstrated [2].

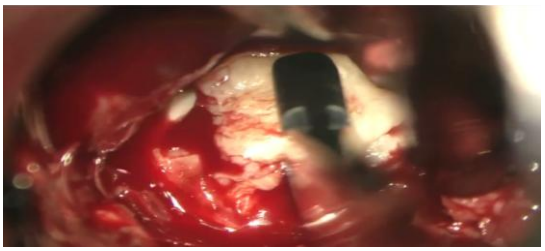

Figure 5: Intraoperative image showing disc excision during microdiscectomy

The positive femoral stretch test is known as a relatively good diagnostic method in 84 to 94% of upper lumbar disc herniation, Pain provocation by the femoral stretch test is believed to be caused by stretching of the femoral nerve. Because the L2, L3, or L4 spinal nerve roots are the main components of the femoral nerve, cases with symptomatic upper lumbar disc herniation may have more opportunities to show positive results for the femoral stretch test compared to cases with lower lumbar disc herniations. Location of the conus medullaris in association with a high lumbar disc herniation may be a cause of urinary affection [3].

MRI helps reveal the location of the conus medullaris and lesions of the upper lumbar level more clearly. Radiographic techniques, including MRI and CT, are essential for the diagnosis of the lesion and identification of the precise location. Therefore, the preoperative careful investigation would be useful for differential diagnosis and prevention of misdiagnosis in cases of upper lumbar disc herniation [3].

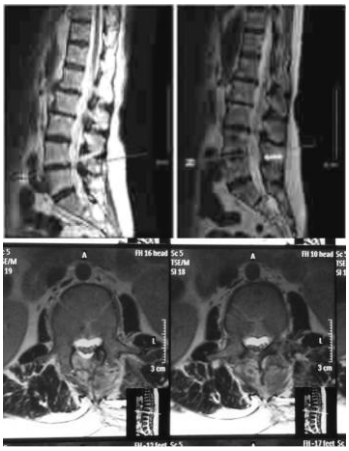

Figure 6: Postoperative MRI T2 axial and sagittal lumbar spine showing L2 hemilaminectomy and L2-3 discectomy

Compared with those of lower levels, upper lumbar disc herniations have a less favourable outcome after surgery. Spinal canals are narrower than those of lower levels, which may compromise multiple spinal nerve roots or conus medullaris. Lengths of the lamina are shorter, the location of the pain varies, and direct cord compression may occur. Because of this unique anatomy, selection of a surgical approach is difficult [4].

The choice of the surgical approach is an important issue when treating patients with disc herniation in the upper lumbar spine [5].

Factors considered important for the determination of the surgical approach include disc size, location, the extent of calcification, surgeon's experience, degree of spinal cord deformation and the general medical condition of the patient. Radiologic findings for L1-L2 and L2-L3 disc herniations are one of the important criteria for the selection of the surgical approach [4].

In the literature, we could not find reports on operation rates in large series of cases being treated by microdiscectomy or open discectomy in management of high-level lumbar disc prolapse, *Sanderson et al.'s* study [2] reported a total of 21 surgeries and *Saberi et al.'s* study [7] reported a total of 28 surgery cases. Otherwise, most papers only reported a small number of cases.

To supplement knowledge in this field, we describe here the results of 10 patients with high-level lumbar disc prolapse where managed in the neurosurgery department, Cairo University Hospitals.

The mean age of this study was 44 years which is close to the study of *Sanderson et al.'s* study [2] , who reported a mean age of 46 years , lower than *Krauss et al.'s* study [6], who reported mean age of 54 years and *Choi et al.'s* study [5] who reported mean

age of 52 years.

In our study, in regard to sex distribution, there are 5 females and 5 males. In *Krauss et al.'s* study [6], there were 11 males and 7 females, in *Saberi et al.'s* study, there were 17 females, 11 males and in *Sanderson SP et al.'s* study [2], there were 8 females and 13 males.

As regards to the clinical picture, in our study we noted that femoralgia was reported in all cases, low back pain in 70%, sensory affection in 60% and motor deficit in 20% compared to the findings of *Choon et al.,* study [8] and *Summers et al.,* study [9] who reported predominance of femoralgia in 87%, back pain in 54%, neurological deficit in 40% in their cases. *Tokuhashi et al.* the study [10] reported femoralgia in 85% of cases.

Sensory manifestations were the most common neurological deficit encountered in our study, while motor deficits were reported in two cases. The indication of surgery was intractable pain in the back and legs, this coincides with *Saberi H et al.,* study [7], who reported that the most common indication for surgery was intractable pain and *AhnY et al.,* study [11], who reported that the most common indication for surgery was intractable pain.

Of 10 cases of the high-level lumbar disc, L2-3 disc prolapse was present in six cases, and L1-2 was present in four cases. *Saberi H et al., the* study [7], reported that L2-3 disc prolapse was present in 75% of cases and *AhnY et al., the* study [11] reported that L2-3 disc prolapse was present in 45% of cases.

The femoral stretch test was present in 70% of our cases. *Krauss WE et al.,* the study [6], reported that femoral stretch test was present in 65% of cases and *Choi et al.,* the study [5], reported that femoral stretch test was present in 70% of cases.

Five of our cases were operated with microdiscectomy and five cases with open discectomy, in *Saberi H et al.,* the study [7] eleven cases operated with microdiscectomy and In *Choi et al., the* study [5] seven cases operated with microdiscectomy.

As regard to perioperative factors, in our study, total laminectomy was done in 100% of cases of open discectomy. Hemilaminectomy was done in 100% of cases of microdiscectomy. Total facetectomy was done in 100% of cases of open discectomy, and medial facetectomy was done in 80% of cases of microdiscectomy.

In *AhnY et al.,* study [11] total laminectomy was done in 65% of cases, hemilaminectomy was done in 35% of cases and total facetectomy was done in 40% of cases, In *Sanderson SP et al.,* study [2] total laminectomy was done in 70% of cases of open discectomy versus 60% in the study of *Choi JW et al.,* study [5].

Shin DA et al., the study [12] reported that microdiscectomy procedure is less invasive than open discectomy, causes less muscle damage and less back pain

Our average blood loss in microdiscectomy was 190 cc versus 510 ccs in open discectomy, in *Kambin P* study [1], average blood loss in microdiscectomy was 230 cc versus 470 ccs in open discectomy, in *Kanayama et al.,* [14] study, average blood loss in microdiscectomy was 150 cc versus 320 cc in open discectomy.

Hospital stay was for our patients was 1,8 days in microdiscectomy versus 2,9 days in open discectomy, in *Ryang A et al.,* study [16] and *Kambin P* study [1], average hospital stay in microdiscectomy was less than average hospital stay in open discectomy, no significant difference was found in *German et al.,* [13] *and Porchet et al.,* [15] studies.

Average time of surgery was 127,4 minutes in microdiscectomy versus 105,2 minutes in open discectomy; this means no statistically significant difference between the two procedures, this is similar to *German et al.,* [13] *and Porchet et al.,* [15] studies.

As regard to perioperative factors, our study was different from the following studies, *Schneider C et al.,* the study [17] who reported that although minimally invasive micro discectomies are appealing to many patients; its superiority over standard open microdiscectomy has not been demonstrated.

Wu et al., the study [18] concluded in their retrospective study that minimally invasive microdiscectomy affords optimal post-operative outcomes and is superior when compared to open microdiscectomy; this is similar to our study.

Harrington and French study [19] founded that preoperative parameters were similar. In their study, the minimally invasive group had less narcotic usage and shorter length of stay, but they did not conclude that one technique was better than the other.

German et al., [13] *and Porchet et al.,* [15] studies show that there is no significant difference between minimally invasive and open micro discectomies.

In *German et al.,* [13] *and Porchet et al.,* [15] studies Forty-nine patients underwent minimally invasive discectomy, and 123 patients underwent open microsurgical discectomy. At baseline, the groups did differ significantly concerning age but did not differ concerning height, weight, sex, body mass index, level of radiculopathy, side of radiculopathy, insurance status or type of preoperative analgesic use.

No, statistically significant differences were identified in operative time, rate of cerebrospinal fluid leak, or need for a physical therapy consultation. Statistically, significant differences were identified in length of stay, estimated blood loss, post-anaesthesia care unit narcotic use, and need for admission to the

hospital [13], [14].

Kanayama et al., the study [14] reported that no significant differences between the 2 surgical procedures in the frequency of use of an analgesic agent after surgery, the pre- and postoperative Japanese Orthopaedic Association scores or postoperative Visual Analogue Scale for sciatica. Statistically significant differences were observed in the operation time, amount of bleeding, duration of hospitalisation, and postoperative VAS for lumbar pain.

Righesso O et al., the study [20]*,* reported statistically significant differences found for size of the incision, length of hospital stays, and operative time between microdiscectomy and open discectomy.

As regard to postoperative pain improvement, in our study, average pain improvement in microdiscectomy was 2 versus 3, 2 in open discectomy according to the analogue pain scale.

Arts MP et al., [the] study [21] reported that both Open discectomy and microdiscectomy lead to a substantial and equivalent long-term improvement in leg pain. Adequate decompression, regardless of the operative approach used, maybe the primary determinant of pain relief — the major complaint of many patients with radiculopathy. Incidental durotomies occurred significantly more frequently during MID, but total complications did not differ between the techniques.

Pain improvement in microdiscectomy was better than open discectomy according to the analogue pain scale. This was similar to results in Cole *4th* [22]*, Ryang et al.,* [16]*, Kambin P* [1] *and Shin DA et al.,* [12] *studies.*

Cole 4th study [22] reported that Lumbar minimally invasive discectomy is our preferred surgical technique for symptomatic disc herniations in this patient population. Decreased incision length and a trend toward reduced infectious complications are the primary reasons. We feel that, given the comorbidities often found in this patient population, a minimally invasive technique will supplant open approaches shortly.

Kambin P study [1] found that advantages of microdiscectomy include: 1) two-hour operative time; 2) negligible blood loss; 3) avoidance of significant scarring in the spinal canal; and 4) anterolateral fenestration of the annulus for continuing relief of intradiscal pressure and nerve root decompression.

Shin et al., the study [12] reported that microdiscectomy procedure is less invasive than open discectomy, and causes less muscle damage and less back pain.

Schizas et al., the study [23] reported that microdiscectomy is at least as effective as open discectomy for the treatment of uncontained or large contained disc herniations, although the advantages over the open technique are short-lived and did not reach significance. Nonetheless, microdiscectomy seems to be a safe procedure.

In our study, the only complications were two cases of a dural tear in two cases of open discectomy; those two complications were managed with the closure of the tear intraoperative, tight closure of the fascia and placement of a drain in the two cases there was no leak postoperatively. This was different from *Ryang et al., the study* [16].

Ryang et al., the study [16] reported that 107 patients (67 males, 40 females) underwent microdiscectomy for the prolapsed lumbar intervertebral disc. Follow up ranged from 2 to 40 months with a mean follow up 12.9 months. Seventy-six patients had an excellent outcome, 22 patients had a good outcome, 5 patients had a fair outcome, and 3 patients had a poor outcome. One patient with a long dural tear required conversion to a standard microdiscectomy and was excluded from outcome assessment. Complications included dural puncture with K-wire (1), dural tear (2), superficial wound infection (3), discitis (4) and recurrent disc prolapse (5).

Righesso et al., the study [20] reported that in microdiscectomy complications were less than those in open discectomy as regard to the occurrence of wound infection and postoperative back pain.

From the above studies our results were close to these in *Ryang A et al.,* study [16], *Shin DA et al.,* study [12], *Kambin P* study [1]*, Righesso O et al.,* study [20]*, Cole 4th* study [22] and *Wu X et al.,* study [18] as regard to hospital stay, postoperative pain, postoperative recovery ,blood loss and time of surgery.

In conclusion, clinical features of upper lumbar disc herniations were different from those of lower lesions. Due to unexpectedly large differences in neurologic findings and clinical manifestations among the herniated disc levels, an accurate workup is needed to avoid misdiagnosis. In our series, a discectomy was successfully performed by hemi or total laminectomy. In upper lumbar disc herniation, favourable clinical outcomes can be expected by adequate selection of surgical methods in consideration of each herniated disc nature such as consistency, direction, and distribution.

We found a significant difference between minimally invasive microdiscectomy and open discectomy for lumbar disc herniation in perioperative factors and outcomes with regards to blood loss, neurological function, complication rate and length of stay in hospital or pain improvement.

Microdiscectomy allows good surgical visualisation and is less traumatic to the involved tissues. Interestingly, the results of this study indicated that microsurgery reduces hospitalisation time, improves the overall surgery-related outcome.

The main differences between the two procedures were the length of the incision and blood loss. We found that lumbar microdiscectomy allows patients earlier return to work and normal life with less reliance on postoperative narcotic analgesic agents.

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Clinical Science

Oxidative Stress and Anti-Oxidant Markers in Premature Infants with Respiratory Distress Syndrome

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Abstract

BACKGROUND: Neonatal respiratory distress syndrome (RDS) caused by decreased surfactant and structural lung immaturity. The imbalance between oxidative status and antioxidant defence system was suggested to be an important trigger for lung affection with RDS.

AIM: The goal of the current research was to elucidate the significance of the oxidant/ antioxidant status in the pathogenesis of RDS in preterm infants.

PATIENTS AND METHODS: This controlled study included 31 preterm neonates with RDS and 36 healthy preterm neonates. Quantification level of oxidative stress biomarkers; malondialdehyde (MDA) & hydrogen peroxide (H_2O_2) along with antioxidant enzymes activity; catalase (CAT) & superoxide dismutase (SOD) in plasma of healthy premature neonates compared with those with RDS.

RESULTS: status of oxidative stress markers (MDA & H_2O_2) showed a significant increase with decreased levels of antioxidant enzymes activity (CAT & SOD) in neonates with RDS when compared to healthy prematures.

CONCLUSION: The results obtained in this study indicate that the increased oxidative stress accompanied by reduced antioxidant defences may play a significant role in the pathogenesis of respiratory distress in preterm newborns.

Introduction

Oxidative stress is recognised by the imbalance between the augmented reactive oxygen/nitrogen species and the defect in the protective ability of the antioxidants. Free radicals with consequent cellular oxidative damage produced by oxidative stress seem to be key players in the pathogenesis of several new-born diseases, like respiratory distress syndrome, bronchopulmonary dysplasia, patent ductus arteriosus, necrotising enterocolitis, and retinopathy of prematurity, periventricular leukomalacia [1].

Negi et al., [2] reasoned the onset of neonatal respiratory distress syndrome (RDS), previously called hyaline membrane disease (HMD) to the decreased surfactant and structural lungs immaturity. Lung tissue damage which occurs during respiratory distress syndrome is not clearly explained yet, but the implication of oxidative damage due to reactive oxygen and hydrogen species is highly appreciated in the etiopathogenesis of this disorder. Preterm neonates are more prone to oxidative deterioration because of the intracellular antioxidant defence system including the anti-oxidant enzymes up-regulates dramatically during the last trimester of pregnancy, and they have low levels of radical scavengers and metal-binding proteins such as transferrin and ceruloplasmin [3]. They also have reduced antioxidant enzymes activity like catalase and glutathione peroxidase. The imbalance between oxidants and antioxidants results in oxidative damage and this problem occurs due to incomplete or abnormal intrauterine development [4].

Immediately after birth, the sudden increase in oxygen supply leads to overproduction of reactive oxygen species (ROS) and down-regulation of antioxidants. This condition induces the enhancement of cytokines and inflammatory mediators (interleukin 6- interleukin 8- Tumor Necrosis factor -α) expression [5].

Preterm infants, in particular, are exposed to many events leading to increased generation of reactive oxygen species (ROS) such as hyperoxia, mechanical ventilation, inflammation and infection [6]. Surfactant is the primary treatment of RDS in neonates as it reduces hyperoxia-induced lung damage. Surfactant replacement has been found to cause antioxidant and anti-inflammatory responses [7].

Superoxide dismutase (SOD) is the most powerful antioxidant and detoxification enzyme. It catalyses the disputation of two molecules of superoxide anion radical ($O_2^{\cdot-}$) to hydrogen peroxide (H_2O_2) and oxygen molecule (O_2) [8]. Several investigators stated that SOD is the crucial enzyme for a proper respiratory function in animal and cellular models and the deficiency of SOD activity almost induces severe hyperoxic lung injury [9]. Endotracheal administration of surfactant increases the activity of SOD in type II alveolar cells, demonstrating enzyme uptake by liposome during the surfactant recycle process [10].

Catalase (CAT) is the common antioxidant enzyme that utilises oxygen. It catalyses the degradation of hydrogen peroxide (H_2O_2). Both superoxide dismutase (SOD) and catalase (CAT) are on the top of the first line of the defence system. This line is essential in the entire antioxidant defence mechanism [8]. Superoxide dismutase (SOD) and catalase (CAT) activities have been demonstrated in several natural surfactants. They also have scavenger activity against hydrogen peroxide (H_2O_2) [11]. Malondialdehyde (MDA) is the major reactive aldehyde that results from peroxidation of lipids in the biological membranes. It can be used as an indicator of tissue damage caused by reactive oxygen species (ROS). MDA reacts with DNA and modifies RNA, proteins and other biological molecules, and this leads to tissue destruction [12].

The goal of the current research was to elucidate the significance of the oxidant / antioxidant status in the pathogenesis of RDS in preterm infants. This could be achieved through estimation of the plasma levels of oxidative stress indicators (MDA, H_2O_2) and detection of plasma activity of the antioxidant indices (SOD, CAT).

Patients and Methods

A case-control study was conducted on a total of 67 neonates recruited from Neonatology Department of El-Galaa Teaching Hospital for Obstetrics and Gynecology, Cairo, Egypt. (Between April 2018 and October 2018). This study was approved by the Medical Ethical Committee of the National Research Centre, Egypt. Written informed consent for all participants was collected from their parents. The case (RDS) group consisted of 31 preterm babies ≤ 37 weeks with a diagnosis of RDS. The control group included 36 healthy preterm newborns. Exclusion criteria of the study were an infection, intracranial haemorrhage, surgical problems and hemolytic diseases.

Respiratory distress syndrome (RDS) was diagnosed with the presence of typical clinical and radiological signs of the disease in preterm infants. Clinically, if they have tachypnea, grunting and cyanosis with several hours of birth required mechanical ventilation and typical radiographic findings on the chest X-ray. The characteristics of the newborns including gestational age, weight, Apgar score, surfactant replacement, were obtained from Hospital sheets.

Two mL of heparinised venous blood samples were withdrawn from the neonates during the first 72 hours after birth. Blood specimens were processed to separate plasma samples which were stored at -20°C.

Determination of plasma levels of oxidative stress markers

I Malondialdehyde (MDA):

Principle: Thiobarbituric acid (TBA) reacts with malondialdehyde (MDA) in the acidic medium at a temperature of 95°C for 30 min to form the thiobarbituric acid reactive product. The absorbance of the resultant pink product can be measured at 534 nm Total thiobarbituric acid reactive materials are expressed as MDA [13].

II Hydrogen Peroxide (H_2O_2):

Principle: In the presence of peroxidase (HRP), H_2O_2 reacts with 3, 5-dichloro -2-hydroxybenzensulfonic (DHBS) acid and 4-aminophenazon (AAP) to form a chromophore, which is measured at 510 nm [14].

$$2H_2O_2 + DHBS + AAP \xrightarrow{HRP} \text{Quinoneimine Dye} + 4H2O$$

Determination of plasma antioxidant enzyme activity

I Catalase (CAT):

Principle: Catalase reacts with a known quantity of H_2O_2. The reaction is stopped after exactly one minute with catalase inhibitor.

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

In the presence of peroxidase (HRP), remaining H_2O_2 reacts with 3, 5-Dichloro -2-hydroxybenzene sulfonic acid (DHBS) and 4-aminophenazone (AAP) to form a chromophore with a colour intensity measured at 510 nm inversely proportional to the activity of catalase in the original samples.

$$2H_2O_2 + DHBS + AAP \xrightarrow{HRP} Quinoneimine\ Dye + 4H_2O$$

II Superoxide Dismutase (SOD)

Principle: This assay relies on the ability of the enzyme to inhibit the phenazine methosulfate – mediated reduction of nitroblue tetrazolium dye. The resultant colour was measured at 560 nm.

Statistical analysis

Data were collected, verified, coded and analysed using the Statistical Package for Social Science (SPSS) version 23 (SSPS Inc., Pennsylvania, and the USA). Descriptive analysis was performed for demographic and clinical characteristics of the cases. MDA, H_2O_2 levels, and CAT, SOD activities were expressed as mean ± SD. The comparison between the cases and control groups was made using student's t-test. A chi-square test was used for comparison of non-parametric data.

Results

The characteristics of all studied neonates and their mothers are illustrated in Table (1). The case (RDS) group composed of 31 preterm neonates with a mean gestational age 31.2 ± 3.2 weeks having a mean birth weight 1740.3 ± 720.0 gm. The control group composed of 36 preterm neonates with mean gestational age 34.3 ± 1.1 weeks–and a mean birth weight 2248.6 ± 147.1 gm. New-borns in RDS group are more premature and have lower birth weight in comparison to the control group (P < 0.005). There is no significant difference in the maternal age between neonates with respiratory distress syndrome and controls (P > 0.05). The mean birth weight and Apgar scores at 1 and 5 minutes and the gestational age are highly significantly lower in cases (RDS) than controls (p < 0.005).

Most preterm neonates with RDS delivered by C.S (80.6%), have no history of premature rupture of membranes (71.0%) and 58.1% of them have a history of multiple pregnancies.

Twenty preterms with RDS (64.5%) received surfactant therapy. Twenty preterms with RDS (64.5%) were on mechanical ventilation.

Table 1: Characteristics of the studied neonates and their mothers

	Case (RDS) group (n = 31)	Control group (n = 36)	t-Test	P
	Mean ± SD	Mean ± SD		
Maternal age (years)	28.97± 7.9	29.4 ± 3.4	*t* = 0.329	0.743
Gestational Age (weeks)	31.2 ± 3.2	34.3 ± 1.1	*t* = 5.348	0.000
Birth weight (gm)	1740.3 ± 720.0	2248.6 ± 147.1	*t* = 4.141	0.000
Apgar score 1st min	3.9 ± 2.05	7.72 ± 0.97	*t* = 10.059	0.000
Apgar score 5 min	6.5 ± 1.9	8.7 ± 097	*t* = 6.248	0.000
	No (%)	No (%)		
Gender:				
Male	24 (77.4%)	14 (38.9%)	χ^2 = 10.073	0.002
Female	7 (22.6%)	22 (61.1%)		
Delivery mode:				
Vaginal	6 (19.4%)	17 (47.2%)	χ^2 = 5.738	0.017
CS	25 (80.6%)	19 (52.8%)		
Multiple pregnancies:				
Yes	13 (41.9%)	6 (16.7%)	χ^2 = 5.235	0.022
No	18 (58.1%)	30 (83.3%)		
Premature rupture of membranes:	9 (29.0%)	2 (5.6%)	χ^2 = 6.690	0.010
Yes	22 (71.0%)	34 (94.4%)		
No				
Surfactant:				
Yes	20 (64.5%)	-	-	-
No	11 (35.5%)			

χ^2 = chi-square; t = t-test.

The data in Table 2 represented the plasma levels of oxidative stress markers and the activity of plasma antioxidant enzymes in neonates with RDS and control counterparts. There is a highly significant elevation in the levels of the oxidative stress markers (MDA, H_2O_2) in the RDS group versus the control one (p < 0.005). The highly significant drop in the activity of the antioxidant enzymes (CAT, SOD) is noted in the RDS group relative to the control group (p < 0.005).

Table 2: The oxidant and antioxidants markers in prematures with RDS and control group

	Case (RDS) group Mean ± SD (n = 31)	Control group Mean ± SD (n = 36)	t	p
Oxidative stress markers:				
MDA mmol/L	7.074 ± 1.88722	2.367± 1.3459	11.753	0.000
H_2O_2 mμ/L	0.78100 ± 0.2498	0.25900 ± 0.109	8.274	0.000
Antioxidants markers:				
SOD U/ml	186.596 ± 47.936	267.244 ± 33.476	-6.218	0.000
Catalase U/L	352.939 ± 68.421	571.217± 117.812	-7.962	0.000

The correlation between clinical parameters of RDS cases is depicted in Table 3. The gestational age shows a highly significant positive correlation with birth weight, maternal age, Apgar score at 1 and 5 minutes (P < 0.005). On the contrary, it shows a highly significant negative correlation with surfactant therapy (P < 0.005). Surfactant therapy shows a highly significant positive correlation with gestational age and birth weight (P < 0.005).

Table 3: Correlation between clinical parameters of RDS cases (31 newborns)

		Gestational Age	surfactant therapy	Birth weight	Maternal age	1st min Apgar score	5min Apgar score
Gestational Age	Pearson Correlation	1	-0.783**	0.910**	0.656**	0.640**	0.638**
	Sig. (2-tailed)		.000	0.000	0.000	0.000	0.000
surfactant therapy	Pearson Correlation	-0.783**	1	-0.638**	-0.367*	-0.450*	-0.390*
	Sig. (2-tailed)	0.000		0.000	0.042	0.011	0.030
1st min Apgar score	Pearson Correlation	0.640**	-0.450*	0.763**	0.685**	1	0.928**
	Sig. (2-tailed)	0.000	0.011	0.000	0.000		0.000
5min Apgar score	Pearson Correlation	0.638**	0.390*	0.711**	0.588**	.928**	1
	Sig. (2-tailed)	0.000	0.030	.000	.001	.000	

**P < 0.005 highly significant; *P < 0.05 significant.

Meanwhile, it shows a significant negative correlation with maternal age and Apgar score at 1 & 5 minutes. The highly significant positive correlation is observed between the Apgar score (at 1 & 5 minutes) and birth weight as well as maternal age ($P < 0.005$).

Discussion

Neonatal respiratory distress syndrome is a major health problem in neonates. It occurs due to immature lungs and requires assisted ventilation with high oxygen concentration. The pathophysiology of RDS is based on the rapid formation of reactive oxygen species (ROS) that inhibit the detoxification capacity of the antioxidative mechanisms [2].

In preterm labour, oxidative stress and other oxidative molecules exceed the antioxidant buffering capacity leading to lung tissue damage [16]. In our study, birth weight and gestational age were significantly lower in RDS cases than controls. The more prematurity, was the less lung surfactant production and more severe RDS. Extremely preterm and very low birth weight infants are considered at particular risk of oxidative stress because of both endogenous and passively acquired exogenous defence systems do not mature enough until late in the third trimester [17].

In the present study, most of the case group (RDS group) were born by caesarean section (CS). The effect of the type of delivery on the oxidative stress experienced by both mother and infant is still not clear. Actually, there is a controversial hypothesis about the mode of delivery and the generation of oxidative stress. Yaacobi et al., [18], stated that there is an increased concentration of malondialdehyde in vaginal delivery and emergency CS after prolonged labour group as compared to elective CS without labour. In contrast, Mutlu et al., [19] demonstrated a conflicting result as their study reported that CS increases total oxidative stress, and oxidative stress indices, and lipid hydroperoxide level.

Gerten et al., [20] concluded that caesarean section is an independent risk factor for RDS development, and the risk is decreased with labour before section. This supports the importance of being certain of neonatal lung maturity before taking the decision of cesarean delivery mainly when done before labor. In our study, as most of the RDS group was born by CS, we were not able to identify whether the increased levels of the oxidative stress were due to the mode of delivery or not. However, Lauire et al., [21] found that distressed neonates born by caesarean section had high MDA concentration, a marker of lipid peroxidation in amniotic fluid and cord blood compared to non-distressed neonates delivered by vaginal route. Nevertheless, we need to investigate the exposure of the mother and neonates to the oxidative stress about the mode of delivery.

In our study, male neonates were more significantly affected by RDS than females. This is in concordance with the study done by Kaltofen et al., [22] who demonstrated that female fetal alveolar cells of the saccular stage of lung development have a higher alveolar Na transport activity compared to age-matched male cells. Besides, male androgens decrease surfactant production and delay lung maturity.

Most neonates in our study had no history of premature rupture of membranes (PROM) and this could be explained by the fact that more than 80% of our neonates were delivered by C.S. Some reactive oxygen species (ROS) during pregnancy may cause direct vasoconstriction or inability to vasodilate placental blood vessels leading to preterm deliveries [23].

Multiple pregnancies in our study were significantly lower than singletons. After considering gestation, twins are not at elevated risk of having RDS except at very early gestation [24].

Most preterm with RDS in our study received surfactant therapy. A study was done by Sardesai et al., [25] demonstrated that surfactant therapy decreases air leaks and neonatal mortality significantly. It also contributed to faster weaning from invasive ventilation. Noninvasive surfactant administration techniques (atomization or aerosolisation) may play an important role in the future. Carty et al., [26] mentioned that reactive oxygen species (ROS) might interact with protein and lipid structures of the lung in addition to pulmonary surfactant, leading to the delay of the normal function of the lung. Therefore, surfactant administration is very important before the start of assisted ventilation [27].

Preterm in the present study showed significantly lower Apgar score at 1st and 5 minutes than controls. These results matched with a study done by Negi et al., [2], who stated that a low Apgar score is indicative of perinatal hypoxia.

At the present study, there was a significant positive correlation between Apgar score and both weight and maternal age. A study done by Jerneck and Herbst [28] registered higher Apgar score with birth weight above 5 kg.

MDA is a final product of lipid peroxidation. The present study showed an increased concentration of MDA and H_2O_2 (oxidative stress markers) in neonates with RDS concerning controls. These findings are in concordance with those of Negi et al., [2]. Furthermore, Zahran et al., [29] recorded an increased level of MDA in neonates with RDS, which is consistent with our results. Our results also fit the study of Dizdar et al., [30] which showed an increase in the total oxidant status in preterm infants. These

investigators mentioned that lower total antioxidant status / total oxidant status is associated with increased severity and mortality in these infants.

Nevertheless, in addition to the elevated levels of MDA and H_2O_2, shown in the present study, there was a significant drop in the activity of the antioxidant enzymes (SOD and CAT). This imbalance between elevated oxidative stress factors and the reduced antioxidant enzymes might be a contributing factor to the RDS in new-born. It is worth noting that the total antioxidant status might serve as a prognostic marker in new-borns with RDS and help to distinguish high-risk infants [31], [32]. This hypothesis is greatly supported by Zahran et al., [33] study, which demonstrated that the suppressive activity of SOD, as an antioxidant, may lead to neonatal RDS.

Several studies proved that SOD is a key enzyme for optimum respiration in animals and cellular models. The absence of SOD3 activity increases massive lung tissue damage, while SOD2 overexpression in type II alveolar cells may lead to prolonged survival in a hyperoxic environment [9].

Aerosol delivered SOD improved alveolar development in RDS patients and reduced the occurrence of bronchopulmonary dysplasia caused by prematurity and mechanical ventilation [33]. A previous study of Dani et al., [34] demonstrated that commercial natural lung surfactants contain a significant concentration of SOD and CAT. These surfactants enhance scavenger activity against H_2O_2 and are effective in reducing oxidative lung damage.

It has been shown that while preterm with RDS developing Bronchopulmonary dysplasia (BPD), they typically exhibit increased CAT enzyme activity in the epithelial lung fluid during the first week of life. Those preterms with simple RDS showed decreasing values of oxyradical inflammation markers during the disease course [35].

Antioxidants have a vital role in defence against free radical-induced lung tissue damage in neonates with RDS [2].

Bahbah et al., [36] found that there is no significant reduction in the activity of CAT, but SOD activity is significantly suppressed in the preterm group as compared to controls.

Trindade et al., [37] obtained similar results as ours; they recorded a decrease in the activity of the antioxidant enzymes and an increased in the susceptibility to oxidative stress among premature neonates in comparison to controls immediately after birth. This could be explained by the development of antioxidant enzyme capacity in the third trimester, so the preterm group is at increased risk of oxidative stress [38].

Pure oxygen use during resuscitation should be avoided. Restriction, the usage of mechanical ventilation by early usage of surfactant and nasal continuous positive air pressure, may decrease respiratory tissue damage in RDS patients [39].

In conclusion, the results obtained in this study indicate that the increased oxidative stress accompanied by reduced antioxidant defenses may play a significant role in the pathogenesis of respiratory distress in preterm new-borns.

Further researches are recommended to study the protective role of antioxidant markers against RDS. Antioxidant modalities may be beneficial in the treatment of RDS cases and prevention of bronchopulmonary dysplasia.

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Case Report

Unfit for Work, Fit for Firearm or driving license - Is that Possible?

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Keywords: Expert witness; Legislation; Military casualty; Psychiatric disorders; Work assessment

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Abstract

BACKGROUND: Psychiatric disorders are not compatible with carrying firearms or with driving a car. Persons with such disorders are often not employed and are persistent in demanding invalidity pensions, but some of them also insist on holding on to the mentioned licenses. In such cases, where persons are already in possession of firearms and driving licences, it never occurs to them, that they should surrender their permits back. AIM Pointing to the importance of OM controlling firearm/car driving licenses.

CASE REPORTS: This paper discusses the problem of three cases that should be widely recognised as it is potentially life-threatening to other people. The first is the case of a war veteran in retirement with PTSD that had his application for firearms licence rejected by the authorities. The second is the case of a labourer who suffers from a depressive disorder, temporarily incapable of work. The third is the case of a war veteran, a chronic alcoholic with toxic epilepsy, who is applying for invalidity retirement but wants to keep his driving license.

CONCLUSION: Occupational medicine assess every single worker by applying advanced methods and psycho tests that enable a thorough assessment of work capacity and fitness for carriage of firearms, driving as well as the assessment of psychiatric disorders, which are the most delicate to assess.

Introduction

Although it has been more than 20 years since the end of the war in Croatia, the consequences are still present. Among the military veterans, the most frequent diagnosis is PTSD, the diagnosis that causes interaction and work-related functioning problems [1]. It could take only seconds to suffer and acquire the PTSD but for the recuperation is needed a very long time [2]. Heavy traumas, long periods in battle zones and other traumatic experiences can lead towards alcoholism which causes changes in a patient that for a clinician are hard to assess [3]. Alcoholism is often the cause, as well as a tragic attempt to solving social problems [4].

Recession, bankruptcy and loss of employment, lead to people developing insecurity, apathy and worry. That leads to a decline in life and work skills, bad perception of oneself and consequently, it leads to depressive disorders [5].

This paper intends to show the effect of some psychiatric disorders on working capacity as well as on capability for carrying firearms / driving cars in patients with a reduced self-criticism due to their disorders. The paper presents three cases, diagnosed with PTSD, depressive disorder and chronic alcoholism (Table 1). Such disorders, if ignored by the community and especially by the medical professionals and competent jurists/judges in charge, can be fatal for the safety of citizens who live in their immediate surroundings.

Table 1: Croatian Institute for Pension Insurance Expert Witness, Independent Second Instance Expert Witness and Board of the Institute Expert Witness for Firearms/Driving licence opinion, regarding disability and licences for firearms and car driving

Cases	Age	Diagnosis	CIPI Expert Witness opinion on work capacity	Independent Second Instance Expert wit. opinion on work capacity	Board of the Institute Exp. witness opinion on firearm/car driving	Independent Second instance expert witness opinion on firearm/car driving
1	59	PTSD	Disabled	Disabled	Disabled	Disabled
2	60	Depressive disorder	Capable - needed for additional treatment	Disabled	Not evaluated	Disabled
3	52	Alcohol abuse	Capable - needed for additional treatment	Capable with contraindications and abuse control	Not evaluated	Temporary disabled 6-12 month up to recovery

CIPI, Croatian Institute for Pension Insurance.

Series of Cases

Case 1

The war veteran, 59 years old, pressed charges against the Board of the Institute for Assessing Firearms / Car driving ability for rejecting his application in 2018 for carrying firearms. He is in military invalidity retirement, due to diagnoses of PTSD and damages of the spinal vertebra. In 2013 his invalidity came up for revision, and according to the experts, his PTSD diminished below 20%. But this same year 2013 his application for firearms was rejected. In 2018 he obtained health certificates from two different private Occupational Medicine practices, where he was assessed as fit and on that basis applied for the firearms licence again. He also obtained a psychiatric report stating that his mental state is regular. However, in their report, the psychiatrist did mention that during the illness of the patient's father, the patient had had mental difficulties, went to psychotherapy and was on medication in the period from 2004 to 2007.

Nevertheless, the patient, after obtaining all the above documentation applied for the firearms licence again. He was one more time rejected, and he then sued the Board of the Institute for assessing Firearms / Car driving capability at the Administrative Court.

He referred to the Firearms Licensing Law, article 5, paragraph 10 that quotes contraindications for carrying firearms: "Neurotic disorders connected with stress and somatic disorders except for mild and feebly expressed disturbances that do not affect secure manipulation of firearms".

The patient at the time of his claim was free of psychiatric disturbances and not on any drug therapy, so he considered himself capable of handling firearms, as stated by the Law [6].

To assess the patient's capabilities to carry firearms, as the Independent Second Instance Expert Witness for this trial was chosen Occupational Medicine specialist.

Case 2

A manual labourer sued the Croatian Institute for Pension Insurance (CIPI) for rejecting his disability claim. The patient considers himself incapable of work because he is diagnosed with recurrent depressive disorders (F 33.3) and organic psycho syndrome (F 07.9). He suffers from hypotony and damages of the spinal vertebra. Additionally, the patient encloses psycho tests dated the years 2013 and 2017 that point to the diminished cognitive capabilities that are according to the psychologist's opinion caused by organic disease. Private OM practice assessed him as incapable of work and issued the patient the certificate of disability, which is contrary to the attitude of the CIPI experts who considered the patient fit to work.

The score of his test for visual motoric capabilities was under average. He encloses tree hospital letters from the psychiatric clinic. The patient is in combination therapy of antidepressants Velafax (*venlafaxine*), Calixta (*mirtazapine*), antipsychotic Nozinan (levomepromazine) and an antiepileptic drug Phenobarbital (*phenobarbital)*. In this case, the Administrative Court engaged the OM specialist, the Independent Second Instance Expert Witness to assess the patient's work capacity.

Case 3

A 52 years old volunteer war veteran, a construction worker with only six years of working experience, sued the CIPI unsatisfied with their refusal to grant him invalidity pension under the Article 39 of the Law for Pension Insurance [7].

As the main reason for his retirement, the patient states his epilepsy (G 40.5), organic psycho syndrome (F 0.7), hypertonic (I 10.0) and vertebrae deformations (M 40). His last major epileptic attack was in 2012, and he is under the therapy of Lamal (*lamotrigine*), twice daily one tablet. In his claim, the patient encloses the letter from the clinic for psychiatry where he was admitted on the 5th of October 2012 and from then received daily hospital treatments until 9 January 2013. The patient was also diagnosed with alcohol addiction (F 10). Electroencephalography test was regular. The psychological test showed simple personality structure, alcohol addiction, anxiety and organic psycho syndrome in development. MR showed multiple small subcortical lesions that imply hypertensive encephalopathy.

The Administrative court engaged the OM specialist, the Independent Second Level Expert Witness to assess if the patient lost his working capacity. The court itself did not raise the question about the patient's capacity for driving, nor did it do that in the case of a patient who suffers from the

depressive disorder (Case 2), under the Article 13, Paragraphs 11 and 22 of the Law for Motor Vehicles Drivers [8]. However, that topic was brought up by the expert witness who considered it his professional duty to raise this question and express his opinion that the lives of the public were in danger.

Discussion

Here are presented three cases of patients with psychiatric disorders, none of whom at the time of assessment were working. There is a well-known fact that such patients are prone to sick – leave and for invalidity pensions. This paper emphasises not only that well-known matter of fact; it indicates problems that influence general safety. Weapon and steering wheel must be in healthy arms, if no, fatal accidents could be expected. Medical practitioners in their surgeries, scientists, politicians and other professionals, who regulate the security of our lives globally, should be conscious of such problems that can generate while issuing licenses for firearms or car driving. Full attention to such problems is needed, so this article is trying to challenge debates and undertake more strict measures and rules for issuing firearm and car driving licenses.

The first patient was retired due to the PTSD and vertebra injuries. Although after the revision of his invalidity the PTSD was found diminished, his incapacity for work remained undeniable. The Independent Second Instance Expert Witness concluded that the patient is incapable of carrying firearms even though he had two certificates from private OM practices that claimed the opposite. It is to be expected that persons who are members of the military and other special units have significant resilience to stress [9]. Generated stress should be solved through stress and recuperation programs [10]. Some current studies point to the fact that negative environmental factors can also trigger genetic materials that lead to PTSD [11]. The harder stress strikes, the longer period for recovery is needed [12]. Although it has been a long time since this patient was exposed to stress and although his condition is stable now, every unpredicted and unpleasant event can awake dormant PTSD (as mentioned above in the event of the death of his father, when the patient visited psychiatrist due to repeated PTSD symptoms). The law that regulates carrying firearms issues firearms permits to persons with mild neurotic disturbances, but neurosis is not the same as PTSD, which is a serious disorder.

The second patient with the recurrent depressive disorder sued CIPI due to rejection of his claim for invalidity retirement. Persons with serious depressive disorders have impairment in cognition and everyday functioning [13], [14]. This is particularly expressed as a negative impact on work productivity [15]. The patient was hospitalised tree times at the clinic for psychiatry, and he is taking psychiatric medication. Due to his impaired cognitive functions, he is not capable of working as a sweeper of metal waste in an industrial plant where heavy vehicles such as tractors, forklifts and lorries pass the whole day. The patient regularly visits psychologist and psychiatrist, where he takes special therapies [16]. For all the reasons above mentioned he is not capable of work at his workplace, but the CIPI demands further analysis and experts sent the patient to do MR as the organic brain damages could be expected to be proven. For the patient is not capable to work, he is also not capable to drive a car, due to significant loss of cognitive functions and with delayed reactions as a consequence of being on psychiatric medicine. Therefore, the Independent Second Instance Expert Witness insists that the judge advises the relevant authorities that the patient’s driving license should be revoked.

The third patient, with only six years of working experience, is applying for invalidity pension. It is well known that alcohol abuse leads to work absence and increases the interest of invalidity pension [17]. Alcohol abuse is connected to reduced work memory capacity [18]. Alcoholics have reduced social sensitivity and capability to solve interpersonal situations [19]. Alcohol consumption/addiction can be proved by changes in the central nervous system [20]. The described patient has small punctual changes on his brain that are more typical for hypertonic encephalopathy. The Independent Second Instance Expert's opinion is that the patient is capable of work regardless of having to lift and carry heavyweight and the work in unfavourable microclimate. He should receive the relevant support trough social structures and refrain from alcohol consumption. The judge should advise the relevant authorities of the reasons for the patient's temporary suspension of his driving license. He should refrain from alcohol consumption for a minimum of 6-12 months before his driving license can be returned. This action is necessary to avoid exposing the life of the public, such as other drivers and the pedestrians, to danger.

In conclusion:

In Case 1, Independent Second Instance Expert Witness agreed with the Board of the Institute for Firearm / Car Driving experts, that the patient, a military veteran is not capable of carrying firearms. As the CIPI experts concluded, he is unfit to work as his state remained unchanged.

In Case 2, Independent Second Instance Expert witness disagreed with CIPI experts where the state of the patient’s health does not permit him further work in his occupation. Still, MR is recommended to prove organic brain damages and continuing psycho tests that show cognition impairments. The patient is not capable of driving a

car; therefore, it is recommended that his driving licence is permanently revoked.

In Case 3, all experts agree that there is no invalidity. The patient's driving license should be temporarily suspended until the patient proves he restrained from alcohol for a minimum of 6 up to 12 months. He is assessed capable of work in spite of all his work requirements.

Occupational Medicine has once again proven its important role in the assessment of work capacity [21]. Here, OM goes a step further pointing to the importance of controlling firearms/car driving licenses parallel with the assessment of work capacity.

In the end, the answer to the question from the paper title: If a person is unfit for work, they are most often also unfit to carry firearms or to drive, especially in cases of the psychiatric disorders.

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Factors Affecting the Learning of Fixed Prosthodontics Course by Students at Kermanshah University of Medical Sciences

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Abstract

AIM: The objective of this study was to investigate the factors affecting the learning of fixed prosthodontics course from the viewpoint of students and faculty members of Kermanshah Dentistry School.

MATERIAL AND METHODS: This research was a descriptive-analytical study conducted using the convenient sampling method. A total of 72 students and 5 faculty members were included in the study. Data were collected using a researcher-made questionnaire containing two sections. The first section consists of demographic information, and the second section consists of 14 questions to evaluate the factors affecting the learning of the fixed prosthodontics course.

RESULTS: From the students' point of view, there was a significant relationship between the effect of using clinical points during a teaching on the learning efficacy of the fixed prosthodontics course and gender (P = 0.028). There was a statistically significant relationship between the level of professor's knowledge regarding the modern educational methods on the learning of fixed prosthodontics course (P = 0.034). The factor of displaying and implementing practical work on the real patient was considered important by students, and having knowledge about modern educational methods was considered important by faculty members.

CONCLUSIONS: It is recommended that appropriate educational planning be implemented to enhance students' practical work on the real patient and increase professors' knowledge about modern educational methods.

Introduction

Education means any pre-designed activity or strategy designed and implemented aiming to create learning in learners and make necessary changes in their behaviour. Each educational program pursues certain goals, based on which they should monitor the education process and evaluate the expected results at the end of the program [1], [2]. Education will be effective if it possesses the following features: being independent and having individual orientation, cooperation and interaction between students and professors; educational orientation should not be long-term and target all aspects of the life of the individual. Moreover, other studies suggest that to improve the level of education, both professors and students should be aware of the objectives of the components of the course and the whole program [3], [4], [5]. Understanding and using a systemic approach are helpful for health educators or other people, whose profession is associated with education in some ways [1], [6]. The goal of educating dentists is to provide preventive and therapeutic services for oral and dental diseases so that dentists can provide these services using theoretical knowledge and clinical skills acquired during their education. To achieve this goal, there is a need for educational planning based on clinical education principles, so that graduated students do not feel unable to perform these skills after graduation [7], [8].

Nowadays, education encounters problems in a theoretical and clinical education setting. Educational planners must attempt to provide the conditions for students so that they can acquire the required knowledge and skill by providing the conditions for optimal use of available resources [9]. In this regard, researchers consider the use of the views of experienced people important in evaluating the curriculum. Many studies have been conducted on

the quality of education and attitudes of dentistry graduates in various aspects of education around the world. They aim to find a solution to improve the quality of education and encourage students to enhance their level of knowledge and scientific performance [10], [11], [12].In this regard, it is necessary to evaluate the current status of education continuously and identify its strengths and weaknesses to achieve effective clinical education. One of the most important and good ways to identify the quality of clinical education is to examine educators' views. In the educational system of medical science universities, teaching is extremely important to the nature of the fields of studies in these universities, and the improvement of the quality of education of medical science students is not possible without changes in teaching methods and techniques.

Hence, the present study aimed to investigate the factors affecting the learning of the fixed prosthodontics course by dentistry students of Kermanshah University of Medical Sciences.

Material and Methods

The present study is a cross-sectional study conducted at the Dentistry School of Kermanshah University of Medical Sciences in Kermanshah province, the largest province in western of Iran with a population of approximately 2 million people [13] in the academic year of 2018-2019. In this research, 5 faculty members and 72 students were interviewed. Convenient sampling was used in this study. The research population included faculty members and students of Kermanshah Dentistry School. Information was collected from the students and faculty members who had completed the fixed prosthodontics courses at the dentistry school. The study inclusion criteria included passing and teaching a fixed prosthodontics course by the participants and having the individual's consent to participate in the study. Also, the exclusion criteria included temporary guest students, students who did not complete the course of fixed prosthodontics, and students who were not willing to participate in the study. In completing the questionnaire, further explanations were provided by the researchers to students and professors, if needed. In this research, a researcher-made questionnaire was used. This questionnaire has 14 questions in addition to the demographic section (gender, age, and university entrance year).

The questions were developed and indexed according to theoretical studies in the field of effective factors in learning, including educational facilities, teaching method, and individual communication. Finally, questions were designed for the questionnaire by considering the indices related to the learning factors appropriate to each of them. The researchers ensured the participants that the questionnaire information was used only for statistical analysis, and it would remain confidential. The study data were analysed in two sections of descriptive statistics and inferential statistics. In the descriptive statistics section, the criteria for central tendency and dispersion were reported along with the table. Non-parametric tests were used in the inferential statistics section owing to the rank nature of the variables. The Mann-Whitney test was used to compare the influential factors between men and women and between faculty members and students. Friedman Test and post hoc test were also used to compare the effective factors in learning the fixed prosthodontics course. SPSS Version 18.0 software (Inc., Chicago, IL, USA) was used to analyse the data. The significance level in this study was considered 0.05.

Results

In the present study, 77 examinees participated, of which 72 (93.5%) were students and 5 (6.5%) were faculty members. Out of the total examinees, 34 (47.2%) students and 4 (80%) faculty members were female, and 38 (52.8%) students and 1 (20%) faculty member were males. The mean age of faculty members was 39.80 ± 8.01, and the mean age of the students was 24.66 ± 1.94. Cronbach's alpha coefficient was used to calculate reliability. The value of this index was obtained at 0.786. There was a statistically significant difference between the female and male students in the level of effect of clinical points during the teaching fixed prosthodontics course (P=0.028) so that the mean of this variable was higher in female students (50%) than in male students (39.5%) (Table 1).

Table 1: Comparison of male and female students in terms of the factors affecting students' learning in fixed prosthodontics

			Mean	SD	Median	Min	Max	P-value*
Q1	Sex	Female	6.03	0.81	6.00	4	7	0.243
		Male	5.80	0.60	6.00	5	7	
Q2	Sex	Female	6.39	0.73	6.50	4	7	0.397
		Male	6.12	1.10	6.00	1	7	
Q3	Sex	Female	6.19	0.89	6.00	4	7	0.028
		Male	5.73	0.84	6.00	4	7	
Q4	Sex	Female	5.75	1.00	6.00	4	7	0.446
		Male	5.54	0.90	6.00	4	7	
Q5	Sex	Female	5.92	0.91	6.00	4	7	0.120
		Male	5.59	0.71	6.00	4	7	
Q6	Sex	Female	5.56	1.08	5.00	3	7	0.460
		Male	5.34	0.82	5.00	4	7	
Q7	Sex	Female	5.42	0.87	5.00	4	7	0.223
		Male	5.15	1.15	5.00	1	7	
Q8	Sex	Female	5.06	1.37	5.00	1	7	0.406
		Male	4.90	0.97	5.00	2	7	
Q9	Sex	Female	5.47	1.08	6.00	3	7	0.073
		Male	5.02	1.17	5.00	1	7	
Q10	Sex	Female	6.22	0.83	6.00	4	7	0.507
		Male	5.98	1.11	6.00	2	7	
Q11	Sex	Female	6.28	0.88	6.50	4	7	0.010
		Male	5.76	0.86	6.00	4	7	
Q12	Sex	Female	6.19	0.89	6.00	4	7	0.995
		Male	6.20	0.95	6.00	3	7	
Q13	Sex	Female	6.39	0.80	7.00	5	7	0.510
		Male	6.32	0.76	6.00	5	7	
Q14	Sex	Female	6.25	1.00	7.00	4	7	0.345
		Male	6.12	0.90	6.00	4	7	

SD: Standard Deviation; Min: Minimum; Max: Maximum; *Mann Whitney Test.

There was a statistically significant difference between faculty members and students in the level of

effect of active participation of students (question and answer) on learning the fixed prosthodontics course (P = 0.039) so that the mean of this variable was higher in faculty members than in students. There was a statistically significant difference between faculty members and students in the level of effectiveness of the exams classified during the semester in learning the prosthodontics course (P = 0.002) so that the mean of this variable was higher in the faculty members than in the students. There was a statistically significant difference between the faculty members and students in terms of the effect of having a preliminary study on the learning of fixed prosthodontics course (P = 0.026) so that the mean of this variable was higher in the faculty members than in the students (Table 2).

Table 2: Comparison of faculty members and students in terms of the factors affecting students' learning in fixed prosthodontics

			Mean	SD	Median	Min	Max	P-value
Q1	Academic Degree	Academic staff	6.40	0.55	6.00	6	7	0.149
		Student	5.88	0.71	6.00	4	7	
Q2	Academic Degree	Academic staff	6.60	0.55	7.00	6	7	0.438
		Student	6.22	0.97	6.00	1	7	
Q3	Academic Degree	Academic staff	6.40	0.55	6.00	6	7	0.297
		Student	5.92	0.90	6.00	4	7	
Q4	Academic Degree	Academic staff	6.20	0.84	6.00	5	7	0.203
		Student	5.60	0.94	6.00	4	7	
Q5	Academic Degree	Academic staff	6.40	0.55	6.00	6	7	0.073
		Student	5.69	0.82	6.00	4	7	
Q6	Academic Degree	Academic staff	5.40	0.89	5.00	5	7	0.818
		Student	5.44	0.96	5.00	3	7	
Q7	Academic Degree	Academic staff	6.20	0.84	6.00	5	7	0.039
		Student	5.21	1.02	5.00	1	7	
Q8	Academic Degree	Academic staff	6.40	0.55	6.00	6	7	0.002
		Student	4.87	1.14	5.00	1	7	
Q9	Academic Degree	Academic staff	6.20	0.45	6.00	6	7	0.026
		Student	5.17	1.15	5.00	1	7	
Q10	Academic Degree	Academic staff	6.60	0.55	7.00	6	7	0.269
		Student	6.06	1.01	6.00	2	7	
Q11	Academic Degree	Academic staff	6.20	0.45	6.00	6	7	0.741
		Student	5.99	0.93	6.00	4	7	
Q12	Academic Degree	Academic staff	6.20	0.45	6.00	6	7	0.756
		Student	6.19	0.94	6.50	3	7	
Q13	Academic Degree	Academic staff	6.60	0.55	7.00	6	7	0.594
		Student	6.33	0.79	7.00	5	7	
Q14	Academic Degree	Academic staff	6.20	0.45	6.00	6	7	0.741
		Student	6.18	0.97	7.00	4	7	

SD: Standard Deviation; Min: Minimum; Max: Maximum; *Mann Whitney Test.

Table 3 presents a comparison of effective factors in learning the prosthodontics course in female students. There was a statistically significant difference among the female students in terms of various factors affecting the learning of the prosthodontics course (P < 0.001, Friedman test). From the viewpoint of female students, the factor of displaying and implementation of practical work on the real patient was the most effective in learning the prosthodontics course. There was a significant difference among the male students in terms of various factors affecting the learning of the prosthodontics course (P < 0.001, Friedman test).

Table 3: Comparison of female students in terms of the effective factors in learning of prosthodontics course

	Median	Range
Q1	6.00^{abcde}	3.00
Q2	6.00^{de}	3.00
Q3	6.00^{bcde}	3.00
Q4	6.00^{abcde}	3.00
Q5	6.00^{abcde}	3.00
Q6	5.00^{abcd}	4.00
Q7	5.00^{ab}	3.00
Q8	5.00^{a}	6.00
Q9	5.50^{abc}	4.00
Q10	6.00^{bcde}	3.00
Q11	6.50^{cde}	3.00
Q12	6.50^{cde}	3.00
Q13	7.00^{e}	2.00
Q14	7.00^{cde}	3.00
P-value*	0.001	

*Non-parametric Friedman Test, followed by Post Hoc test; Medians followed by different letters, express a statistically significant difference (*P-value* < 0.05).

From the viewpoint of the male students, the factors of appropriate presentation of the professor, the way the professor interact with the students and the displaying and implementation of practical work on the real patient were the most effective factors in learning the fixed prosthodontics course (Table 4).

Table 4: Table 4: Comparison of male students in terms of the effective factors in learning of prosthodontics course

	Median	Range
Q1	6.00^{bcd}	2.00
Q2	6.00^{d}	6.00
Q3	6.00^{bcd}	3.00
Q4	5.50^{abcd}	3.00
Q5	6.00^{abcd}	3.00
Q6	5.00^{abc}	3.00
Q7	5.00^{ab}	6.00
Q8	5.00^{a}	5.00
Q9	5.00^{ab}	6.00
Q10	6.00^{cd}	5.00
Q11	6.00^{bcd}	3.00
Q12	6.50^{d}	4.00
Q13	6.00^{d}	2.00
Q14	6.00^{cd}	3.00
P-value*	0.001	

*Non-parametric Friedman Test, followed by Post Hoc test; Medians followed by different letters, express a statistically significant difference (*P-value* < 0.05).

There was no significant difference among the faculty members in terms of various factors affecting learning the prosthodontics course (P = 0.181, Friedman test) (Table 5).

Table 5: Comparison of faculty members in terms of the factors affecting the learning of prosthodontics course

	Median	Range
Q1	6.00	1.00
Q2	7.00	1.00
Q3	6.00	1.00
Q4	6.00	2.00
Q5	6.00	1.00
Q6	5.00	2.00
Q7	6.00	2.00
Q8	6.00	1.00
Q9	6.00	1.00
Q10	7.00	1.00
Q11	6.00	1.00
Q12	6.00	1.00
Q13	7.00	1.00
Q14	6.00	1.00
P-value*	0.181	

*Non-parametric Friedman.

Discussion

The results of this study showed that the female students considered the displaying and implementation of practical work on the real patient as

the most important factor affecting the learning of fixed prosthodontics course, which could be justified given the clinical nature of this course. From the viewpoint of the male students, the displaying and implementation of practical work on the real patient as well as the professors' presentation skills and the interaction with the students were one of the most important factors influencing the learning of the prosthodontics course. Furthermore, the views of the professors participating in the present study demonstrated that all the evaluated factors had equal importance, and there was no significant difference between these factors. Midgley (2006) and Karimi *et al*., (2010) in separate studies indicated that the most important determinant ineffective education was the performance and knowledge of professors, since professors can transfer their knowledge and experience better to the student by having characteristics such as self-confidence, high skill and effective communication skills [14], [15]. These results were consistent with the results of the present study from the viewpoint of the male students (a good presentation by the professor and his or her interaction with the student).

In line with the present study, the study conducted by Mehralizadeh *et al*., (2013) showed that the students' opinion of lecturers' good presentation skills is the most influential in learning [11]. The results of other study indicated that from the viewpoint of the students, good behaviour of the officials, the professor and the student play an effective role in enhancing the level of learning [16]. It was consistent with the result of the present study. Elkan and Robinson (2000) have stated that the university should provide opportunities for students to be prepared for providing services in the future. In this regard, teaching consistent with the clinical needs and the use of a safe clinical, educational setting by the experienced and skilled professors are extremely important [9]. Maginnis *et al*. (2010) also stated in their study that students were not able to transfer what they were learning in an academic setting to the clinical setting. There is a cognitive dissonance here. This cognitive dissonance makes students unable to integrate the ideals of the academic setting with the realities of the clinical setting [17]. It is more highlighted in medical science courses, particularly specialised courses of dentistry. Achievement of effective learning requires using the existing clinical facilities as much as possible and enhancing the student's access to more practical settings. Thus, the educational setting reform should be considered by educational officials to standardise it in supplying educational facilities and equipment. The results of this research revealed that all students, including male and female students, considered displaying and implementation of practical work on the real patient as the most important factors influencing the learning of the fixed prosthodontics course.

In the present study, the effects of factors such as patience and calmness of the professor in the classroom, the exams classified during the semester, active participation of students (question and answer), having a preliminary study, reviewing theoretical topics in the practical class, using dental moulage, using educational illustrations, including video and animation, and holding educational seminars on learning the fixed prosthodontics course were studied. The results did not reveal a significant difference in the groups studied. The only significant difference based on the students' gender was related to the use of clinical points during the teaching on learning the fixed prosthodontics course. Ramazani *et al*. (2013) investigated the practical education and the factors affecting it. The results of their study revealed a significant difference in the mean score of students' viewpoints in terms of age ($P = 0.048$) and gender ($P = 0.040$) so that the score of viewpoints was higher for students older than thirty-five years. Involvedness in things such as out-of-school treatment work, life pursuits, has drawn such a conclusion. Also, compared to male and female students, male students' viewpoints indicate a more favourable situation in practical education. This may be owing to emotions and self-esteem in boys [18]. In the research conducted by Kelsey *et al*., (2009), there was no difference between the genders in evaluating the academic setting of the dentistry school [19]. Regardless of using clinical point during the teaching, it was consistent with the result of the present study.

Moreover, in the present study, the viewpoints of professors and students differed only in the level of the effect of professor's knowledge about modern educational methods on learning the fixed prosthodontics course. Karimi *et al*., (2011) also showed that applying new teaching methods, enhancing the academic and practical levels of professors, using active educational methods, and establishing consistency between theoretical education and professional needs could be effective in improving the quality of education [15]. Akbari *et al*., (2014) stated that the views of professors and students matched each other, except for four cases (the importance of timely presence of the professor in the classroom, the importance of paying attention to the secondary cases, such as communication with the patient, the importance of presenting pamphlets in the classroom and the importance of giving high scores to the students) [20]. In the present study, this difference was observed only regarding the effect of the professor's knowledge about modern educational methods. Rieger *et al*., (2009) reported that applying new educational methods could be effective in improving the educational process and showed that the new and blended educational method compared to traditional education would increase the success rate of students by 10% [21]. Previous studies showed that the use of new and innovative educational methods was effective in teaching the fixed prosthodontics course [22], [23]. Kavadella *et al*. (2012) reported the effect of the blended method on

teaching oral and dental radiology to dentistry students [24].

According to the present study results, it can be stated that the factor of displaying and implementing the practical work on the real patient was considered important by the students and having knowledge on the modern educational methods was considered important by the faculty members. Thus, it is recommended that appropriate educational planning be implemented in this regard to enhance and improve these two factors. Healthcare services aiming at improving the health of individuals and the community depend on the extent to which the goals of the educational programs are realised. If the educational programs are not designed and implemented well, it can cause irreparable damage and social, economic and cultural harms to the learners and the people in the community. Thus, it is necessary to consider specialised courses like the fixed prosthodontics course, which has a more practical nature and requires providing an appropriate clinical setting for education.

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Appendix

Questionnaire Form

This questionnaire was designed to investigate the factors affecting learning of fixed prosthesis from the viewpoints of students and faculty members of Kermanshah Dental School. Dear students and faculty members, please complete the questionnaire and help us in this regard.

Academic degree : □ Faculty members □ Student
Gender : □ Female □ Man

Age: ..... Year of entry: .....

1) How is the impact of using dental molasses on learning a fixed prosthesis lesson?
   a) excellent b) very good c) good d) average e) relatively bad f) not bad g) very bad
2) How is the effect of using a good teacher's expression on learning a fixed denture lesson?
   a) excellent b) very good c) good d) average e) relatively bad f) not bad g) very bad
3) How effective are the clinical points during the teaching of fixed prosthesis?
   a) excellent b) very good c) good d) average e) relatively bad f) not bad g) very bad
4) How effective is the review of theoretical issues in the practical classroom of fixed prosthesis lessons?
   a) excellent b) very good c) good d) average e) relatively bad f) not bad g) very bad
5) How effective are educational images like animation videos in learning a fixed denture lesson?
   a) excellent b) very good c) good d) average e) relatively bad f) not bad g) very bad
6) How effective is the conduct of educational seminars on learning a fixed denture lesson?
   a) excellent b) very good c) good d) average e) relatively bad f) not bad g) very bad
7) How effective is the active presence (question and answer) of students in learning a fixed denture lesson?
   a) excellent b) very good c) good d) average e) relatively bad f) not bad g) very bad
8) How effective are the exams classified during the semester of taking a fixed prosthesis course?
   a) excellent b) very good c) good d) average e) relatively bad f) not bad g) very bad
9) How effective is having a pre-study course on fixed denture learning?
   a) excellent b) very good c) good d) average e) relatively bad f) not bad g) very bad
10) How effective is the teacher's patience and calmness in the classroom on learning a fixed denture lesson?
   a) excellent b) very good c) good d) average e) relatively bad f) not bad g) very bad
11) How effective is the use of clinical tips when teaching a fixed prosthesis lesson?
   a) excellent b) very good c) good d) average e) relatively bad f) not bad g) very bad
12) How effective is the way a teacher deals with students in learning a fixed denture lesson?
   a) excellent b) very good c) good d) average e) relatively bad f) not bad g) very bad
13) How effective is the demonstration and execution of practical work on a real patient in learning a fixed prosthesis lesson?
   a) excellent b) very good c) good d) average e) relatively bad f) not bad g) very bad
14) How is the effect of a teacher's familiarity with new teaching methods on learning a fixed prosthesis lesson?
   a) excellent b) very good c) good d) average e) relatively bad f) not bad g) very bad

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Relationship between Sanitation Hygiene and Health Care with Healthy Family Security of the Family of Smokers at Berastagi Subdistrict

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Keywords: Hygiene Sanitation; Health Care; Healthy Family Security; Smoker

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Abstract

BACKGROUND: Healthy family security means the strength and the ability of a family to meet health needs and to be free from health problems. The health problem itself is various and numerous, especially related to sanitation hygiene and health care.

AIM: This research aims at analysing the relationship between sanitation hygiene and health care with healthy family security of the family of smokers.

METHODS: It involved 120 families of smokers living at Berastagi Subdistrict, North Sumatera Province, Indonesia, as the sample of the research. Data collection was done by doing observation and interview with a structured questionnaire instrument. Weight and height of the family members of the smokers were recorded as the measurement of nutrition status as well as health status for the measurement of healthy family security.

RESULTS: The research found that there was a significant relationship between sanitation hygiene and health care with healthy family security.

CONCLUSION: The research concluded that sanitation hygiene and health care performed by the family of smokers could increase healthy family security.

Introduction

Health is one of human's rights, and it has become one of the main issues related to social development. Generally, it deals with the concept of disease prevention and health improvement [1]. Health improvement itself can be made by decreasing the factors leading to health problems, especially in the family. Family health problems developing in society recently are frequently related to family behaviours without ignoring community behaviours. One of the family health problems is a smoking problem. Smoking problem has been worrying and increasing all the year rapidly. According to the report on the result of National Basic Health Research [2], it is stated that the number of daily smokers in Indonesia reached around 24.3% of the total 10 aged population, meanwhile, in North Sumatera province it reached around 24.2%. It means that evenly a smoker spends around 12.3 cigarettes (around one pack) every day. Smoking can lead to illness and death, but actually, it can be prevented. The impact of smoking further can also destroy the future and the economy of a country as well as a family, especially family security. Authors stated that family security could be assessed through physical security assessment, for examples, morbidity and disease frequency in a family

[3]. Moreover, [4] stated that the components of family security include the component of systematical approach: input (physical and non-physical resources), process (both physical and non-physical problem and solving) and output (physical and non-physical welfare).

Authors further explained that family security covers external and internal factors [4]. External factors include disaster management, rural development, for examples, food security, social security, infrastructure, natural resource management and live hood pattern; meanwhile, internal factors include income, education, health and stress management. Internal factors are used to determine the human development index. Environmental factors which become one of the leading causes of unhealthy children in the family and affect growth disorders on children also determine life quality and human's health [5]. These growth disorders certainly decrease the human's quality and healthy family security. Also, [6] stated that environmental factors affect a child's growth after birth, for instance, race, sex, age, nutrition, health care, and infectious disease.

Family as the smallest unit in a community plays important roles to create qualified generations as a family is a system which involves interaction leading to conflict or vice versa and also gives benefits to support the quality of family members inside. Other has studied the growth of 380 children aged 6-24 months in low-income families [7]. The result indicated that the children of low-income families experienced growing disorders by 18-24 months. These disorders represent the low quality of generations and frequent problems occurring on children under 2 (two) years old, which are also influenced by the factors within the family itself.

It was pointed out that the most frequent family problems occur are financial difficulties (economic problem), agricultural production, food supply, and marketing difficulties of agricultural products [3]. Moreover, [8] stated that family plays important roles for children as they have responsibility for personal socialisation, especially during the growing period. Some authors emphasised that the participation of family members is an important action to prevent disease in the community, and it must be done continually [9].

The quality of human resources becomes more important towards the family's roles. A healthy family will support its members to be positive for health by empowering the available resources and considering healthy living behaviour [10]. Certainly, these families will try to overcome the health problems occurring due to smoking, for examples, reducing the cigarette spending and substituting it with health and education investment as it often ignores another spending, i.e. nutritional food. This situation absolutely will affect children's growth. Thus, enhancing the social awareness that 'not smoking is an investment' must be implemented, whether for yourself, family, community, or country.

Every family member is at risk to be a smoker; however, the family significantly must try to maintain positive condition, especially for health to create a secured family as [11] defined, "Security means success in life despite being in a high-risk state. Meanwhile, according to [12], the notion of security from the point of behaviour is the pattern of positive behaviour and functional ability of the individual or family to overcome stress and difficulty. Furthermore, [13] stated that security begins in the absence of pathology (disease) to the ability to overcome, find the meaning and continues despite the difficulties occur.

Security is the ability to deal with the significant developmental challenges facing people in their growth process and also added that family security is strengthened by the ability of family members to assume their responsibilities and roles [14]. They have to commit and work together to increase their family health. Support from surroundings is also required to empower the family security. Some authors argued that security is used to describe a process, in which people not only manage the efforts to overcome life difficulties but also create, maintain and contribute meaningful life to the people around them [15]. Moreover, the family's tight relationship and understanding each other also support family security to be healthier. Thus, this research was done to analyse the relationship between sanitation hygiene and health care with healthy family security of the family of smokers.

Methods

This research was observational research with a cross-sectional design. It was done at Berastagi subdistrict, North Sumatera Province, involving 120 families as the sample. The number of samples was determined by using a sample calculation formula for survey research. The simple random technique was used to select families whose one of their family members was a smoker. Data collection was done by doing observation and interview using structured questionnaire instrument toward the variable of hygiene, sanitation and health service. Previously, the instrument was tested to measure validity and reliability.

Measurement of healthy family security was done through physical measurement of the anthropometric index (nutritional status) and disease history of the family. The anthropometric index was seen from data collected by weighing (BB) and measuring the height of the body (TB) and collecting age data (U) of all family members. Anthropometric

index used was different for each family member: for the age group 0-24 months used the index of BB/U, TB/U and BB/TB; for the age group 2-18 years used the index of IMT/U, and for the age group of 18 years up used the body mass index (BMI). Furthermore, the composite value of nutritional status and family health status was given, so that the variable can be categorised into good (secured-healthy) and less (less-secured-healthy). Variable of hygiene sanitation and health cares, each was also categorised into dichotomous, into two levels: good and less. Further, data was analysed and tested by using C-square test.

Results

Healthy family security in the family of smokers was measured physically by the composite size of nutritional and health status of the family. Measurement of healthy family security was done through physical measurement of the anthropometric index (nutritional status) and disease history of the family. The following figure shows the research result related to the proportion of healthy family security, as displayed in Figure 1.

Figure 1: The Proportion of Smoker's Family Based on Healthy Family Security at Berastagi Sub-District

Based on the above figure, it can be seen that there is 37.5% of the family of smokers with less healthy family security and 62.5% with good healthy family security. This healthy family security also has a closed relationship to sanitation and hygiene. Based on the research done, it was found that 71 families (59.2%) have good sanitation and hygiene and the remaining, 49 families (40.8%) has less sanitation and hygiene, as shown in Figure 2.

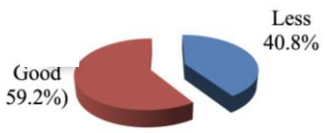

Figure 2: The Proportion of Smokers' Family Based on Hygiene and Sanitation at Berastagi Sub-District

Also, the description of the proportion of the family of smokers based on hygiene and sanitation at Berastagi Subdistrict can be seen in Table 1.

Table 1: The Proportion of Smokers' Family Based on Hygiene at Berastagi Sub-District

Hygiene	n	%
Washing hands before eating		
Yes	111	92.5
No	9	7.5
Taking a bath every day		
Yes	98	81.7
No	22	18.4
Washing hands with soap after using a toilet		
Yes, always	66	55.0
Sometimes	49	40.8
No	5	4.2
Cutting nails		
Yes, always	85	70,8
Sometimes	31	25.8
No	4	3.3
Changing clothes every day		
Yes	105	87.5
No	15	12.5
Draining the bathtub once a week		
Yes	63	52.5
Sometimes	24	20.0
No	33	27.5
Total	120	100

Health cares also affect the security of the family of smokers, as shown in Table 2 and illustrated in Figure 3.

Table 2: The proportion of Smokers' Family Based on Sanitation at Berastagi Sub-District

Sanitation	n	%
Family water supply for drinking		
Local Water Supply (PDAM)	98	81.7
Shallow/dug well	5	4.2
Drilled-well	4	3.3
Spring	6	5.0
Others	7	5.9
Construction of opened-household sewerage (SPAL)		
Opened	71	54.1
Closed	49	40.8
Trash bin availability		
Yes	83	69.2
No	37	30.8
Trash bin condition		
Opened	73	60.8
Closed	47	39.1
Latrine meets health conditions		
Yes	104	86.7
No	16	13.3
The distance between livestock and water supply		
No livestock	93	77.5
≤ 11 metre	22	18.5
> 11 metre	5	4.2
Total	120	100,0

Figure 3 describes health cares in the family of smokers in which 85 families (70.8%) are in the less category, and 35 families (29.2%) are in a good category.

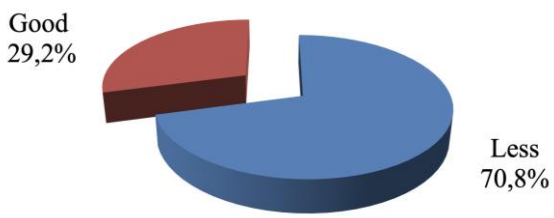

Figure 3: The proportion of Smoker's Family-Based Health Care at Berastagi Sub-District

Thus, there must be more intensive efforts to support the implementation of health care for healthy living, specifically toward the family of smokers, as shown in Table 3.

Table 3: The Efforts of Smoker's Family in Health Care for Healthy Living

The Efforts in Health Care	No		Sometimes		Yes	
	N	%	N	%	N	%
Keeping the environment cleanliness	2	1.7	27	22.5	91	75.8
Maintaining a clean and healthy life every day	2	1.7	29	24.2	89	74.2
Doing exercises regularly	85	70.8	19	15.8	16	13.3
Maintaining personal health	3	2.5	16	13.3	101	84.2
Avoiding consuming excessive fats	47	39.2	30	25.0	43	35.8
Having a balanced diet	6	5	36	30.0	78	65.0
Reducing/avoiding smoking or drinking alcohol	59	49.2	18	15.0	43	35.8
Seeing/consulting a doctor immediately whenever getting ill.	13	10.8	24	20.0	83	69.2
Breastfeeding the baby	71	59.2	19	15.8	30	25.0
Consuming iodized salt	11	9.2	2	1.7	107	89.2
Weighing regularly	72	60.0	25	20.8	23	19.2
Asking health staff whenever having a problem	20	16.7	30	25.0	70	58.3

The efforts done by the family of smokers mostly related to health cares are: consuming iodised salt (89.2%), maintaining personal health (84.2%), keeping the environment cleanliness (75.8%) and maintaining clean and healthy life every day (74.2%). In contrast, the least efforts done by the smoker's families are: reducing smoking (35.8%), weighing regularly (19.2%) and doing exercise regularly (13.3%). Despite, the smoker's families have been trying to implement health care, i.e. having a balanced diet (65%) and seeing/consulting a doctor immediately whenever getting ill.

The result of the C-square test indicated that sanitation hygiene and health cares have a relationship with healthy family security. The value of p showed a significant relationship, as illustrated in Table 4.

Table 4: The Relationship between Sanitation Hygiene and Health Cares with the Healthy Family Security of Family of Smokers at Berastagi Sub-District

Variable	Healthy Family Security				P
	Less		Good		
	n	%	n	%	
Sanitation Hygiene					
Less	25	51.0	24	49.0	0.011
Good	20	28.2	51	71.8	
Health Care					
Less	37	43.5	48	56.5	0.033
Good	8	22.9	27	77.1	

Table 4 shows that there is a significant relationship between sanitation hygiene with healthy family security with p-value = 0.011, and also there is a significant relationship between health cares with healthy family security with p = 0.033. It means that good sanitation hygiene and health care contribute to the healthy family security of the family of smokers. It is shown in Table 4 that if sanitation hygiene is in less category, so the healthy family security will be less, too. Also, if health care is categorised good, healthy family security will be categorised well. These conditions make families tend to be infected by disease easily and give impact to the health and nutrition status of the family members. The proportion of family of smokers for less health service implementation is high. Therefore, the family of smokers trying to implement health care better will be categorised as a secured, healthy family.

Discussion

The higher level of good healthy family security (62.5%) compared with less healthy family security (37.5%) indicates that there are still smoker's families who are less trying to improve their health. It is caused by the fact that smoking becomes a part of custom activities for Karonese, predominantly tribes living at Berastagi subdistrict. Cigarette and betel are always served and must be consumed on family occasions, family meeting, family gathering, etc. It will be an honour for the family if their guests or relatives consume the cigarette or betel served. Therefore, it contributed to the level of healthy family security.

It is stated previously that family security has closed relationship to sanitation and hygiene. Healthy family security related to hygiene and sanitation includes the absence of environment-based diseases, for examples, diarrhoea, helminthiasis, Upper Respiratory Infection (ARI), pneumonia, etc. According to the result of the research, it was found that there is a relationship between sanitation cleanliness and healthy family safety (p = 0.011). Further, the result of the research found that 71 families (59.2%) have good sanitation and hygiene and the remaining, 49 families (40.8%) has less sanitation and hygiene as shown in Figure 2. The worrisome circumstances related to the hygiene of smokers' family are washing hands with soap after using a toilet and draining the bathtub once a week, while for sanitation; the worrisome circumstances are opened-household sewerage (SPAL) and opened-trash bin.

The wastes pass through opened-household sewerage (SPAL) will build up and make it being stopped up and stagnant. Opened-trash bin will cause bad smell and invite the vector of diseases, such as flies, cockroaches, or mosquitoes. Both can help the spreading of the disease rapidly. These conditions will affect the health status of smokers' family. Therefore, they must be improved and well-managed. It is supported by the research conducted by [16] finding that physical sanitation of the house (lighting, ventilation, smoking behaviour) that does not meet the requirements becomes a risk factor for the incidence of ARI in infants. This research also proved that there is a relationship between basic sanitation facilities (clean water facilities, garbage disposal, wastewater disposal facilities and family latrines) and personal hygiene with the incidence of diarrhoea.

Moreover, correlated to health cares in the family of smokers in which 85 families (70.8%) is in the less category and 35 families (29.2%) is in a good category, the main problem is caused by their ignorance of the importance of implementing healthy living. The most common effort done by the families is only consuming iodised salt as it is the easiest to be obtained, even in the small shops. They use it every day in their cooked food. This situation can also

represent the good performance of local government in providing and promoting the iodised salt. However, the local government must be more intensive to promote other activities supporting health security, for instance, doing physical exercise regularly. This situation represents that hygiene sanitation and health care are related to healthy family security, for example, growth in infants and overall quality of life. Based on the results of the study, it was also found that there is a relationship between health care and healthy family security (p = 0.033). It is strengthened by the research conducted by [17] stating that the variable feeding practices and hygiene practices are significantly related to the incidence of stunting. The results of this study explained that the practice of feeding and good hygiene practice could prevent stunting. The incidence of stunting during the growth of children can reduce the quality of life until they are adults. Consequently, the impacts that occur can reduce the quality of health.

In conclusion, there are more healthy-secured families in case of smoker's families (62.5%), but for sanitation hygiene and health cares, both are less. The correlation between sanitation hygiene and health cares with healthy family security shows significantly; bad sanitation hygiene and health cares in the smoker's families tend to create an unhealthy family.

It is expected that there will be training or coaching for smoker's families to increase sanitation hygiene and health cares through health promotion activity, which will change the way of thinking of the smoker' s families to do many activities for preventing disease as well as improving and empowering healthy family security.

The community support and cross-sectoral activities from Karo District Government, Health Department, Social Department, or Education Department are expected to implement health promotion activity and prevent disease-related to sanitation hygiene and health cares in the smoker's families. Moreover, the cooperation and participation among the parts involved in the health community, for examples, regional government, department of health, social department, or educational department are required to support health promotion and disease prevention related to sanitation hygiene and health cares of smokers' family.

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Public Health

Low Maternal Vitamin D and Calcium Food Intake during Pregnancy Associated with Place of Residence: A Cross-Sectional Study in West Sumatran Women, Indonesia

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Abstract

BACKGROUND: There is a high prevalence of vitamin D deficiency in pregnancy worldwide, and variable availability of vitamin D-rich foods may affect the adequacy of vitamin D food intake in different regions.

AIM: We analysed the relationship between place of residence and maternal food intake of vitamin D and calcium in West Sumatra, Indonesia.

METHODS: This cross-sectional study was conducted in 203 pregnant women. Data collection was carried out in four districts in West Sumatra – two in coastal locations and two in mountainous locations – with subjects divided into groups based on their place of residence. The dietary intakes of pregnant women were assessed through a semi-quantitative food-frequency questionnaire (SQ-FFQ).

RESULTS: The means of maternal vitamin D and calcium food intake were 7.92 ± 5.26 µg/day and 784.88 ± 409.77 mg/day, respectively. There were no reports of vitamin D supplement intake during pregnancy. A total of 86.7% and 89.7% of the study subjects had low vitamin D and calcium food intake status, respectively. There was a significant association between maternal vitamin D intake and place of residence ($p = 0.02$) and significant different mean levels of vitamin D food intake with the place of residence (9.04 vs 6.55 µg/day; $p = 0.01$). Mothers who had higher education levels had adequate calcium food intake ($p = 0.015$; OR: 0.295; 0.116–0.751).

CONCLUSION: Low maternal vitamin D and calcium food intake were found to be common in West Sumatra, Indonesia and their differed between those residents in mountainous and in coastal areas.

Introduction

Vitamin D deficiency and insufficiency during pregnancy is a global public health problem. According to our recent studies, the pregnant Indonesian women investigated had inadequate vitamin D and calcium intakes [1], [2], [3]. Adequacy of food intake during pregnancy determines the adequacy of maternal nutrition, and in terms of vitamin D intake, foods rich in this nutrient are not as common as other vitamin sources. Recent studies showed that low vitamin D food intake during pregnancy was positively but weakly correlated with maternal serum vitamin D level (25-hydroxyvitamin D) [4]. Our previous studies had no association between vitamin D and calcium food intake status. However, pregnant women who had low vitamin D levels were 60-70% in the inadequate vitamin D and calcium food intake status. There were 30-40% of pregnant women in the normal vitamin D level [5]. Vitamin D and calcium food intake were extremely low during the first trimester of pregnancy with more than 90% of inadequate vitamin D and calcium food intake status [6]. This high number of inadequate status for both vitamin D and calcium food intake status might due to the quantity of

supplementation and intake of vitamin D-fortified food policies during pregnancy. The limited availability of such foods and the difficulty of accessing them are challenges for people in meeting their daily vitamin D requirements.

Dietary sources of vitamin D can be obtained from natural or fortified foods such as dairy products, fish and supplements [7]. However, the main source of vitamin D is exposure to sunlight, and lifestyle changes such as limited sunlight exposure resulting from the full coverage of the skin by clothing, use of sunscreen/sunblock and lack of outdoor physical activities may restrict sun exposure [8], [9]. Vitamin D helps to maintain calcium homeostasis and a healthy, mineralised skeleton, making it important in the process of foetal development. It can also affect the activity of the immune system, cell proliferation and differentiation and insulin cell synthesis, and is thus one of the essential micronutrients for maintaining maternal and foetal health during pregnancy [10]. Several previous studies have reported that pregnant women with vitamin D deficiency are at risk of low birth weight babies, preterm births, autoimmune disorders such as type I diabetes mellitus [8], [9], [10], [11], [12] and impaired blood vessel function. The latter may lead to pre-eclampsia, which is a common cause of maternal death [16].

There are some areas of debate about adequate food intake of vitamin D and optimal vitamin D food intake levels. The recommended food intake of vitamin D varies by country: in Scandinavia, all pregnant women are advised to take 10 mcg/day to maintain an adequate level of vitamin D food intake. It is estimated that the food intake of vitamin D-rich foods at this level may maintain a serum 25OHD level of 25 nmol/L [17]. In contrast, Indonesia's recommended dietary allowance (RDA) for vitamin D food intake level is 15 mcg/day [18]. Calcium also plays an important role in foetal growth during pregnancy and vitamin D assists the absorption of calcium in the small intestine. Thus, the fulfilment of vitamin D and calcium food intake requirements are important for foetal development during pregnancy [19]. Place of residence is one of the factors which determines the availability of food. It is easier for pregnant women who live in coastal areas to access vitamin D-rich foods in seafood such as fatty fish (mackerel, tuna, salmon and sardines), fish oil, oysters, prawns and cod liver oil than for those who live in mountainous areas. A recent study showed that 37% of vitamin D food intake source was coming from seafood, followed by starchy, meat, eggs, dairy, and sweet and pastry foods [4].

This study analysed the relationship between place of residence and maternal intake of vitamin D and calcium in West Sumatra, Indonesia.

Methods

Study design and participants

Of 215 initial candidates for this cross-sectional study, 203 were enrolled while 12 refused to participate, giving a total enrolled cohort of 203. This cross-sectional study was conducted between September and December 2016 in two coastal districts (Pariaman and Pasaman Barat) and two mountainous areas (Solok and Tanah Datar) of West Sumatra, Indonesia. This study was part of The Vitamin D Pregnant Mother (VDPM) cohort study [6]. Inclusion criteria for the women taking part were as follows: (1) > 28 weeks of pregnancy attending routine antenatal reviews in the primary health service; (2) no history of taking any kind of drug that could interact with vitamin D and calcium metabolism; (3) absence of chronic disease; (4) living in close proximity to the research area; and (5) agreement to participate in the study.

Solok, Tanah Datar, Pariaman and Pasaman Barat are distinct districts of West Sumatra which demonstrate variety concerning geography, social life, eating habits and living conditions. Pariaman and Pasaman Barat are located on the the Indian Ocean coast at altitudes of between 0 and 15 m above sea level. According to the Indonesia Directorate of Meteorology, the average daily temperature in these locations varies between 20°C and 26°C. Fish consumption rates are higher in coastal areas such as these, as fish is relatively abundant. In contrast, Solok and Tanah Datar are located in a mountainous area of West Sumatra. The study protocol was reviewed and approved by the local ethics committee at the Faculty of Medicine, Andalas University, Indonesia (No: 108/KEP/FK/2016) and written informed consent was obtained from all participants before their recruitment and their identity anonymity was preserved.

Data collection

Participants gave written informed consent and were then asked to complete two questionnaires: one addressing socio-demographic characteristics and the other a validated semi-quantitative food-frequency questionnaire (SQ-FFQ). Anthropometric data such as maternal body weight and height without shoes were measured to the nearest 0.1 kg and 0.1 cm, respectively. Pre-pregnancy BMI was calculated as weight (kg)/height $(m)^{2,}$ and then participants were categorised into four groups based on current World Health Organization classifications: underweight (BMI < 18.5), normal (BMI 18.5 – 24.99), overweight (BMI 25 – 29.99) and obese (BMI 30 or higher) [20]. The questionnaires collected information regarding age, educational level, marital status, occupation, family income, drug history, maternal health history and dietary behaviour, and blood pressure was recorded using a sphygmomanometer.

Dietary assessment

Dietary intake was assessed using a semi-quantitative food-frequency questionnaire (SQ-FFQ) developed and validated by Lipoeto [21]. This questionnaire was adapted for Minangkabau food habits. Minangkabau people, also known as *Minang*, comprise an ethnic group indigenous to the Minangkabau Highlands of West Sumatra. The validated SQ-FFQ gathered information on foods fortified with vitamin D, natural foods rich in vitamin D and dietary supplements, and referenced more than 223 general food items available in West Sumatra, including potential sources of vitamin D. Daily energy and nutritional intakes were calculated and compared with RDAs for pregnant women [15]. Based on their calcium and vitamin D intakes, the pregnant women were divided into two groups: inadequate intake (< 15 µg/day) and adequate intake (≥ 15 µg/day) for vitamin D and inadequate intake (< 1300 mg/day) and adequate intake (≥ 1300 mg/day) for calcium.

Statistical analysis

All data were analysed using IBM SPSS Statistics for Windows (SPSS version 23.0, IBM Inc., Chicago, IL, USA). Descriptive analysis was presented as frequency and percentage for categorical variables and as mean and standard deviation (SD) for continuous variables to show respondents' characteristic measurements. Continuous variables with normal distribution were presented as mean ± SD. Student *t*-test and chi-square test were used to test the relationship between place of residence and vitamin D and calcium intake levels. The level of significance was set at $p < 0.05$ to determine the relationship.

Results

Participant characteristics

The study group comprised of 203 women above 28 weeks of pregnancy. Table 1 shows the socio-demographic and anthropometric data for the subjects. Ages varied from 16 to 45 years old, with a mean age of 29.96 ± 6.13 years. The mean gestational week was 31.77 ± 3.4 in the coastal area group and 31.90 ± 3.6 in the mountainous area group. Many of the subjects were high school graduates (47.3%), and more than 70% were housewives. Mean monthly income was Rp 3,179,000 ± 340,000, with 142 (70%) earning above the average minimum wage of Rp 1,800,000 and 61 (30%) earning below it. Most of the subjects (138) were multiparous (68%), with the remaining 65 (32%) being nulliparous.

Table 1: Socio-demographic and anthropometric data for participants (n = 203)

Variables		Value (%)	Mean ± SD
Socio-demographic data			
Age in years			29.96 ± 6.13
a.	< 20	5 (2.480)	
b.	20-29	92 (45.32)	
c.	30-34	53 (26.10)	
d.	≥35	53 (26.10)	
Parity			
a.	Nulliparous	65 (32)	
b.	Multiparous	138 (68)	
Working status			
a.	Housewife	143 (70.40)	
b.	Non-housewife	60 (29.60)	
Monthly income, Rp			3,179,000 ± 340,000
a.	≥ Minimum wages	142 (70)	
b.	< Minimum wages	61 (30)	
Education level			
a.	< High school	72 (35.40)	
b.	≥ High school	131 (64.60)	
Physical activity			7.252 ± 0.826
a.	Low	23 (11.30)	
b.	Medium	79 (38.90)	
c.	High	101 (49.80)	
Anthropometric data			
Height (cm)			152.74 ± 5.370
a.	≥ 145	194 (95.60)	
b.	< 145	9 (4.40)	
Pre-pregnancy weight (kg)			51.03 ± 9.380
Weight (kg)			61.05 ± 10.110
Pre-pregnancy BMI (kg/m^2)			21.85 ± 3.727
a.	Underweight (< 18.50)	37 (18.20)	
b.	Normal (18.50–22.99)	91 (44.80)	
c.	Pre-obese (23.00–24.99)	35 (17.20)	
		30 (14.80)	
d.	Obese I (25.00–29.99)	10 (4.90)	
e.	Obese II (≥ 30)		
Weight gain (kg)			10.01 ± 5.265
MUAC (cm)			26.93 ± 3.459
a.	Low (< 23.50)	27 (13.30)	
b.	Normal (23.50–25.00)	38 (18.70)	
c.	Obese (> 25.00)	138 (68)	
Average blood pressure			
a.	Systolic		115.60 ± 12.04
b.	Diastolic		74.52 ± 8.69

SD: standard deviation; BMI: body mass index; MUAC: mid-upper arm circumference.

Anthropometric characteristics

The means (SD) of height, weight, BMI, weight gain during pregnancy, mid-upper-arm circumference (MUAC) and systolic and diastolic blood pressures were 152.74 ± 5.370 cm, 61.05 ± 10.11 kg, 21.85 ± 3.727 kg/m^2, 10.01 ± 5.265 kg, 26.93 ± 3.459 cm, 115.60 ± 12.04 mmHg and 74.52 ± 8.69 mmHg, respectively. Based on MUAC measurements, 18.5% of the pregnant women were classified as underweight, 19.7% as normal and more than 60% as obese.

Dietary intake of pregnant women

The mean (± SD) values for the intake of vitamin D and calcium were 7.92 ± 5.26 mcg/day and 782.67 ± 408.84 mg/day, respectively. The mean daily energy intake for the pregnant women was 2,443.77 ± 479.996 kcal, 106.33 ± 30.789 g of protein, 109.56 ± 26.092 g of fat and 266.577 ± 57.977 g of carbohydrates. The percentages of RDAs intake of pregnant women are presented in Table 2.

Table 2: Dietary intake of subjects (n = 203)

Variables	Min-max	Mean ± SD	RDA*	%RDA
Energy (kcal)	1,577.97–3,484.23	2,443.77 ± 479.996	2,550	95.8
Total carbohydrate (g)	137.07–440.42	266.577 ± 57.977	363	73.2
Total protein (g)	54.37–198.52	106.33 ± 30.789	79	134
Total fat (g)	56.08–180.29	109.56 ± 26.092	85	128
Calcium (mg)	181.73–2,993.79	784.88 ± 409.77	1,300	60
Vitamin D (µg)	0.39–29.28	7.92 ± 5.26	15	52.8

RDA: recommended dietary allowance. *RDA adopted from Indonesia Ministry of Health [18].

Percentages of RDAs consumed were 60% for calcium, 52.8% for vitamin D, 95.8% for energy, 134% for protein, 128% for fat and 73.2% for carbohydrates. None of the pregnant women in the

study consumed vitamin D supplements during pregnancy.

Nutrient intake data were compared with RDAs for pregnant women. Energy, total protein and total fat intakes were adequate (≥ 77% of RDA) while calcium, vitamin D and total carbohydrate intake were inadequate (< 77% of RDA) [18]. This study found that more than half of the pregnant women had inadequate intakes of vitamin D (86.7%) and calcium (89.7%). We did not find any subjects took vitamin D as their dietary supplements during pregnancy. Means (± SD) of vitamin D and calcium intake were 7.92 ± 5.26 µg/day and 784.88 ± 409.77 mg/day, respectively.

Association between the place of residence and maternal intake of vitamin D and calcium

Comparison of mean dietary intakes of vitamin D and calcium in pregnant women based on place of residence is shown in Table 3. Maternal vitamin D intake compared to the place of residence is significantly different (p = 0.001), with the average of vitamin D intake being higher for those living in coastal areas than in mountainous areas. In contrast, although the average intake of calcium in the coastal areas is higher than in the mountainous areas, the difference, at p = 0.09, is not significant.

Table 3: Association between the place of residence and vitamin D and calcium intake (n = 203)

Variables	Place of residence		Odds ratio (95% CI)	P-value
	Coastal (%)	Mountainous (%)		
Vitamin D intake (µg)*	9.04 ± 5.92	6.55 ± 3.92		0.001
Vitamin D status**			0.306 (0.118–0.794)	0.02
Adequate (≥ 15 µg/day)	21 (18.80)	6 (6.60)		
Inadequate (< 15 µg/day)	91 (81.20)	85 (93.40)		
Calcium intake (mg)*	812.385 ± 434.840	751.043 ± 376.263		0.09
Calcium intake status **			0.583 (0.225–1.513)	0.37
Adequate (≥ 1,300 mg/day)	14 (12.50)	7 (7.70%)		
Inadequate (< 1,300 mg/day)	98 (87.50)	84 (92.30)		
Energy (kcal)*	2476.26 ± 490.63	2406.70 ± 467.69		0.329
Carbohydrate (g)*	297.86 ± 89.87	273.94 ± 76.93		0.046
Fat (g)*	119.90 ± 37.26	113.60 ± 30.42		0.195
Protein (g)*	118.03 ± 42.38	107.62 ± 30.87		0.044

Analysis of the relationship between place of residence and vitamin D and calcium intake found that 18.8% of the pregnant women in the coastal areas had adequate vitamin intake levels compared with only 6.6% in the mountainous areas. Thus, inadequate intake of vitamin D is more common in mountainous areas (93.4%) than in coastal areas (81.2%). Our study results showed that the intake of maternal vitamin D was significantly associated with place of residence (p = 0.02; OR: 0306; 95% CI: 0.118 to 0.794). In contrast, calcium intake and place of residence had no significant relationship (P = 0.37; OR: 0583; 95% CI: 0.225 to 1.513), but adequate intake of calcium is more common in coastal areas than in mountainous areas, at 12.5% and 7.7%, respectively.

Discussion

To our knowledge, this is the first study to investigate the relationship between place of residence and maternal vitamin D and calcium intake in the population of West Sumatra. The study found that the level of maternal vitamin D intake was associated with place of residence, in that women who lived in mountainous areas had less adequate vitamin D intake than those who lived in coastal areas. In contrast, we found no association between maternal calcium intake and place of residence, but we did find that women who lived in both locations had inadequate intake. High prevalence of low vitamin D and calcium intake need to be considered by health services because of the vital role of these nutrients in foetal growth and development. Our findings, if replicated in further studies, may have significant public-health implications for raising awareness about the need to fulfil nutritional needs during pregnancy, especially in terms of micronutrients such as vitamin D and calcium, to maintain a healthy pregnancy.

Vitamin D and calcium are essential nutrients for the human body throughout life [22]' and inadequate vitamin D intake may reduce calcium absorption in the intestine while low calcium intake can greatly increase vitamin D catabolism [23], [24]. This study has shown that an inadequate intake of vitamin D and calcium is a serious health problem for pregnant women in West Sumatra, Indonesia, that must be addressed. Based on the recommended level suggested by the Ministry of Health in Indonesia [18], almost 90% of pregnant women in our study had inadequate intakes of vitamin D and calcium. The standard recommendation for dietary allowances applied in Indonesia of 15 µg/day is the same as that used by the US Institute of Medicine [25].

The prevalence of inadequate intake of vitamin D and calcium found in this study is similar to the results of other recent research in Indonesia. A study conducted into healthy first-trimester pregnant women (n = 143) living in Jakarta showed that intake of vitamin D and calcium in pregnant women was below RDAs by 100% and 97.9%, with an average intake of vitamin D and calcium of 1.1 µg and 433.3 mg, respectively [3]. These average intakes of vitamin D and calcium were thus even lower than in this present study.

Two additional studies in women of childbearing age in North Sumatra and West Sumatra showed that more than 80% had inadequate vitamin D intake, with an average intake of vitamin D being between 5.24 and 7.29 µg/day [5], [26]. Another study reported that there was no significant association between the intake of vitamin D and body mass index in adult women aged 20 – 50 years [27]. A systematic review of three different studies into the nutritional status of pregnant women in Indonesia found that the average calcium intake of pregnant women was 45%

– 80% below the estimated average requirement (EAR). The average values for calcium intake in these studies were 536.23 mg, 614.41 mg and 360 mg, respectively [28].

Our findings indicate a significant difference between the average intake of vitamin D and calcium of pregnant women who live in coastal and of those living in mountainous areas. However, no significant differences were found in calcium intake between those living in these contrasting areas. Thus, only the status of vitamin D intake was found to differ significantly. Research conducted in North Sumatra has reported that vitamin D intake was significantly different between urban and rural groups, and these findings match those of the present study because they are similarly based on an environmental divided between coastal areas, which are urban, and mountainous areas, which are predominantly rural [29]. The effects of lifestyle choices can also be among several risk factors in determining the dietary intake of pregnant women. Pregnant women's choices related to physical activity, food habits, clothing style, occupation and the daily use of sunscreen can affect their quality of food intake and exposure to sunlight [29], [30].

Place of residence plays an important role in dietary health, in this case, because women who live in coastal areas tend to have better access to food sources rich in vitamin D such as fish or seafood than women who live in mountainous areas [29], [31], [32]. Also, a cardiovascular cohort study (MESA) conducted by Franco et al. at the Baltimore site of the Multi-Ethnic Study of Atherosclerosis showed that family economic status, as seen from the aspect of the average minimum wage, is associated with vitamin D intake. Reasons for this include difficulty in accessing healthy food sources, consumer buying power and the availability of food [33]. Women who live in low-income communities have less available income to purchase healthy foods because prices in their neighbourhoods exceed their incomes. The prices of food sources rich in vitamin D such as fish and dairy products tend to be especially high in such markets [34], [35]. This present study shows that pregnant women living in coastal areas have better consumption of vitamin D-rich foods than those in mountainous areas, but that their intake is still inadequate.

More than 80% of vitamin D is obtained through the synthesis in the skin from sunlight exposure, with the remaining 20% being obtained from the diet [23]; however, in this study, the frequency of sunlight exposure was not measured. The daily activities of Indonesian women feature limited exposure to sunlight due to the wearing of full-length dress and veils, sunscreen application and a tendency to avoid sunlight exposure during pregnancy. Changes in lifestyle, such as reducing outdoor activities, increasing the use of sheltered transportation and increased consumption of fast food, also reduce sunlight exposure [26]. A recent study among 160 third-trimester pregnant women in West Sumatra reported that living in mountainous areas and having low levels of physical activity were significantly associated with low vitamin D intake [5]. Very low intake of vitamin D contributes to the high prevalence of hypovitaminosis for vitamin D found in immigrant East Asian women living in Sydney [36]. In the study, subjects had 9.04 ± 5.92 mcg/day of vitamin D intake, and this is below the 15 mcg/day EAR [25]. Also, this study revealed an average calcium intake by women during pregnancy of 784.88 ± 409.77 mg/day, a level which is significantly below the RDA (60.37% of RDA).

Vitamin D intake is a predictor of circulating levels of 25OHD, and regular use of prenatal multivitamins increases vitamin D levels in pregnant women [37]. The role of vitamin D should not be underestimated: during the preconception period, vitamin D intake affects IVF success; during pregnancy, vitamin D is involved in the bone formation of the foetus through increasing absorption of calcium in the intestines and influencing systemic immune functions (adaptive and innate) vital to the function of the placenta and nephrogenesis; and in the perinatal period, vitamin D affects early life immunomodulation [38]. Also, our previous findings reported that maternal vitamin D intake was associated with blood pressure levels in the third trimester [1], and Shin et al. suggested that inadequate vitamin D intake during pregnancy is related to low neonatal birth weight and shorter infant height [39]. In light of these contributions to healthy foetal development and maternal health, additional intake of vitamin D from supplements may be important to meet the recommended dietary levels for pregnant women.

There are some limitations to this study. Firstly, we consider the number of subjects to be insufficient to represent vitamin D intake in the wider community, and this small sample size did not represent all of West Sumatra vitamin D and calcium food intake status. Secondly, the use of SQ-FFQ must be validated for measuring serum levels for vitamin D and calcium to ensure more accurate findings. Thirdly, we were not accounted for some confounding variables such as sunlight exposure, vitamin D from supplementation, and vitamin D-fortified foods which has an impact on maternal vitamin D status and vitamin D intake. Finally, the identified relationship may have been stronger if the study had been designed as a cohort or randomised clinical trial and a higher level of statistical analysis. In the future studies, the association between maternal vitamin D and calcium intake and pregnancy outcomes needs to be considered and involving more holistic approach which could be explaining clearer the association between vitamin D and calcium food intake status on pregnancy health outcomes.

In conclusion, low maternal vitamin D and calcium intake are common in West Sumatra,

Indonesia. Vitamin D and calcium intake may differ between those residents in mountainous and coastal areas. Adult women should understand the importance of vitamin D and calcium supplementation for meeting their daily needs and should be aware of proper food sources for these nutrients. Further large studies are required to confirm our findings before considering strategies for the implementation of vitamin D supplementation programs in Indonesia. Our results demonstrated that strategies for promoting the increase of vitamin D and calcium food sources and support for reducing a high prevalence of vitamin D deficiency status in the Indonesian population.

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Effect of Counteracting Lifestyle Barriers through Health Education in Egyptian Type 2 Diabetic Patients

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Abstract

BACKGROUND: Egypt is among the world top 10 countries in diabetes prevalence. It is the first country among the MENA region. Healthy lifestyle education and support help people with diabetes to improve health outcomes. Many physical and psychological barriers can hinder patients from following a healthy lifestyle.

AIM: This study aimed to examine the effect of lifestyle modification educational sessions in helping Egyptian patients to overcome main barriers of diabetes self-management through improving nutritional behaviours, physical activity, medication compliance, and blood glucose monitoring.

METHODS: A cohort study included 205 patients with type 2 diabetes. Baseline assessment of patients' lifestyle behaviours and barriers using personal diabetes questionnaire of Louisville University, with both anthropometric and blood glucose assessment. Interventional lifestyle health education was provided weekly through multiple integrated techniques, followed by a post-intervention assessment to evaluate the effect of the health education sessions. Statistical analysis was done to identify any statistically significant difference before and after the health education intervention.

RESULTS: There was a significant improvement of the post-education mean scores of the studied behaviours when compared with the pre-education scores of the participants' behaviours ($p < 0.001$). There was also a significant reduction in the barriers facing patients to diabetes self-management including nutritional barriers ($P < 0.001$), medication compliance barriers ($P < 0.001$) with a percent change (43%), physical activity barriers ($p < 0.001$), and blood glucose monitoring ($p < 0.001$) with a percent change (44%).There was a statistically significant positive correlation between improvement of medication compliance ($P = 0.027$), blood glucose monitoring($P = 0.045$), and glycated haemoglobin of the study participants

CONCLUSION: lifestyle modification education of type 2 diabetic patients can overcome the main barriers of following a healthy lifestyle and improve their anthropometric measures and blood glucose level.

Introduction

According to the International Diabetes Federation and the World Health Organization, Diabetes is recognised as a significant and growing health problem [1]. The global prevalence of diabetes among adults over 18 years of age has risen from 4.7% in 1980 to 8.5% in 2014. In 2013, 382 million adults were diagnosed with diabetes worldwide. This number is expected to grow to 592 million in 2035 [2]. In the MENA region (the Middle East & North Africa), about 40 million have diabetes (18-99 years) which is expected to be 84 million in 2045 [3]. Egypt is among the world top 10 countries with diabetes prevalence (15.6%). Furthermore, reports indicate that further 4.5 million patients are undiagnosed [2], [3]. In Egypt, annual cost analysis estimated that the economic burden of type 2 diabetes was $1.29 billion in 2010. This number excluded the cost associated with

prediabetes and the cost associated with loss of productivity. This figure will be doubled by the year 2030 adjusted for inflation [4]. Type 2 diabetes is the result of excess body weight and physical inactivity. Patients with type 2 diabetes constitute around 90%-95% of all diabetic patients worldwide which represent a growing epidemic [5].

Symptoms include polyuria (excessive excretion of urine), thirst (polydipsia), constant hunger, weight loss, vision changes and fatigue [5].

Diabetes is a major cause of many complications including; blindness, kidney failure, heart attacks, stroke and lower limb amputation. In 2012, an estimated 1.5 million deaths were directly caused by diabetes, and another 2.2 million deaths were attributable to high blood glucose. People with diabetes are at increased risk of macrovascular and microvascular complications, as well as early mortality. For instance, patients with diabetes are 2 to 4 times more likely to have fatal or nonfatal coronary events or a stroke. Almost 70%-80% of patients with T2D die from 1 of these two conditions. Almost half of all deaths attributable to high blood glucose occur before the age of 70 years. WHO projects that diabetes will be the 7th leading cause of death in 2030 [2]. Diabetes is a chronic disease that requires the patient to make a multitude of daily self-management decisions and perform complex care activities. Diabetes self-management education and support help people with diabetes to navigate these decisions and activities and has been shown to improve health outcomes and prevent complications. Diabetes self-management education is the process of facilitating the knowledge, skill, and ability necessary for diabetes self-care [6]. Different members of the health care team and community can contribute to this process, it is important for health care providers to have the knowledge, resources, and a systematic referral process to ensure that patients with type 2 diabetes consistently receive their care [6]. In Egypt, although patient education is part of the Ministry of Health (MOH) hospital accreditation scheme, it is not widely implemented or unsatisfactory implemented via brief, uninformative discussions. Moreover, diabetic patients often have difficulty in following healthy lifestyles due to many barriers such as irregular working hours, food cravings, or lack of motivation to exercise. Studies revealed that diabetic patients are also prone to forget their doctor’s advice or possibly ignore it after leaving the clinic. [7]. Easy, understandable, cultural-modified health education messages should be available to the Egyptian patients in good quality to promote their health outcome, prevent complications and counteract any barriers of lifestyle modification.

The objective of this study is to examine the effect of lifestyle modification educational sessions in helping Egyptian patients to overcome main barriers of diabetes self-management through improving nutritional behaviours, physical activity, medication compliance, and blood glucose monitoring.

Study design: A cohort study of Egyptian diabetic patients. Their lifestyle behaviours and barriers were assessed with both anthropometric and blood glucose assessment.

Inclusion criteria: Patients were enrolled if they were adults over 18 years and have type 2 diabetes, belonging to the low socioeconomic village under the study.

Exclusion criteria: Patients were excluded if they are taking insulin or had severe complications.

Study setting: Patients were recruited from low socioeconomic Egyptian village from January 2017 until September 2018 .

Sample Size Calculations

Using OpenEpi version 3 [8], two hundred and five adult (18-70 years) diabetic patients with an average age 52.6 years were randomly selected to achieve 95% confidence interval and 5% error with the following equation:

Sample size $n = [Np(1-p)] / [(d^2/Z^2_{1-\alpha/2}*(N-1) + p*(1-p)]$

N: Population size

p: prevalence in the population

d: Confidence limits

Taking into consideration that the total adult population is 5000. The prevalence of diabetes is 15.6% with a 5% drop out. The study was started including a total of 205 patients, but there were 8 patients' dropouts along the intervention period and follow up to be 197 patients at the end of the study .

Methods

Socio-demographic assessment: Updated validated a socioeconomic status scale for health research in Egypt [9] was used to assess age, sex, educational level, occupation for matching the socioeconomic data.

Assessment of nutritional behaviours, physical activity, proper medication use and level of blood glucose monitoring: Standard personal diabetes questionnaire of Louisville University with the standard scoring system [10] to assess three main parts including; the level of proper medication use and level of blood glucose monitoring.

Assessment of glycated haemoglobin and anthropometric measures were used to assess the effect of lifestyle education on the participants' health.

- Laboratory tools to measure the blood

glucose of the participants.

- Anthropometric tools: Calibrated SECA digital scale, HOLTEN stadiometer, a stretch-resistant tape was used to measure weight, height, waist and hip circumference.

Different motivational health education tools were used in this study, and the health education messages were delivered through multiple integrated techniques:

- Appropriate and culturally sensitive educational sessions.

- Written educational materials (diet plans, healthy nutrition booklet).

- Individualised health education sessions to every patient with patient-doctor consultation. Patients' family and friends were invited to attend these sessions. Problem-solving techniques were taught to help patients to solve their problems with lifestyle modification.

- Peer education: patients who perfectly followed a healthy lifestyle were invited to teach other patients in a simple, easy way.

- Group therapy: patients were encouraged to make small teamwork groups, walk together, ensure medication compliance of each other, and psychologically support each other.

- Community committee involvement in the health education sessions ensures the sustainability of the study.

Health education site: The health education was done in an equipped room with a data show, a microphone, and a sound magnification system .

Health education schedule: The patients were divided into groups. Each group received 48 sessions (one session per week for one year). Each patient selected a regular appointment for an individualised educational session.

Health education topics include the following items:

Contents were adapted from the American Diabetes Association recommendations [11].

Educational materials were translated, simplified, modified to adapt the Egyptian diabetic patients' culture and economic status. Health education materials were then finally reviewed and then pretested for easiness, clarity, and applicability. The main items for health education included:

- Diabetes: Definition, symptoms, types, causes, complications, and lines of treatment.

- Healthy nutrition for diabetics: The importance of diet regulation in ameliorating symptoms, and preventing complications: including healthy eating principles, and how to put it into practice recommended quantity, quality, and frequency of meals. Diet was prescribed based upon energy requirements, physical activity, and type of anti-hyperglycemic medication.

- Medication compliance: Patients were motivated to take their medication as prescribed regularly by a specialist doctor in diabetes as a part of their self-management strategy. The messages discussed the most common types of oral hypoglycemic drugs, their action in diabetes, their side effects, how to overcome these side effects.

- Blood glucose monitoring: including the importance of blood glucose monitoring compliance, types of blood glucose tests, and target levels.

- Physical activity as a part of treatment: benefits, how much, and what sort of exercise should be incorporated, and maintained. A minimum level of regular walking for 30 minutes at least five days a week was advised.

- Psychological support: how to deal with stress, and anxiety, problem-solving techniques.

- Barriers of lifestyle modification: what are the main barriers of the participants, available solutions to overcome them, how to select the most suitable solution to each patient according to his surroundings.

- Hypoglycemia, Hyperglycemia, Diabetic ketoacidosis: Causes, symptoms, and proper management.

- General health care: for the long-term complications of diabetes on health.

- Foot care: the importance of foot care, and tips to avoid diabetic foot.

- Kidney care: how to avoid diabetic nephropathy, and the importance of urine testing for microalbuminuria once a year.

- Eye care: Frequency of recommended fundus examination.

Health education package was divided so that the patient received one package each visit. The next visit included reinforcement of the previous health education package in addition to the provision of the new package. Reinforcement was performed in the form of questions to assure retention of knowledge each visit. Questions on the new health education package were also asked as a method of the baseline assessment for the patient's knowledge about the new information covered by the next package. The following visits also covered the same questions to assess retention of knowledge, and to detect changes in attitude and practice.

Data analysis

After data cleaning, the statistical package of social sciences (SPSS-18) was used for data

processing [12]. Descriptive statistics were done for data summarisation in the form of frequency, and percentage for qualitative variables, and means ± SD for quantitative variables. Scoring of the patients' answers was done by using standard scoring of Louisville personal diabetes questionnaire scoring [10]. Inferential statistics were done for comparing the participant groups using Mcnemar test for qualitative variables and paired t-test for parametric quantitative data. With p-value < 0.05 considered significant, and p-value < 0.01 considered highly significant.

Ethical consideration: Informed consents were obtained from all participants. Approval of the research protocol was taken from the ethical committee of the National Research Centre. The research was conducted according to the World Medical Association Declaration of Helsinki [13].

Ethical approval: The Research and Ethical Committee of NRC cleared the study protocol. The number of ethical approvals was 16466

Informed consent: It was obtained from the parents enrolled in the study Confidentiality: Mothers and children were identified by a serial number, and the information at the individual level was kept strictly confidential.

Results

The present study showed that the socio-demographic characteristics of the study population were as follows: The mean age of the study participants was 52.6 years old. About 46.7% of them were men, and 53.2% were women. The majority of the participants were married (80.2%) (Table1).

Table 1: Sociodemographic characteristics of the study participants

Character	Mean	SD
Age	52.61	10.63
Crowdness Index*	1.44	0.75
Sex	Frequency	Percent (%)
Male	92	46.71
Female	105	53.29
Total	197	100
Marital status		
Single	2	1.01
Married	158	80.22
Widow	32	16.24
Divorced	5	2.53
Total	197	100.0
Education		
Illiterate	80	40.63
Read & write	25	12.69
Primary	17	8.62
Preparatory	16	8.12
Secondary	34	17.25
University	25	12.69
Total	197	100.0
Occupation		
Doesn't work	100	50.76
Unskilled manual worker	15	7.63
Skilled manual worker/farmer	30	15.22
Have trades/ business	8	4.06
Semi-professional/ clerk	25	12.69
Professional	19	9.64
Total	197	100.0
Economic status		
Doesn't meet necessary expenses (in debt)	67	34.03
Only meet necessary expenses	114	57.86
Meet necessary expenses &emergencies	13	6.59
Meet necessary expenses &able to save/ invest money	3	1.52
Total	197	100.0

* Crowdedness index: number of individuals living in the house divided by the number of rooms.

There was an overall significant improvement in medication compliance among the study participants (P < 0.001) with a mean pre-education score (2.1 ± 1.5) versus post-education mean score (5 ± 0.15). More than 90% of the study participants seek the doctor for only medication prescription and not for health education.

There was an overall significant reduction in barriers of medication non-compliance (< 0.001) with a percent change (43%) in post-education assessment compared to pre-education assessment (Figure 1). The reduction included absence of family support (percent change 57.8%), expensive drugs (11.5%), feeling anxious or stressed (70.8%), being busy (47.5%), feeling discouraged due to lack of results (61.8%), being away from home (25.8%), with the least barrier in reduction was unpleasant side effects with only 1.4% percent change.

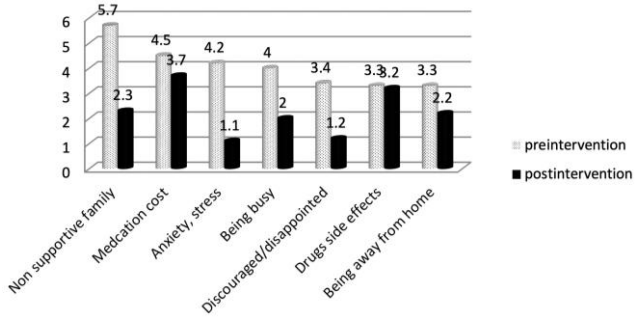

Figure 1: Comparison of pre-intervention and post-intervention score of barriers to medication compliance among the participants

There was a major significant improvement in blood glucose monitoring (p < 0.001). There was an overall significant reduction in barriers of blood glucose monitoring (< 0.001) with a percent change (44%) in post-education assessment compared to pre-education assessment (Figure 2). The reduction includes absence of family support (percent change 67.5%), expensive drugs (15.6%), feeling anxious or stressed (71.7%), being busy (38.2%), feeling discouraged due to lack of results (46.2%), with the least two barriers in reduction were painful test (23.8%), and expensive test with only 15.6% percent change**.**

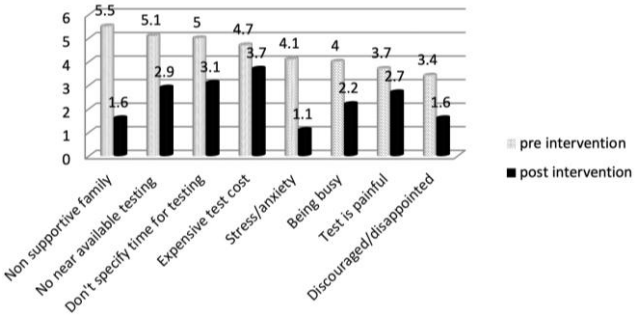

Figure 2: Comparison between pre-education and post-education score of barriers of blood glucose monitoring among the participants

There was an overall statistically significant improvement in the healthy nutritional behaviours' score (P < 0.001) in post-education assessment compared to pre-education assessment. The behaviour improvement included: increase using information about calories, carbohydrates, fat, using a diet plan, and resist the temptation to eat unhealthy food.

There was an overall statistically significant reduction in barriers of healthy nutritional behaviours' score (P < 0.001) in post-education assessment compared to pre-education assessment (Figure 3).

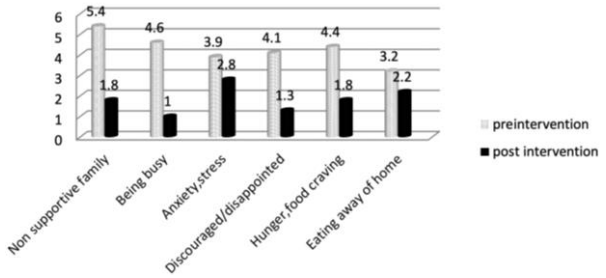

Figure 3: Comparison of pre-education and post-education score of barriers of healthy nutritional behaviours among the participants

There was a statistically significant increase in walking and bicycling among the participants (Table 2).

Table 2: Comparison between pre-education and post-education physical activities among the participants

Activity	Pre-education		Post education		P
	Yes	No	Yes	No	
Walking	103 (52.28%)	94 (47.72%)	190 (96.44%)	7 (3.56%)	< 0.001
Bicycling	5 (2.53%)	192 (97.46%)	20 (10.15%)	177 (89.84)	< 0.01
Going to youth sports center	9 (4.88%)	188 (95.12%)	17 (8.62%)	180 (91.37%)	0.17

There was an overall statistically significant reduction in barriers to physical activity (Figure 4).

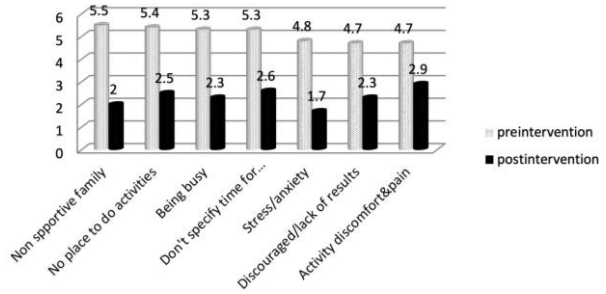

Figure 4: Comparison of Pre education and Post education Score of Physical Activity Barriers among the Study Participants

There was a statistically significant improvement after the lifestyle educational education; the improvement included glycated haemoglobin (HBA1C) of the patients, weight, waist, hip circumference, Waist/Hip Ratio, and BMI of the patients (Table 3).

Table 3: Comparison between pre-education and post-education indicators (anthropometric measures and HBA1C) among the participants

Variable	Pre education		Post education		P	Percent change (%)
	Mean	SD	Mean	SD		
HBA1C	11.33	2.02	8.45	2.46	< 0.001	25.04
Weight	94.28	14.87	79.23	17.38	< 0.001	13.82
Waist circumference	115.58	12.59	103.26	21.05	< 0.001	9.03
Hip circumference	118.56	13.96	102.30	21.58	< 0.001	12.05
Waist/Hip ratio	1.02	0.19	0.97	0.07	< 0.001	5.49
BMI	35.13	6.69	30.35	7.32	< 0.001	11.44

There was a statistically significant positive correlation between improvement of medication compliance, actual blood glucose monitoring, and glycated haemoglobin of the study participants (Table 4).

Table 4: Correlation between improvement percent of the studied behaviours and HBA1C improvement percent among the Study Participants

		Healthy nutritional behaviours	Medication compliance	blood glucose monitoring	Physical activity
HBA1C	Correlation coefficient	0.155	0.158	0.143	0.537
	P value	0.030	0.027	0.045	< 0.001

NB: Improvement percent of HBA1C means reduction in its estimated level from basal assessment

Discussion

Shared care defined as care for patients with a chronic condition provided in cooperation between primary and secondary healthcare has been promoted and developed to reduce complications [14]. It was found that 70% of diabetic patients spend 15 minutes or less with their health care providers and more than 90% of office visits of diabetic patients are delivered by health care providers without special training in diabetes management [15].

Research showed that patient-doctor communication is essential for patient compliance and satisfaction with care [16]. In the current study; however, the role of the village physicians in modifying patient's lifestyle was deficient; their role in prescribing the diabetes medication made the majority of the current study participants seek the doctor for medication prescription which made them feel secure .

Proper compliance to medication refers to the degree or extent of conformity to the recommendations about day-to-day treatment by the provider concerning the timing, dosage, and frequency [17]. There was a significant improvement in medication compliance which is consistent with Ward study [18].

The barriers are defined as the perception of individuals toward the levels of how challenging is the diverse obstacles to the accomplishment of specific behaviour [19]. To manage diabetes effectively, individuals must utilise knowledge and decision-

making skills in the context of barriers [20]. There was an overall significant reduction in barriers of medication compliance which is consistent with an American study aimed to assess the impact of medication adherence on diabetes control in Type 2 diabetic patients with the results showed significant improvement among the education group [21].

The barriers in the current study were more prominent than Cheng study which done among type 2 diabetic Chinese patients [22]. This difference may be due to lower accessibility to qualified health care service in the current study, unavailability of a diabetes monitoring program which emphasises on the importance of medication compliance and the fact that many of the study participants had no health insurance coverage.

In the current study, the best barrier in overcoming was anxiety with a significant reduction in medication noncompliance due to anxiety or stress which reflected the good effect of the psychological support delivered to the participants through problem-solving and group therapy sessions. The least barrier in overcoming was drugs' side effects due to the resistance of the participants to change their medication or add some drugs to reduce the side effects.

The main obstacle to blood glucose monitoring in Egypt is the relatively poor structure and the process of care in government hospitals and primary health care units, as measurement of glycated haemoglobin, blood glucose level, and microalbuminuria is not performed routinely for all patients [23]. Incorporating these processes into the health system and training health care providers in the areas of information, education, and communication should be a vital cornerstone in the control of diabetes in Egypt [24].

In the current study, there was a major significant improvement in blood glucose monitoring after lifestyle educational sessions. This was similar to the results of a Korean study which was conducted to test the effects of diabetic education on blood glucose control among type 2 diabetic patients. The results indicated that patient education improved blood glucose monitoring [25].

As indicated by the prominent theories and extensive reviews, the perceived barriers are the strongest predictors of health behaviours [26], [27], [28]. Situational barriers, which arise from one's situation in life, can directly or indirectly impede self-management activities [29].

The current study showed that there was an overall significant reduction in barriers of blood glucose monitoring in post-education assessment compared to pre-education assessment. The reduction included barriers such as the absence of family support, expensive drugs, feeling anxious or stressed, being busy, feeling discouraged due to lack of results, painful test and the least barrier in overcoming was the expensive cost of the test.

The results showed higher barriers in the current study than the Cheng study. This difference may be due to the bad socioeconomic status in the current study compared to the participants in Cheng study; they had no extra money to test their blood glucose, they considered it as a luxury among their difficult socioeconomic status.

Regarding the healthy nutritional behaviours such as using information about calories, carbohydrates and fat, there was a statistically significant improvement in mean total post-education score of the healthy nutritional behaviours. The results of the current study showed that the mean total pre-education score was slightly lower than Cheng study which showed that mean total score [19]. This difference may be because the food labels which enables the patients to know information about the food ingredients are uncommon in Egypt especially in the village under study.

Following a healthy diet plan help the patient to manage their diabetes, Ward study assessed the mean score of using a diet plan among the participants after health education sessions [18]. Its score is slightly lower than the score in the current study, which may be due to the condensed multiple integrated nutritional education in this study which provided a healthy diet plan to each participant [30].

With the help of the educational sessions in the current study, the nutritional barriers showed an overall reduction which is consistent with an English study aimed to assess the impact of the nutritional education on diabetes control [31].

There was an overall statistically significant improvement in physical activities as walking and bicycling, which is consistent with an Iranian study showed statistically significant improvement of glycemic control among the education group after lifestyle education [32].

There was no statistically significant improvement in going to the sports centre as the rural culture of the current study participants made it a shame for women and older men to go to the youth sports centre.

The results showed higher barriers in the current study than Cheng study [19]. This difference may be due to the lower degree of education among the current study participants as many of the participants are illiterate who had little idea about the importance of physical activity for control of diabetes.

In the current study, the hardest barrier to overcome was the pain and discomfort associated with physical exercise. This may be due to unavailability of qualified trainers to teach the diabetic patients how to exercise safely, and due to the presence of co-morbidities in diabetic patients which

made exercise painful such as early-onset osteoarthritis of the knee, and peripheral neuropathy.

According to the WHO, nearly 70% of adults in Egypt are overweight or obese [33], which is consistent with the current study. There was a statistically significant reduction in the weight and the BMI of the participants after the health education sessions. This improvement may be because health education was afforded to the patients with different ways which make the nutritional information easy and understandable to all the participants.

The health education tools included group sessions, doctor-patient consultation, and written materials. The results of the current study are consistent with the Look ahead study, which proved that intensive lifestyle education for one year resulted in a statistically significant reduction in weight after health education [34].

Glycated haemoglobin (HBA1C) is an indicator that reflects the average plasma glucose level over the past 2 to 3 months. The HBA1C test is relatively stable and has less variability [35]. There was a statistically significant reduction in the post-education measurement than the pre-education assessment which is similar to the results found in an education study carried out on 122 diabetic patients attending diabetes outpatient clinic in Zagazig university, with a statistically significant improvement was found in their levels of glycated hemoglobin (HBA1C) after application of educational messages [36].

In the current study, there was a statistically significant positive correlation (P = 0.03) between improvement of HBA1C (reduction) and improvement of healthy nutritional behaviours. This is consistent with an Australian cohort study which stated that greater healthful food intake reduces HbA1C concentration [37]. This may be because of the effect of nutritional education supporting high fibre low glycemic diet in decreasing glycated haemoglobin and decreasing degree of obesity.

In the current study, there was a statistically significant positive correlation between improvement percent of HBA1C and improvement of medication compliance. This result is consistent with a cross-sectional study conducted in three Malaysian public health clinics, which reported that medication adherence was associated with an improved level of HbA1C [38]. This may be because medication compliance allows the diabetic patient to benefit from its active substances.

The results of the current study are consistent with the Diamond study, which revealed that continuous blood glucose monitoring effectively lowers glycated haemoglobin (HbA1C) in diabetic patients [39]. In the current study, there was a significant positive correlation between blood glucose monitoring and HBA1C which may be due to the alarming effect of health education about continuous blood glucose monitoring which results in more glycemic control.

There was also consistency in the current study with the results of a systematic review study which revealed the effectiveness of physical activity in reducing HbA1c levels [40]. This may be due to the effect of physical activity in reducing insulin resistance.

In the current study, the maximum impact of lifestyle modification education on the glycated haemoglobin was due to the improvement of physical activities, followed by medication compliance, healthy nutritional behaviours then blood glucose monitoring. These results may be due to that physical activity is the most cost-effective lifestyle modification with no specific time, place, or cost. Meanwhile, the other lifestyle modifications need a multifactorial approach to change them.

For example, nutritional modification needs an available budget to buy healthy food, available market, and accessibility to that market. Medication compliance needs the availability of medication cost, accessibility of drugs, and a good memory to remember the time of administration. Blood glucose monitoring also needs accessibility, availability of the test and its kits which is to some extent difficult in the village.

In conclusion, lifestyle education of type 2 diabetes patients counteract the barriers against following healthy nutrition, regular physical activity, medication compliance, and blood glucose monitoring behaviours which appear in the post-education assessment. It is also beneficial in improving their health status, blood glucose level, and body mass index. Health education should be an integral part of diabetes management and should be included in the Standards of Practice (SOP) of diabetes care to be implemented at all levels of health care in Egypt.

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Knowledge and Attitude Regarding Sleep Medicine among Medical Students at Qassim University, Saudi Arabia

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Abstract

BACKGROUNDS: Sleep disorders and sleep medicine are underrecognized by both the general public and health care workers. Lack of education and training in sleep medicine has resulted in a culture of physicians who have very limited knowledge about sleep disorders and, as a result, are likely to underdiagnose and under-treat patients.

AIM: This study aimed to assess the knowledge of and attitude regarding sleep medicine among medical students at Qassim University.

METHODS: This was a cross-sectional study of 4th and 5th-year medical students, conducted at Qassim University (Central and Unaizah branches), Saudi Arabia. We used a self-administered data collection tool to collect personal information (age, name, sex, medical school), and assessed general attitude toward sleep medicine and the students' current knowledge about sleep medicine using the Assessment of Sleep Knowledge in Medical Education (ASKME) survey.

RESULTS: The prevalence of medical students who had a special interest in sleep medicine was 23.3%. Poor knowledge about sleep medicine was detected in 94.8% of students, while good knowledge was observed in only 5.2%. The attitude of the students toward sleep medicine was negative among 40.5% and positive among 59.5%. University branches, gender, and preferred speciality were all significantly associated with attitude score, whereas interest in sleep medicine and knowledge of sleep disorders were associated with both knowledge and attitude scores.

CONCLUSION: This study found that medical students' knowledge of sleep medicine was very low, despite the majority of them having a positive attitude toward it.

Introduction

Sleep is known to influence the physical and emotional wellbeing of adolescents by exerting substantial control over biological and psychosocial processes [1]. Sleep not only impacts physical growth and emotional development but also influences cognitive function and learning [2], [3]. Despite the magnitude and clinical importance of sleep issues, several studies have documented poor recognition of sleep disorders [3], [4].

In Saudi Arabia, the first certification exam for sleep medicine as an independent speciality was approved in 2009 [5], and sleep medicine was recognised as an independent speciality in 2012 [6], though studies investigating the prevalence of sleep disorders in the Kingdom of Saudi Arabia (KSA) are limited. Based on available data and waitlists for participation in sleep studies [7], however, it appears that sleep disorders are prevalent among Saudis. Using the Berlin questionnaire to assess the prevalence of obstructive sleep apnea (OSA) risk and symptoms among middle-aged Saudi men and women in their primary care setting, it was revealed that 3 out of 10 Saudi men and 4 out of 10 Saudi women are at a high risk of OSA [8], [9]. A recent national survey quantitatively assessed sleep medicine service in the KSA [7], revealing that the field is nascent compared to developed countries. The survey identified nine sleep disorder facilities; seven

were defined as sleep disorder centres that provide clinical diagnostics and therapeutic services for patients with different sleep disorders, and two were defined as sleep laboratories that provide diagnostic and therapeutic services limited to sleep-related breathing disorders such as OSA [7], [10].

Sleep disorders and sleep medicine as a speciality are underrecognized by both the general public and health care workers. Members of the public in the KSA accept information on sleep disorders from any source without criticism, even if the information is not validated; therefore, they have developed their ideas and myths about sleep. Some of the public believe that sleep problems are natural and do not think of them as legitimate medical issues that can be treated [11]. Most patients seen in the clinic have gone to many doctors before visiting the sleep disorders clinic, thinking that all sleep problems are handled by mental health therapists, particularly psychiatrists. Due to the under-recognition of the seriousness of disorders like OSA, patient compliance with Continuous Positive Airway Pressure (CPAP) in the KSA is low compared to developed countries [12].

Similarly, the knowledge of practising physicians, particularly primary care physicians, about sleep disorders is limited [13]. Salem et al. reported that only 33.6% of the primary care physicians assessed knew sleep medicine [14]. A survey of primary health care (PHC) physicians in all primary care centres in Riyadh revealed that PHC physicians do not completely recognise the importance and impact of OSA and other sleep disorders [13]. Forty-three per cent of the participants did not realise the existence of sleep medicine as a speciality; 40% felt that sleep disorders are not common, and 38% did not know to whom they should refer their patients [13]. In general, medical students in the KSA rarely have a chance to learn sleep medicine in medical schools.

Similarly, postgraduate teaching of sleep disorders during residency training seems to be limited as well [12]. This lack of education and training in sleep medicine has resulted in a culture of physicians who have very limited knowledge about sleep disorders and, as a result, are likely to underdiagnose and under-treat patients [12]. A study published in Riyadh, KSA documented that only 27.7% of the medical students evaluated using the Assessment of Sleep Knowledge in Medical Education (ASKME) survey displayed awareness of sleep medicine [15].

The health system in the KSA relies on a referral system, where the patient's first exposure is usually to a PHC physician, who assesses and decides the patient's management plan. Thus, early detection and treatment of patients with sleep disorders depend, considerably, on the knowledge and awareness of PHC physicians. PHC physicians have limited knowledge about sleep disorders, sleep disorders among their patients are likely under-recognised, and patients with these disorders may be inaccurately diagnosed and may receive inappropriate treatment [16]. Studies in the KSA and Western countries have shown that OSA is common among patients attending PHC clinics [8], [9], [17]. With the limited number of sleep medicine specialists in the KSA, it is impractical to expect that they will be able to be the primary caregivers for all patients with sleep disorders. Therefore, an alliance of sleep medicine specialists, PHC physicians, and general physicians (including those in internal medicine and paediatrics) becomes essential. Educational interventions are effective in increasing the rates of recognition of OSA among PHC physicians [18], though the level of knowledge and attitude regarding sleep medicine among medical students of the Qassim region is still unknown.

Hence, the present study aimed to determine the knowledge of and attitude towards sleep medicine among medical students at Qassim University, KSA.

Methods

Selection and description of participants

This was a cross-sectional study based on a survey conducted at two medical branches of Qassim University in Saudi Arabia. The study population consisted of 4th and 5th-year medical students in the two medical colleges of Qassim University (main campus and Unaizah campus). Random number generator software was used to randomly select participants from a list of all upper-year medical students to reach the required sample size to ensure adequate statistical power. Inclusion criteria were 4th and 5th-year medical students. Exclusion criteria were 1st, 2nd, and 3rd-year medical students as well as those whose data was incomplete.

Sample size calculation

Based on the article published by Almohaya et al. [15], the prevalence of medical students who were aware of sleep medicine was 27.7%. This prevalence was used to determine the required sample size using the categorical formula: $n = z^2(pq)/e^2$, where

- n = sample size
- z = score from z distribution associated with confidence level (1.96 for 95% confidence)
- p = estimated proportion of the event in the population
- q = 100 – p
- e = margin of error (0.05)

The sample size required to detect a

statistically significant result with 95% confidence and a narrow margin of error of 0.05, should be 158. Since Qassim's main campus has a bigger population, about 70% of the calculated sample size was recruited from the main campus; 30% were recruited from Unaizah campus.

Ethical review and confidentiality

The study was approved by the Regional Research Ethics Committee, registered at the National Committee of Bio & Med. Ethics [NCBE] (Registration No. H-04-Q-001). All information is kept strictly confidential and used only for research purposes.

Data collection

A self-administered questionnaire collected demographic data of the participants (age, name, sex, medical school) and their attitude toward sleep medicine [19], [20]; knowledge about sleep medicine was assessed using the Assessment of Sleep Knowledge in Medical Education (ASKME) survey [19]. The ASKME survey is a 30-item questionnaire regarding the knowledge of sleep medicine. The questionnaire was previously tested in a pilot study of 10 medical students to identify the optimal time for questionnaire completion by participants. The ASKME survey has face validity, and the statistical analysis for internal consistency revealed a Cronbach's alpha value of 76%, which indicates a moderately good internal consistency.

Items were presented in a "true," "false" or "I don't know" format. For the ease of analysis, the knowledge questionnaire was re-coded into two categories where the correct answer for each question was identified and coded with 1, whereas the wrong answer was coded with 0. The total knowledge score was obtained by summing up the 30 questions. The minimum score was 0 and the maximum score was 30; a higher score signifies greater knowledge, and by using the cutoff points of 18/30 (60% of the total score), participants were classified as having poor knowledge if the score range was from 0-17 points, whereas good knowledge was determined if the score range was from 18-30 points.

Attitude toward sleep medicine was assessed using the 10-question attitude questionnaire adapted from ASKME [19], [20]. This questionnaire is scored on a 5-point Likert scale ranging from 1 (strongly disagree) to 5 (strongly agree). The lowest possible score was 10, and the highest score was 50 points. Attitude scores were obtained by taking the sum of the scores of the ten questions. We then categorised participants who scored 30 (60% of the total score) or more as having a positive attitude and those whose score was less than 30 were categorized as having a negative attitude.

Statistics

Statistical Package for Social Sciences (SPSS) version 21 (Armonk, NY: IBM Corp.) was used for all data analyses. Descriptive statistics are presented as numbers and percentages for all qualitative variables, while the mean ± standard deviation is presented for all quantitative variables. Knowledge and attitude scores among sociodemographic characteristics of participants were compared using independent t-tests (two-tailed). A p-value cut off point of 0.05 at 95% CI was deemed statistically significant. The normality of the distribution of data was assessed using Shapiro Wilk and Kolmogorov-Smirnov tests. Data violating assumptions of normality were instead compared using non-parametric tests.

Results

A total of 158 questionnaires were distributed randomly among the students (4^{th} and 5^{th} years); 116 were returned (response rate of 73.4%). Table 1 presents the sociodemographic characteristics of students who were involved in this study. Students' ages ranged from 22 to 27 years; a majority was of a younger age (22 - 23 years age group). The majority of the students were recruited from the Qassim main campus (62.1%), and the rest were from the Unaizah branch (37.9%); most respondents were male (56.9% vs 43.1% female).

Table 1: Sociodemographic characteristics of respondents (n=116)

Study Variables	N (%)
University branch	
• Unaizah branch	44 (37.9%)
• Main campus branch	72 (62.1%)
Age group	
• 22 – 23 years	76 (65.5%)
• 24 – 27 years	40 (34.5%)
Year of study	
• 4^{th} year	65 (56.0%)
• 5^{th} year	51 (44.0%)
Gender	
• Male	66 (56.9%)
• Female	50 (43.1%)
GPA	
• 2.4 – 4.0	64 (55.2%)
• 4.1 – 5.0	52 (44.8%)
Preferred speciality	
• Medicine	42 (36.2%)
• Surgery	47 (40.5%)
• Others	27 (23.3%)
Specific interest in sleep medicine	
• Yes	27 (23.3%)
• No	89 (76.7%)
Importance of sleep medicine	
• Absolutely not important	03 (02.6%)
• Not important	13 (11.2%)
• Average	48 (41.4%)
• Important	41 (35.3%)
• Very important	11 (09.5%)

A high proportion of students were in their 4th-year level (56%), and most had a 4.0 or lower grade point average (GPA) (55.2%) (the highest GPA in our institution was 5.0). Based on the preferred area of specialisation, 40.5% of students preferred surgery, 36.2% preferred medicine, and the rest expressed mixed speciality interests. Most of them had no interest in sleep medicine (76.7%), with 41.4% having an average knowledge of sleep disorders.

Table 2: General knowledge toward sleep medicine (n=116)

Knowledge statement	Correct answer N (%)
K1. The need for sleep decreases in persons above 50 years of age.	54 (46.6%)
K2. Melatonin is a natural body hormone that typically increases during nighttime hours.	87 (75.0%)
K3. Dream sleep (REM) occurs more in the second half of the night.	41 (35.3%)
K4. Sleeping longer on weekends is recommended as a regular practice to make up for the loss of sleep during the workweek.	34 (29.3%)
K5. Newborn infants spend about 16-18 hours per 24-hour period sleeping.	94 (81.0%)
K6. Incidence of insomnia is twice as common in older men as in older women.	20 (17.2%)
K7. A young (pre-adolescent) child who regularly has trouble getting to sleep at night should be allowed to sleep later in the morning.	45 (38.8%)
K8. The typical age of symptom onset for narcolepsy is 40 years or older.	25 (21.6%)
K9. The ability to sleep increases in persons above 50 years of age.	39 (33.6%)
K10. Slow-wave sleep is more prominent in the second half of the night.	17 (14.7%)
K11. The amount of slow-wave sleep increases in persons above 50 years of age.	13 (11.2%)
K12. Episodes of sleepwalking tend to occur in the last third of the night.	13 (11.2%)
K13. Episodes of REM sleep tend to lengthen throughout the night.	28 (24.1%)
K14. Periodic limb movements during sleep are typically decreased in REM sleep.	29 (25.0%)
K15. Hyperactivity in children can be exacerbated by inadequate sleep.	49 (42.2%)
K16. In alcoholics in recovery, sleep normalises within one month of alcohol abstention.	12 (10.3%)
K17. Daytime napping is recommended for patients with difficulty initiating sleep.	31 (26.7%)
K18. Weight loss is often indicated in the treatment of primary snoring or mild OSA.	70 (60.3%)
K19. Slow-wave sleep is enhanced following daytime exercise.	30 (25.9%)
K20. Chronic bedwetting in children responds to treatment with anticholinergic drugs.	11 (09.5%)
K21. Nightmares are more common within the first two hours of sleep.	22 (19.0%)
K22. Heart rate, respiration, and blood pressure are more variable during REM sleep compared to non-REM sleep.	53 (45.7%)
K23. Antihypertensive drugs may cause sleeping difficulties as a side effect.	27 (23.3%)
K24. Early morning awakenings in the elderly are often associated with changes in the timing of their biological rhythms.	39 (33.6%)
K25. Alcohol can be beneficial in reducing the effects of jet lag.	24 (20.7%)
K26. Night shift workers are more likely to fall asleep on the job compared to employees with regular, daytime hours.	56 (48.3%)
K27. Episodes of sleepwalking commonly occur during REM sleep.	12 (10.3%)
K28. Menopausal women are at higher risk for developing symptoms of sleep apnea compared to pre-menopausal women.	29 (25.0%)
K29. Irregular sleep scheduling can increase the incidence of sleepwalking in children.	43 (37.1%)
K30. Symptoms of narcolepsy are related to seizure activity in the brain.	15 (12.9%)

Results of the ASKME questionnaires are shown in Table 2**,** which also indicates the correct answer for each question asked. Based on students' responses, the most commonly agreed with statements were: "newborn infants spend about 16-18 hours per 24-hour period sleeping", "melatonin is a natural body hormone that typically increases during nighttime hours", and "weight loss is often indicated in the treatment of primary snoring or mild OSA". Students exhibited the least knowledge concerning the statement, "chronic bedwetting in children responds to treatment with anticholinergic drugs."

Details of the general attitude of medical students toward sleep medicine are shown in Figure 1**;** nearly all students strongly agreed/agreed with the statement, "asking about symptoms of sleep disturbances is an essential component of a comprehensive medical evaluation", whereas most strongly disagreed/disagreed with the statement, "compared to other medical problems, sleep disturbances are usually less important for a patient's health"

Details of the general attitude of medical students toward sleep medicine are displayed in Figure 1. The results showed that nearly all respondents either agreed or strongly agreed with the statement, "Asking about symptoms of sleep disturbances is an essential component of a comprehensive medical evaluation", whereas most students correctly disagreed with the following statements: "Compared to other medical problems, sleep disturbances are usually less important for a patient's health?", "Sleep disorders are individual problems that are not important for a wide population", and "Inadequate sleep is a lifestyle issue, not a medical problem".

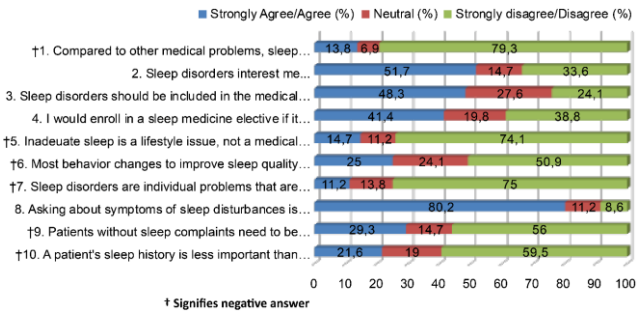

Figure 1: General attitude of the students toward sleep medicine

Table 3 shows the characteristics of the students' knowledge and attitude toward sleep medicine. The mean knowledge score was 9.2 (± 4.9). The prevalence of poor knowledge in this study was high (94.8%) as opposed to good knowledge (5.2%). The mean attitude score was 37.9 (± 7.2) out of a possible total score of 50. Of these students, 40.5% viewed it with a negative attitude, while 59.5% viewed it with a positive attitude.

Table 3: Knowledge and attitude toward sleep medicine (n = 116)

Predictor variable	N (%)
Level of knowledge	
• Poor	110 (94.8%)
• Good	6 (5.2%)
Attitude	
• Negative	47 (40.5%)
• Positive	69 (59.5%)
	Mean ± SD
Knowledge score (mean ± SD)	9.2 ± 4.9
Attitude total score (mean ± SD)	37.9 ± 7.2

When comparing the knowledge and attitude scores among sociodemographic characteristics of participants, we found that the attitude of the students in Unaizah branch was significantly higher compared to the main campus branch (T = 2.622, p = 0.010), where female students had a better attitude compared to males (T = -2.867, p = 0.005). The attitude of those students expressing interest in a non-internal medicine speciality was higher compared to those students with internal medicine speciality (T = -2.491, p = 0.014). Additionally, those students with interest in sleep medicine had better knowledge (T = 2.098, p = 0.038) and better attitude (T= 2.585, p = 0.011), and those who responded that knowledge of sleep disorders was important had significantly higher levels of current knowledge (T= -2.800, p = 0.006) and more positive attitudes (T = -3.209, p = 0.002) (Table 4).

Table 4: Comparison between knowledge and attitude scores among sociodemographic characteristics of students (n=116)

Factor	Knowledge Score Total Score (/30)		Attitude Score Total Score (/50)	
	Mean ± SD	*T* test; P-value §	Mean ± SD	*T* test; P-value §
University branches				
Unaizah branch	08.6 ± 04.0	-1.015; 0.312	40.1 ± 06.9	2.622; 0.010 **
Main campus branch	09.5 ± 05.3		36.6 ± 07.0	
Age group				
22 – 23 years	08.9 ± 04.9	-0.512; 0.610	38.1 ± 06.9	0.450; 0.654
24 – 27 years	09.5 ± 04.9		37.5 ± 07.8	
Year of study				
4th Year	08.5 ± 04.2	-1.589; 0.115	37.9 ± 06.7	-0.062; 0.950
5th Year	09.9 ± 05.5		37.9 ± 07.8	
Gender				
Male	09.3 ± 04.8	0.259; 0.796	36.3 ± 07.1	-2.867; 0.005 **
Female	09.0 ± 05.0		40.0 ± 06.8	
Grade point average (GPA)				
2.4 – 4.0	08.8 ± 04.5	-0.879; 0.381	38.2 ± 06.7	0.403; 0.688
4.1 – 5.0	09.6 ± 05.3		37.6 ± 07.8	
Preferred speciality				
Medicine	08.6 ± 05.1	-0.933; 0.353	35.8 ± 08.0	-2.491; 0.014 **
Non-Medicine	09.5 ± 04.8		39.1 ± 06.4	
Interest in sleep medicine				
Yes	10.9 ± 04.8	2.098; 0.038 **	40.9 ± 07.8	2.585; 0.011 **
No	08.6 ± 04.8		36.9 ± 06.8	
Importance of sleep disorders				
Not important	08.0 ± 04.4	-2.800; 0.006 **	36.1 ± 06.8	-3.209; 0.002 **
Important	10.5 ± 05.1		40.2 ± 07.0	

** Significant at p ≤ 0.05.

Discussion

The purpose of the present study was to assess the knowledge and attitude regarding sleep medicine among medical students at Qassim University. This study revealed that the medical students' knowledge toward sleep medicine was relatively poor (94.8%), highlighting the need for sleep medicine to be added to the medical school's curriculum, which could be beneficial and will improve their knowledge of the subject. Our finding is consistent with a previous study conducted by Almohaya and colleagues [15]. Using the ASKME questionnaire, respondents were identified as having poor (score of < 60%) or good (score of ≥ 60%) knowledge of sleep issues. Among 348 recruited students in their study, the mean score was 10.4 (± 4.4), with more than 80 per cent of them being classified as having poor knowledge. In Croatia [21], among 112 respondents surveyed, poor knowledge of sleep issues was identified in most of the medical students, postgraduate physicians and specialists, which corroborates our results. In another study published locally (in the KSA), Saleem et al. [14] surveyed 88 primary care centres comprising 223 primary care physicians (PCP). They reported that the mean knowledge of PCP based on the ASKME questionnaire was 14.4 (± 4). The majority of PCPs obtained a score between 11 and 20. These scores were higher than we observed, though we only investigated medical students, not physicians. In Egypt, Zaki et al. assessed the knowledge of final year medical students and house-officers about normal sleep and sleep disorders [22], also using the ASKME questionnaire. They reported that 91 per cent of the participants exhibited a low level of knowledge, which was also in agreement with our results. However, in China, Luo and associates reported poor knowledge about sleep in more than 60 per cent of students, and the majority of them were not aware of any medical school offering sleep medicine-related courses [23].

In this study, less than half of the students expressed a negative attitude; more than 60% viewed sleep science in a positive light. The mean score was 37.9 out of 50 points. In Singapore, Mahendran et al. reported the mean attitude of the medical students was 35 (±4.3) [24]. This result was comparable to our study outcome. However, they measured the attitude of the students by Medical Education (MED) sleep survey. Also, Kovaèiæ et al. reported that the majority of respondents exhibited positive attitudes toward sleep medicine, which was consistent with our study results [21]. We also discovered that even though Unaizah branch has fewer students, they demonstrated a significantly better attitude compared to students from the main campus branch. Females exhibited a better attitude than males, and those who preferred non-medicine specialities also had a more positive attitude toward sleep medicine. We also

found out that those with interest in and those who believed knowledge of sleep medicine was important had significantly greater levels of knowledge and more positive attitudes toward the subject. Various studies have reported negative associations between knowledge and attitude based on sociodemographic factors [14], [15], [21], [22], [24]. Salem et al. [14] observed that participants who attended lectures about medicine scored significantly better for the level of knowledge, but that knowledge had no relation to gender or the number of years of practice, whereas Almohaya and colleagues reported that the knowledge score did not differ by sex, GPA, level of academic achievement, or university attended [15]. A study from Egypt [22] reported that the knowledge score did significantly differ based on faculty location and gender, but no significant difference was observed based on years of study. In Singapore [24], researchers found no significant effect of age, gender, or medical qualifications on knowledge of basic sleep medicine, whereas, in Croatia, significant differences were found between the level of knowledge, but the attitude toward sleep medicine did not differ [21].

Our study demonstrates that one out of four students was interested in sleep medicine. This finding is by papers published locally [14], [15] Saleem et al. [14] reported more than 30 percent of the PCPs were interested in the subject, as did Almohaya et al. [15]. However, in China, more than 80 percent of medical students have demonstrated a strong interest in coursework related to sleep medicine, though most of them were not aware of any medical schools teaching the subject [23].

It is recommended that replication of this study be carried out with larger sample size, involving multiple institutions to better assess the knowledge and attitude of medical students toward sleep medicine in a broader, more generalizable context.

In summary, medical student knowledge regarding sleep medicine was very low, even though a majority of students expressed a positive attitude toward it. In this regard, faculty members such as lecturers, professors, and other related teaching staff should provide better sleep medicine education to increase their awareness. Although this subject might not be popular in the Saudi medical school, it is expected that better education on this subject will be part of the curriculum within the foreseeable future.

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Occupational Burnout and Its Related Factors Among Iranian Nurses: A Cross-Sectional Study in Shahroud, Northeast of Iran

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Abstract

BACKGROUND: Good physical and mental health of employees is one of the major characteristics of a healthy organisation.

AIM: Due to the importance of occupational burnout and its potentially negative consequences, the aim of this study was to assess occupational burnout and its related factors among Iranian nurses.

METHODS: In this cross-sectional study, 205 nurses who were working in two educational hospitals affiliated with Shahroud University of Medical Sciences were included. Forty-two of the nurses were male, and 163 were female (mean age: 31 years). Maslach Occupational Burnout Inventory, demographic and job characteristics questionnaires were completed for each nurse. Questionnaires were then collected, and data were analysed statistically by SPSS, version 22.

RESULTS: In the assessment of occupational burnout, the highest level was observed in emotional exhaustion and then in personal performance. There was a significant difference between the frequency of nurses' emotional exhaustion domain in terms of gender ($P < 0.05$), so that, women suffered more from emotional exhaustion. There was a significant difference between the frequencies of nurses' performance in terms of marital status ($P < 0.05$) so that married people had more personal performance disorder.

CONCLUSION: The results of this study showed that nurses suffered from low occupational burnout. Female gender, sleeping disorders, awakening at night and employment in stressful wards such as the emergency ward was associated with a higher level of occupational burnout in nurses.

Introduction

Employees' physical and mental health is one of the major characteristics of a healthy organisation. In a healthy society, the responsibility of manufacturing organizations is not limited to producing as much as profitable goods and services, and the managers of the organizations of such communities know that more production is the result and outcome of effective management; this important issue is not obtained without the attention and believing in the employees' mental health [1], [2], [3]. So, one of the duties of every competent, forward-looking and resourceful manager is to provide the mental health of the employees in the organisation. Therefore, mental health in the workplace means the prevention of psychological distresses and behavioural disorders in employees due to the pathogenic factors in the workplace; making the mental work environment and space healthy is also very important in the workplaces [4], [5], [6], [7].

One of the concepts that in recent years has attracted the attention of psychologists to itself is being exhausted, disability, lethargy, weakness, immobility in employed individuals, so-called occupational burnout [8], [9], [10]. Occupational burnout is a phenomenon in which the cumulative effects of workplace stress gradually make individuals desperate, forcing them to withdraw mentally [11], [12]. Occupational burnout is a common syndrome in occupations that most of their time is spent on supporting others and has been widely studied among the employees of medical professions [13], [14], [15], [16]. Occupational burnout has been associated with specific reports of decreased ability in paying attention

to the patients. Psychological distress harms professional satisfaction feeling and also the quality of patient care [17], [18], [19], [20]. Personal, interpersonal, and organisational factors have a relationship with occupational tension and burnout [21], [22], [23].

It has previously been shown that occupational burnout is common among nurses. However, the intensity of occupational burnout is varied among nurses working in various fields. The comparison made between various wards of the hospital, including the operating room, pediatric, gynaecology and surgery wards indicate the impact of the environment on the occupational burnout [24], [25], [26]. Nurses are more prone to occupational burnout progress, and its reason is mainly the nature of the job and their emotional demand [27], [28], [29]. As occupational burnout develops in response to the chronic emotional stress, it disrupts the nurses' relationship with patients, colleagues, family, and the social environment [30], [31]. Also, occupational burnout is closely related to the work absence of nurses and abandoning nursing job which ultimately leads to the decreased attention and cares for patients [32], [33]. Due to importance of occupational burnout and available controversies in the literature in this regard among nurses, as well as lack of sufficient evidence among nurses in northeast Iran, the present study has been conducted aiming to assess occupational burnout and its related factors among the nurses who worked in Northeast Iran.

Material and Methods

In a cross-sectional study, a total of 205 nurses who were working in two educational hospitals affiliated with Shahroud University of Medical Sciences (Imam Hossein and Bahar hospitals) in 2017, were included. Forty-two of the nurses were male, and 163 were female (mean age: 31 years). After obtaining informed consent, Maslach Occupational Burnout Inventory, demographic and job characteristics questionnaires were completed for each nurse. The questionnaires were completed by the nurse him/herself, and preferably when it did not disrupt the work of the nurse and also when the nurse was not psychologically and mentally tired.

Maslach Occupational Burnout Inventory is the most common tool to assess occupational burnout among various people with professional and career backgrounds has been used [34]. The validity and reliability of this questionnaire have been confirmed in previous studies [35], [36]. This questionnaire has 25 questions and is especially applied for measuring occupational burnout follow-up in professional groups, such as nurses. This questionnaire has three main scales of emotional exhaustion (9 questions), depersonalization (5 questions), personal performance (8 questions), and a subtest called involvement (3 questions). Subjects are asked to read each sentence and describe themselves in front of it concerning the frequency of the characteristic`s proposed and also its intensity in the desired sentence. Two points are assigned for a person for scoring the scale in each question, the frequency score and the intensity score. Each individual gets a score from 1 to 6 in frequency and a score from 1 to 7 in intensity. Finally, according to the questions of each subtest, the scores of each subtest are calculated separately, and the mean of scores is obtained [34], [35], [36]. The data were statistically analysed using SPSS, version 22. Mann Whitney U test and independent-sample t-test were used to compare the quantitative variables in the two groups. Significance level was considered at $p < 0.05$.

Results

In this study, 147 (71.7%) of nurses were married, and 58 (28.3%) were single. The educational level of 186 (90.7%) of the nurses was a bachelor, and 19 people (9.3%) had mastered. The employment status of 67 nurses (32.7%) was formal, 76 people (37.1%) had treaty status, 48 people (23.4%) had project status, 6 people (2.9%) had contractual status, and 8 people (3.9%) had another status. The time duration of nurses' work experience was between 1 - 31 years with the mean and standard deviation of 6.76 ± 5.77 years. The time duration of nurses' working experience in the emergency ward was between 1 to 20 years (mean: 2.97 ± 2.78 years). The nurses' working hours in a day were between 6-12 hours (mean: 7.19 ± 1.2 hours). The mean time of nurses' working hours at night was 10.8 ± 2.37 hours.194 (94.6%) of the nurses had a rotating shift, and 163 (79.5%) of the nurses had the physical activity of more than half an hour in a day.

The sleeping time duration of nurses was between 3-12 hours (mean: 6.48 ± 1.48 hours). The desired sleeping time duration of nurses was between 5-14 hours (mean: 8.51 ± 1.56 hours). In 159 (77.5%) of the nurses, the usual hour of beginning to sleep at night was between 10 PM and 1 AM. In 158 (77%) of the nurses, the usual wake-up time in the morning was between 5 AM to 8 AM. The length of time nurses fall asleep was between 3 to 120 minutes (mean: 30.41 ± 19.58 minutes). 155 (75.6%) of the nurses had an afternoon nap. Nurses' sleeping time duration during the weekend and holidays was between 5-12 hours (mean: 8.17 ± 1.71 hours). Nurses' satisfaction with their recent sleep pattern was as follow: 35 (17.1%) were very dissatisfied, 62 (30.2%) were dissatisfied, 73 (35.6%) were somewhat satisfied, 23 (11.2%) were highly satisfied, and 12 (5.9%) were

very satisfied. The interference of nurses' sleeping difficulties with their daily activities was as follows: 47 (22.9%) had rarely, 76 (37.1%) had somewhat, 51 (24.9%) had high, 25 (12.2%) had very high and the rest, 6 (2.9%), did not have difficulties. Dimensions of nurses' burnout have been described in Table 1.

Table 1: Descriptive Characteristics of Frequency and Intensity of Four Dimensions of Nurses' Occupational Burnout

Occupational Burnout Dimensions	Scale	Minimum	Maximum	Median	Mean	Standard Deviation
Emotional Exhaustion	Frequency	0	55	19	20.9	12.74
	Intensity	0	70	23	23.6	14.66
Personal Performance	Frequency	3	86	31	30.57	10.07
	Intensity	6	56	33	33.24	10.63
Depersonalization	Frequency	0	30	6	8.14	6.69
	Intensity	0	35	7	9.14	7.48
Involvement	Frequency	0	18	5	6.02	3.9
	Intensity	0	21	7	6.92	4.28

According to Table 2, except for gender and employment status in emotional exhaustion, marital status, working hours at night and number of waking up while sleeping during the night in personal performance, and work experience duration in ED in depersonalization and usual hour of beginning sleep at night, number of waking up while sleeping during the night and time duration of being awake during the night in involvement dimension ($P < 0.05$).

Table 2: Descriptive characteristics and comparison of nurses' occupational burnout frequency in terms of demographic characteristics and sleeping status variables

Variables		Occupational Burnout Domain			
		Emotional Exhaustion	Personal Performance	Depersonalization	Involvement
Age	30 >	20.52 ± 13.38	29.81 ± 11.42	7.4 ± 5.85	5.61 ± 3.62
	≥ 30	21.29 ± 12.09	31.35 ± 8.46	8.9 ± 7.42	6.44 ± 4.14
	P-Value	0.447	0.22	0.267	0.153
Gender	Male	15.57 ± 10.87	31.54 ± 13.26	8.47 ± 6.95	6.73 ± 4.21
	Female	22.28 ± 12.85	30.32 ± 9.11	8.05 ± 6.65	5.84 ± 3.81
	P-Value	0.001	0.713	0.742	0.217
Marital Status	Married	20.89 ± 12.68	31.48 ± 10.66	8.14 ± 6.8	5.82 ± 4.09
	Single	20.93 ± 12.98	28.27 ± 8.05	8.13 ± 6.47	6.53 ± 3.35
	P-Value	0.968	0.02	0.995	0.163
Educational Level	Bachelor	20.54 ± 12.54	30.5 ± 10.2	8.15 ± 6.7	6.01 ± 3.92
	Master	24.47 ± 14.41	31.31 ± 8.9	8.05 ± 6.8	6.15 ± 3.74
	P-Value	0.234	0.803	0.938	0.777
Employment Status	Formal	22.05 ± 12.73	30.95 ± 9.05	8.42 ± 6.98	5.85 ± 3.98
	Contractual & others	18.25 ± 12.46	29.69 ± 12.16	7.48 ± 6	6.41 ± 3.71
	P-Value	0.04	0.21	0.513	0.294
Work Experience Duration	< 5	20.35 ± 13.27	29.9 ± 10.19	6.98 ± 5.77	5.54 ± 3.69
	≥ 5	12.32 ± 21.35	21.35 ± 12.32	9.07 ± 7.25	6.41 ± 4.04
	P-Value	0.477	0.596	0.053	0.124
Work Experience Duration in ED	≤ 2	21.77 ± 12.64	30.32 ± 10.93	6.7 ± 5.88	5.46 ± 3.6
	> 2	19.7 ± 12.84	30.91 ± 8.8	10.12 ± 7.26	6.8 ± 4.17
	P-Value	0.181	0.758	0.001	0.027
Working Hours in the Day	< 7	22.23 ± 13.13	32.16 ± 10.91	8.59 ± 7.26	5.92 ± 6.42
	≥ 7	20.26 ± 12.54	29.8 ± 9.59	7.92 ± 6.42	6.07 ± 3.9
	P-Value	0.297	0.304	0.683	0.842
Working Hours at Night	< 12	19.24 ± 10.79	28.39 ± 8.14	9.11 ± 6.68	7.7 ± 3.8
	≥ 12	21.61 ± 13.45	31.5 ± 10.68	7.72 ± 6.68	5.31 ± 3.73
	P-Value	0.355	0.018	0.123	0.001 >
Rotating Shift	Has	21.15 ± 12.88	30.7 ± 10.29	8.08 ± 6.79	5.94 ± 3.9
	Does Not Have	16.45 ± 9.19	28.36 ± 4.8	9.18 ± 4.89	7.36 ± 3.88
	P-Value	0.24	0.376	0.259	0.23
Physical Activity	Has	21.12 ± 12.71	30.96 ± 10.1	7.84 ± 6.52	5.87 ± 3.89
	Does Not Have	23.95 ± 12.51	29.07 ± 9.95	9.3 ± 7.29	6.61 ± 3.93
	P-Value	0.065	0.421	0.266	0.32
Sleeping Time Duration	< 7	22.11 ± 14.18	30.89 ± 9.24	7.79 ± 6.89	5.98 ± 3.67
	≥ 7	19.92 ± 11.4	30.31 ± 10.74	8.42 ± 6.55	6.05 ± 4.09
	P-Value	0.584	0.441	0.314	0.961
Desired Sleeping Time Duration	< 9	20.89 ± 12.93	31.03 ± 9.14	7.98 ± 6.92	6.08 ± 3.78
	≥ 9	20.92 ± 12.51	29.86 ± 11.4	8.38 ± 6.36	5.93 ± 4.1
	P-Value	0.816	0.242	0.504	0.789
Usual Hour of Beginning Sleep at Night	< 12	19.43 ± 11.76	28.8 ± 9.21	9.16 ± 6.89	7.31 ± 3.92
	≥ 12	21.51 ± 13.11	31.31 ± 10.35	7.71 ± 6.59	5.48 ± 3.77
	P-Value	0.341	0.152	0.14	0.003
Usual Hour of Beginning Sleep in the Morning	< 7	19.55 ± 11.41	31.24 ± 10.46	8.07 ± 6.64	6.33 ± 3.81
	≥ 7	22.67 ± 14.15	29.7 ± 9.54	8.22 ± 6.8	5.61 ± 3.99
	P-Value	0.214	0.298	0.959	0.174
Number of Waking up while Sleeping During the Night	< 2	21.4 ± 13.05	31.99 ± 10.5	7.62 ± 6.98	5.44 ± 4.06
	≥ 2	20.13 ± 12.27	28.36 ± 9	8.95 ± 6.18	6.92 ± 3.46
	P-Value	0.451	0.009	0.052	0.003
Length of time to Fall Sleep	< 25	19.23 ± 11.64	31.6 ± 9.02	8.02 ± 6.89	6.03 ± 3.96
	≥ 25	21.93 ± 13.31	29.94 ± 10.66	8.21 ± 6.59	6.01 ± 3.88
	P-Value	0.238	0.213	0.813	0.847
Time Duration of Being Awake during the Night	< 15	21.99 ± 13.67	32.15 ± 10.81	7.73 ± 6.94	5.42 ± 3.9
	≥ 15	19.62 ± 11.47	28.71 ± 8.82	8.61 ± 6.39	6.73 ± 3.8
	P-Value	0.257	0.015	0.201	0.008

There was no significant difference between the frequency of all the nurses' occupational burnout dimensions in terms of age, level of education, working experience duration, working hours per day, working shift, physical activity, sleeping time duration, desired sleeping time duration, usual waking-up o'clock in the morning and time duration to fall sleep ($P > 0.05$).

Table 3: Descriptive Characteristics and Comparison of the Nurses' Occupational Burnout Intensity in Terms of Demographic Characteristics and Sleeping Status Variables

Characteristic		Occupational Burnout Domains			
		Emotional Exhaustion	Personal Performance	Depersonalization	Involvement
Age	30 >	23.87 ± 15.75	32.75 ± 11	8.38 ± 6.43	6.44 ± 4.07
	≥ 30	23.31 ± 13.52	33.76 ± 10.25	9.93 ± 8.38	7.41 ± 4.45
	P-Value	0.909	0.682	0.372	0.077
Gender	Male	17.64 ± 12.94	33.16 ± 11.24	9.88 ± 7.5	7.66 ± 4.66
	Female	25.13 ± 14.73	33.26 ± 10.5	8.95 ± 7.48	6.73 ± 4.16
	P-Value	0.002	0.946	0.349	0.3
Marital Status	Married	23.34 ± 13.26	33.97 ± 11.1	9.02 ± 7.41	6.72 ± 4.44
	Single	24.25 ± 15.96	31.41 ± 9.17	9.44 ± 7.71	7.41 ± 3.83
	P-Value	0.893	0.1	0.834	0.288
Educational Level	Bachelor	23.36 ± 14.5	33.22 ± 10.56	9.09 ± 7.25	6.93 ± 4.28
	Master	25.94 ± 16.46	33.52 ± 11.58	9.68 ± 9.67	6.78 ± 4.35
	P-Value	0.579	0.979	0.759	0.945
Employment Status	Formal	25.31 ± 14.69	33.85 ± 10.21	9.48 ± 7.89	6.89 ± 4.32
	Contractual &Others	19.64 ± 13.93	31.85 ± 11.49	8.35 ± 6.42	6.98 ± 4.2
	P-Value	0.007	0.335	0.507	0.962
Work Experience Duration	< 5	23.28 ± 15.79	33.03 ± 11.54	7.81 ± 6.16	6.18 ± 3.98
	≥ 5	23.85 ± 13.75	33.42 ± 9.87	10.23 ± 8.27	7.52 ± 4.43
	P-Value	0.537	0.902	0.065	0.022
Work Experience Duration in the Emergency Ward	≤ 2	25.21 ± 14.99	33.25 ± 11.3	7.62 ± 6.48	6.31 ± 4.17
	> 2	21.37 ± 13.98	33.24 ± 9.68	11.18 ± 8.28	7.76 ± 4.29
	P-Value	0.071	0.664	0.002	0.014
Working Hours in the Day	< 7	26.05 ± 15.75	34.59 ± 9.98	9.47 ± 8.58	6.62 ± 4.14
	≥ 7	22.4 ± 14.01	32.59 ± 10.91	8.98 ± 6.91	7.06 ± 4.35
	P-Value	0.099	0.242	0.789	0.616
Working Hours at Night	< 12	19.06 ± 10.99	30.18 ± 10.51	9.68 ± 6.74	8.18 ± 3.97
	≥ 12	25.52 ± 15.61	34.54 ± 10.44	8.91 ± 7.78	6.38 ± 4.3
	P-Value	0.007	0.002	0.196	0.001
Rotating Shift	Has	24.12 ± 14.76	33.51 ± 10.75	9.09 ± 7.63	6.86 ± 4.29
	Does Not Have	14.36 ± 9.03	28.54 ± 6.93	10 ± 4.12	7.9 ± 4.06
	P-Value	0.24	0.376	0.259	0.23
Physical Activity	Has	22.28 ± 14.55	33.75 ± 10.58	8.68 ± 7.21	6.71 ± 4.23
	Does Not Have	28.69 ± 14.15	31.28 ± 10.71	10.95 ± 8.26	7.73 ± 4.39
	P-Value	0.008	0.219	0.108	0.324
Sleeping Time Duration	< 7	25.98 ± 16.37	34.67 ± 9.73	8.86 ± 8.03	6.98 ± 3.93
	≥ 7	21.65 ± 12.86	32.08 ± 11.22	9.37 ± 7.02	6.86 ± 4.56
	P-Value	0.108	0.074	0.276	0.716
Desired Sleeping Time Duration	< 9	24.48 ± 15.22	34.08 ± 9.81	9.14 ± 7.87	7.23 ± 4.12
	≥ 9	22.22 ± 13.72	31.95 ± 11.73	9.15 ± 6.87	6.43 ± 4.49
	P-Value	0.395	0.132	0.583	0.172
Usual Hour of Beginning Sleep at Night	< 12	21.05 ± 14.27	30.65 ± 10.78	10.58 ± 7.11	8.33 ± 4.52
	≥ 12	24.65 ± 14.74	34.32 ± 10.41	8.55 ± 7.56	6.33 ± 4.04
	P-Value	0.061	0.016	0.031	0.007
Usual Hour of Beginning Sleep in the Morning	< 7	21.77 ± 13.32	33.69 ± 10.93	9.12 ± 7.23	7.4 ± 4.37
	≥ 7	25.97 ± 16.02	32.66 ± 10.25	9.16 ± 7.83	6.29 ± 4.09
	P-Value	0.084	0.482	0.755	0.092
Number of Waking up while Sleeping During the Night	< 2	23.74 ± 14.21	34.36 ± 10.79	8.2 ± 7.45	6.21 ± 4.24
	≥ 2	23.37 ± 15.44	31.5 ± 10.2	10.61 ± 7.32	8.02 ± 4.11
	P-Value	0.537	0.025	0.006	0.003
Length of time to Fall Sleep	< 25	21.64 ± 13.7	34.88 ± 10.17	9.05 ± 7.46	6.91 ± 4.22
	≥ 25	24.8 ± 15.15	32.24 ± 10.81	9.2 ± 7.52	6.92 ± 4.33
	P-Value	0.161	0.095	0.966	0.798
Time Duration of Being Awake during the Night	< 15	25.56 ± 15.49	34.93 ± 10.31	8.82 ± 8.12	6.42 ± 4.37
	≥ 15	21.27 ± 13.33	31.25 ± 10.71	9.52 ± 6.66	7.51 ± 4.11
	P-Value	0.048	0.007	0.183	0.031

Based on Table 3, there was no significant difference between all dimensions of nurses' occupational burnout intensity in terms of age, marital status, educational level, working hours per day, working shift, sleeping time duration, desired sleeping time duration, usual waking-up o'clock in the morning, and the time duration to fall sleep ($P > 0.05$), except for time duration of being awake during the night,

number of waking up while sleeping during the night, usual hour of beginning sleep at night and working hours at night ($P < 0.05$).

Discussion

The results of the present study indicate that most Iranian nurses in the northeast of Iran experienced low occupational burnout; with the highest disorder in personal performance and then in the emotional exhaustion dimensions. Also, it has been revealed that different factors had a significant effect on domains of occupational burnout in nurses. Among these cases was nurses' gender, that the male nurses experienced less emotional exhaustion than females. In line with the results of our study, it has been shown that female healthcare providers experienced more frequency and intensity of burnout compared to male healthcare providers [37]. Another study in Sweden showed that in general working population, women had a higher level of burnout compared to men [38] that is consistent with the results of our study. The higher burnout in females compared to males may be due to their gender properties, situational life factors, unsuitable working conditions and the male's higher resistance and resilience in the workplace [39], [40]. Therefore, developing a gender-specific program for reducing nurses' occupational burnout is reasonable.

In the dimension of personal performance, married people had more disorder than single people, which can be attributed to more responsibilities and concerns of the married people. The results of a meta-analytic study indicate that single or divorced nurses experience a higher level of burnout compared to married nurses. The lower burnout level in married nurses may be due to the support and security provided by their family which can consequently protect them from a negative attitude towards their work environment and colleagues [41]. In other words, spousal and family support can be protective and mitigate some of the adverse effects posed by work-life conflict in nurses. The formal nurses suffered from more emotional exhaustion that might be reasonable due to more responsibility and higher age and higher work experience. Nurses with more working experience in the ED suffered significantly more from involvement and depersonalization, which was justifiable regarding very high working stress in the emergency and the unexpected arrival of patients in the emergency ward [42], [43]. Working at night in the emergency ward was among the important disrupting factors in the area of personal performance and involvement, in a way that people with more working at night had more interference, which was justifiable regarding the previous studies and the disruption of sleeping cycle [44], [45]. The highest impact of physical activity has been on the emotional exhaustion in a way that those who were exercising regularly suffered less from emotional exhaustion. The results of a recent meta-analysis do not support the efficacy of exercise therapy in managing the symptoms of burnout; although exercise therapy had some positive impacts. Lack of high-quality studies has been suggested as a possible reason for this result [46]. Nevertheless, another systematic review confirmed the efficacy of yoga in managing burnout in healthcare providers [47]. Further well-designed studies are required to evaluate the effect of exercise on the burnout of nurses.

Regarding the hour of sleeping, those nurses who fell sleep late suffered more from involvement in their hospitalised patients' care during the day to reach a feeling of competence and achievement in one's work. Nurses who did not have good sleep quality and woke up at night often suffered more from depersonalization and involvement, which was a justifiable point. Considering similar studies conducted on different occupational burnout individuals and groups, this study can be compared with the study, in which the emergency medical assistants of Iran were included. According to the conducted study, among the indicators investigated about the causes of occupational burnout, the most important factors investigated in the causes of occupational burnout have been residency year, gender, age, marital status, having child, study location city, other sources of income, doing overtime work, supervisory status, choice of course, chronic illness, sedative drug consumption, doing regular exercise, sense of humor, religiousness, flexibility, and hope for job prospect that the causes of hope for job prospect and having chronic illness and interest in the field and having the source of income and the age of individuals have been the factors affecting more burnout in the residents of emergency medicine field. Based on the above points, it was concluded that the lack of hope for a job prospect, having a chronic illness, the lack of interest in the field of study and the lack of income resources and high age during the residency period has led to more occupational burnout [48]. In our study, the effect of nurses' age on the ratio of occupational burnout and other factors such as job prospect and chronic illness, and so on has not been calculated. Unlike our study, in another study, no significant relationship has been observed between gender and occupational burnout. Moreover, contrary to our study, a strong, significant and direct relationship has been found between the age of individuals and their ratio of burnout, so that older people suffered from higher burnout than younger people, which was in contrast with our study that the age has not had any effect on any of the burnout indicators [49], [50].

In conclusion, according to the present study, it can be concluded that the Iranian nurses in the northeast of Iran suffer from a low level of

occupational burnout. However, female gender, sleeping disorders, night awakening and working in stressful wards such as ED are associated with a higher level of occupational burnout in nurses.

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Public Health

Prevalence of Depression and Its Associated Risk Factors among Young Adult Patients Attending the Primary Health Centers in Tabuk, Saudi Arabia

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Keywords: Depression; Primary health centres Tabuk

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Abstract

BACKGROUND: The number of young depressive patients approaching the primary health care centres in Saudi Arabia for supportive care and treatment has enormously increased, but more cases of depression are not being diagnosed properly at the primary health care level.

AIM: To assess the prevalence and associated factors of depression among young adult patients attending the primary health centres in Tabuk, Kingdom of Saudi Arabia.

METHODS: A cross-sectional study was done in selected primary health care centres in Tabuk city from March 2018 to June 2018. Patient Health Questionnaire- PHQ-2 and PHQ-9 and a modified questionnaire were used to collect the necessary information and data were analysed by using SPSS (Version 25.0, SPSS Inc. Chicago, IL, USA).

RESULTS: The study included 384 patients aged between 20 and 40 years. Most of them (76.6%) were females. The prevalence of depression was 74%; mild among 37.8%, moderate among 20.8% whereas moderately severe to severe among 15.4% of them. Multivariate logistic regression analysis revealed that married patients were at lower risk for depression (Adjusted odds ratio "AOR" was 0.36, 95% confidence interval "CI" was 0.20-0.93), $p < 0.001$. Patients who reported a lack of social support were more likely to be depressed than those with social support (AOR = 2.05, 95% CI = 1.03-4.07), $p = 0.041$. Patients who reported disturbed marriage were at almost four-folded risk of depression compared to those without disturbed marriage (AOR = 3.50, 95% CI = 1.23-9.98), $p = 0.019$. Patients with financial problems were at almost double risk for developing depression compared to those without financial problems (AOR = 2.37, 95% CI = 1.16-4.85), $p = 0.019$. Those with stressful experience were significantly more likely to have depression compared to those without stressful experience (AOR = 4.75, 95% CI = 2.58-8.71), $p < 0.001$. Opposed to patients without a family history of depression, those with such history were at higher significant risk for depression (AOR = 2.75, 95% CI = 1.23-6.14), $p = 0.014$. Also, patients who reported sleep disorders were at nearly double folds of having depression compared to those without such disorders (AOR = 2.24, 95% CI = 1.16-4.30), $p = 0.016$.

CONCLUSION: Depression among young adult patients (20-40 years) attending primary healthcare centres in Tabuk is very high. However, it is mostly mild to moderate in its severity. Suicidal thoughts were reported by one-tenth of the participants. Some predictors for depression among them were identified.

Introduction

Mental illnesses are one of the major public health issues across the world, which affects a hundred millions of people. The concepts of westernisation, socialisation and globalisation tremendously altered the lifestyle of the population across the globe and it has become the main reason for the increase in the burden of psychiatric disorders. The mental health problems are anticipated to accounts for 14% of the disease burden worldwide [1].

Depression is one of the most common mental health problems all over the universe that disturb the physical, mental and social well-being of the population [2]. The people with depression always feel unhappy, frustrated and hopeless. Depressed persons don't have interest in any things, and most importantly, those people have a very low self-esteem level [3]. Around 70% of the depressive patients do have somatic complaints [4].

There are multiple predisposing factors for depression that includes chronic medical condition, stress, chronic pain, family history, female gender, low income, Jobless, substance abuse, low self-esteem, lack of social support, history, being single, divorced or widow, traumatic brain injury and younger age [5]. It is projected that the burden of depression will be more by the year 2020 and it will be the second contributing disease for the global burden as measured by the Disability-adjusted life years (DALY) [6].

According to the world health report 2011 of the world health organisation, 15 % of major depressive patients are more likely to commit suicide [7]. According to 2012 Statistics, one in every twenty persons is likely to have an episode of depression across the globe [2]. In the United States, the prevalence of depression is about 9% among the general population [5], [8], [9] whereas the European countries have the prevalence rate of about 8.5% [10].

The prevalence rate of depression has sharply increased universally during the last 10 years [11], especially in developing countries, the prevalence rate even reached around 44% [12]. The Primary Health Center (PHC) is the first point of contact between the people and the health care delivery system, and the prevalence of depression is high among the patients attending the primary health centres [13]. The prevalence rate of depression among the patients in the primary health centres varies between 15-22% [14], [15].

In Saudi Arabia, the prevalence of depression among the attendees of primary health centres was estimated to be between 30 to 46% in the year 1995 [16] whereas in 2002 the prevalence rate of depression in Saudi Arabia was found to be 18% among the adult population [17]. The prevalence rate of depression among adult patients visiting the primary health centres in the United States ranges from 5 to 13% [18]. It is evident that the prevalence of depression in the kingdom of Saudi Arabia is quite high and also increase in the risk factors which can enhance the depression level such as stress, chronic diseases, sedentary lifestyle, social isolation and social stigmas in terms of psychiatric illnesses [19].

The research studies found that the causes of depression are often undiagnosed in the primary health centres and it showed that one third to one-half of the cases of depression remain undiagnosed at the primary health care level [13], [20]. World Health organisation report says that between 20 and 40 years of age, ie among the young adults the onset of major depression occurs [21]. Therefore, the current study aims to determine the prevalence of depression and its associated factors among young adult patients attending the primary health centres in Tabuk, Kingdom of Saudi Arabia.

Material and Methods

Study design: This is a cross-sectional study.

Study setting: This study was conducted in the selected primary health centres in Tabuk, Saudi Arabia.

Study Period: The study was conducted from Nov 2018 to April 2019.

Sample Size: The sample size was calculated by the Epi Info statistical program. Based on the previously published literature [33] prevalence of depression was 49.9%, at a significance level of 0.05, a design effect of 1 and confidence interval of 95% ended with a minimum sample size equal 384.

Sampling Strategy: Multistage cluster sampling was followed, and the Tabuk city will be divided into East, West, North and South administrations and the number of primary health centres was listed based on these four administrative directions. Three Primary health centres from each administrative direction were selected by using a simple random sampling technique. Patients from the selected primary health centres were enrolled in the study by using the simple random sampling technique.

Ethical permission: Ethical permission was obtained from the Research Ethics Committee, NWAFH, Tabuk, Saudi Arabia.

Inclusion criteria: Patients with the age group between 20 and 40 years at the selected primary health centre on the day of visit and agreed to participate in the study with written informed consent were included.

Exclusion criteria: Patients with special needs and patients < 20 years and > 40 years were excluded.

Potential risk: Nil.

Potential benefit: The participants were benefited by knowing their level of depression and got appropriate medical and social care.

Tools for data collection: Data related to the depression were collected by using standardised questionnaire Patient Health Questionnaire-PHQ-2, and PHQ-9 Arabic version validated questionnaires [34].

PHQ-9 is completed by the participant in a few minutes and is rapidly scored by the researcher [35]. Liu et al., [36] reported that the PHQ-9 had a good internal consistency (α = .80) and test-retest reliability (intra-class correlation coefficient = 0.87).

Scoring of PHQ-9 responses: The PHQ-9 has 9 questions with a score ranging from 0 to 3 for each setting. The following Table describes the provisional diagnoses for scoring classes [37] (Table 1).

Table 1: Provisional diagnoses for scoring classes [37]

PHQ-9 score	Provisional diagnosis
0-4	None
5-9	Mild depression
10-14	Moderate depression
15-19	Moderately severe depression
20-27	nSevere depressio

A self-administered modified questionnaire was developed by conducting an extensive literature search from various databases and the previously published scholarly literature on depression and its risk factors among young adult patients visiting the primary health centres were identified, selected and used to frame the questionnaire.

Each patient was interviewed by using a structured questionnaire which contains information about the socio-demographic characteristics such as nationality, age, marital status, occupation, educational level, family income, type of family, crowding index, and family history of psychiatric disease. The questionnaire was framed in English, translated in Arabic (local language) and back-translated in English to check the translation.

Validity and Reliability: The questionnaire was validated by conducting a pilot study, and test-retest reliability will be done by Alpha (Cronbach's) test reliability for internal consistency.

Data Analysis: The data analysis was carried out by using SPSS (Version 25.0, SPSS Inc. Chicago, IL, USA).

Frequency tables were used to describe the prevalence rate of depression and Socio-demographic variables. Qualitative data were summarised in percentages, and non-parametric test of significance (Chi-square test) was applied for nominal scale. Multivariate logistic regression analysis was applied to identify predictors of depression among the participants after controlling for confounders. The p-value is two-tailed and statistical significance is set at < 0.05 (Table 2 and 3).

Management Plan

Table 2: Work Schedule-Gann Chart

Research Items	Dec 2017	Jan 2018	Feb 2018	Mar 2018	Apr 2018	May 2018	June 2018
Identify Research Areas							
Formulate Research Questions							
Formulate Research Strategy, Research Design and Research Methods							
Literature Search							
Write a Research Proposal							
Finalise the Research Proposal							
Literature Review							
Data Collection							
Data entry and Data Cleaning							
Data Analysis							
Report writing							
Final Report Submission							

Utilisation

The study results will be used to give a valid recommendation to the Ministry of health for implementing the systematic screening for depression among the young adult patients attending the primary health centres based on the prevalence rate. It also alarms the primary health care physicians and motivates them to screen the patients for depression and other psychiatric illness. Based on the statistical association between the depression and Socio-demographic variables, the awareness can be created among the patients for minimising the potential risk. The study results will also help the policymakers in proposing a new policy about mental disorders. The study results also serve as baseline data for future researchers.

Table 3: Budget/Funding

S.No	Budget Items	Numbers	Cost per Item (In SAR)	Total Cost (In SAR)
1	Man power	2	1000	2000
2	Instruments (Print out of Questionnaires)	4X 500 = 2000	1	2000
3	Equipment's (Laptop)	1	1500	1500
4	Consumables (Papers, pens, stapler, pins, writing pads etc)		500	500
5	SPSS Software original version	1	2000	2000
	Total Cost			8000

Results

The study included 384 patients aged between 20 and 40 years, with 46.3% aged between 20 and 25 years. Most of them (76.6%) were females. More than half of the patients (53.1%) were singles whereas 43.2% were married. Majority of them (88.5%) were belonging to nuclear families, at least university graduated (82.8%), employed (78.7%) and having high income (> 8000 SR/month) (73.8%), Table 4.

Table 4: Socio-demographic characteristics of the participants

Variables	Categories	Frequency	Percentage
Gender	Male	90	23.4
	female	294	76.6
Age (years)	20-25	178	46.3
	26-30	64	16.7
	31-35	49	12.8
	36-40	93	24.2
Marital status	Single	204	53.1
	Married	166	43.2
	Divorced/widowed	14	3.7
Type of family	Nuclear	340	88.5
	Joint	44	11.5
Highest education qualification	High school or lower	66	17.2
	College	292	76.0
	Postgraduate	26	6.8
Employment	Employed	187	78.7
	Not employed	171	44.5
	Housewife	26	6.8
Income (SR / month)*	< 3000	8	4.3
	3000-5000	21	11.2
	5001-8000	20	10.7
	> 8000	138	73.8

* For employed only (n = 187).

Habitual and social characteristics

The negative experience of childhood abuse among the participants was mentioned by 28.1% of the participants as illustrated in Figure 1 (left).

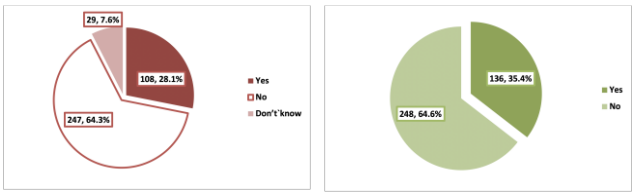

Figure 1: Negative experience of childhood abuse among the participants (left); Facing a lack of social support among the participants (right)

Facing a lack of social support was cited by 35.4% of the participant's Figure 1 (right).

History of death of near relative among the participants was observed among 47.7% of the patients (Figure 2 left) whereas the history of disturbed marriage life or marital breakdown was reported among 20% of the participants Figure 2 (right).

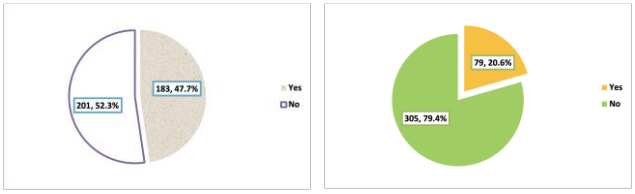

Figure 2: History of death of near relative among the participants (left); History of disturbed marriage life or marital breakdown among the participants (right)

History of family/neighbour quarrel was mentioned by 18.2% of the participants (Figure 3 left) whereas the history of having a financial problem was reported by 31.8% of them as shown in Figure 6(right).

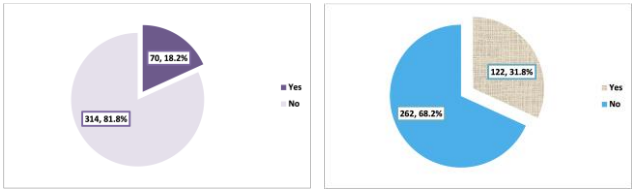

Figure 3: History of family/neighbour quarrel among the participants (left); History of having a financial problem among the participants (right)

History of stressful experience was mentioned by almost half of the participants (53.1%) as displayed in Figure 4 (left). Prevalence of patients` smoking was 15.9%, Figure 4 (right).

Figure 4: History of stressful experience among the participants (left); History of smoking among the participants (right)

Medical characteristics

Sleeping disorders were mentioned by almost one-third of the patients (36.2%) as obvious from Figure 5 (left).

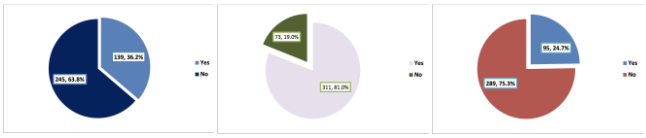

Figure 5: History of having sleep disorders among the participants (left); History of serious illness among the participants (middle); Family history of depression among the participants (right)

History of serious illness was mentioned by 19% of the patient's Figure 5 (middle).

Nearly one-quarter of the participants (24.7%) had a family history of depression as seen in Figure 5 (right).

Suicidal thoughts

Suicidal thoughts were reported by 10.2% of the patients as clear from Figure 6 (left).

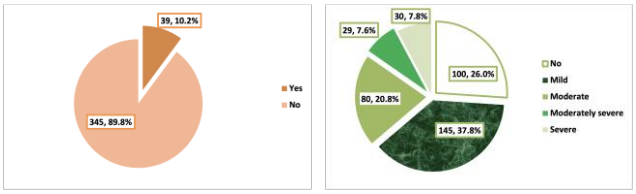

Figure 6: History of suicidal thoughts among the participants (left); Prevalence of depression among the participants, based on PHQ-9 (right)

Prevalence of depression

From Figure 6 (right), it is shown that the prevalence of depression was 74%; mild among 37.8%, moderate among 20.8% whereas moderately severe to severe among 15.4% of them.

Socio-demographic factors

Depression was more reported among single and divorced/widowed than married patients (81.4% and 78.6% versus 64.5%) p = 0.001. Regarding the severity of depression, moderately severe was more

observed among divorced/widowed patients (21.4%) compared to singles (5.9%) and married (8.4%) whereas severe depression was more reported among single patients than married (11.3% versus 4.2%), p = 0.001. Depression was significantly more observed among not employed patients compared to both employed and homemakers (81.3% versus 67.9% and 69.2%, respectively), p = 0.013. Patients` gender, age, type of family, highest qualification and income were not significantly associated with depression Table 5.

Table 5: Socio-demographic factors associated with depression among patients attending primary healthcare centres, Tabuk

	Depression					P1	P2
	No	Mild	Moderate	Moderately severe	Severe		
	N (%)	N (%)	N (%)	N (%)	N (%)		
Gender							
Male (n = 90)	30 (33.3)	33 (36.7)	17 (18.9)	6 (6.7)	4 (4.4)		
Female (n = 294)	70 (23.8)	112 (38.1)	63 (21.4)	23 (7.8)	26 (8.8)	0.072	0.345
Age (years)							
20-25 (n = 178)	46 (25.8)	74 (41.6)	37 (20.8)	9 (5.1)	12 (6.7)		
26-30(n = 64)	15 (23.4)	22 (34.4)	14 (21.9)	7 (10.9)	6 (9.4)		
31-35 (n = 49)	8 (16.3)	16 (32.7)	14 (28.6)	6 (12.2)	5 (10.2)		
36-40 (n = 93)	31 (33.3)	33 (35.5)	15 (16.1)	7 (7.5)	7 (7.5)	0.158	0.421
Marital status							
Single (n = 204)	38 (18.6)	80 (39.2)	51 (25.0)	12 (5.9)	23 (11.3)		
Married (n = 166)	59 (35.5)	60 (36.1)	26 (15.7)	14 (8.4)	7 (4.2)		
Divorced/widowed (n = 14)	3 (21.4)	5 (35.7)	3 (21.4)	3 (21.4)	0 (0.0)	0.001	0.001
Type of family							
Nuclear (n = 340)	88 (25.9)	125 (36.8)	75 (22.1)	25 (7.4)	27 (7.9)		
Joint (n = 44)	12 (27.3)	20 (45.5)	5 (11.4)	4 (9.1)	3 (6.8)	0.743	0.530
Highest qualification							
High school or lower (n = 66)	18 (27.3)	23 (34.8)	14 (21.2)	8 (12.1)	3 (4.5)		
College (n = 292)	73 (25.0)	113 (38.7)	63 (21.6)	18 (6.2)	25 (8.6)		
Postgraduate (n = 26)	9 (34.6)	9 (34.6)	3 (11.5)	3 (11.5)	2 (7.7)	0.546	0.584
Employment							
Employed (n = 187)	60 (32.1)	73 (39.0)	29 (15.5)	14 (7.5)	11 (5.9)		
Not employed (n = 171)	32 (18.7)	66 (38.6)	44 (25.7)	13 (7.6)	16 (9.4)		
House wife (n = 26)	8 (30.8)	6 (23.1)	7 (26.9)	2 (7.7)	3 (11.5)	0.013	0.063
Income (SR / month)*	1 (12.5)	2 (25.0)	2 (25.0)	2 (25.0)	1 (12.5)		
< 3000 (n = 8)	5 (23.8)	11 (52.4)	2 (14.3)	2 (9.5)	0 (0.0)		
3000-5000 (n = 21)	7 (35.0)	10 (50.0)	1 (5.0)	0 (0.0)	0 (0.0)		
5001-8000 (n = 20)	47 (34.1)	49 (35.5)	24 (17.4)	10 (7.2)	2 (100) 8 (5.8)	0.495	0.334
> 8000 (n = 138)							

*For employed only (n = 187); P1: Depression versus non-depression; P2: Severity of depression.

Habitual and social risk factors

Histories of child abuse, lack of social support, disturbed marriage, financial problems and stressful experience were significantly associated with depression and its severity among the participants, p < 0.001. Also, the history of family/neighbour quarrel was significantly associated with depression, p = 0.001. Smoking and death of near relative were not significantly associated with depression or its severity among the participant's Table 6.

Table 6: Habitual and social factors associated with depression among patients attending primary healthcare centres, Tabuk

	Depression					P1	P2
	No	Mild	Moderate	Moderately severe	Severe		
	N (%)	N (%)	N (%)	N (%)	N (%)		
Smoking							
No (n = 323)	87 (26.9)	120 (37.2)	67 (20.7)	22 (6.8)	27 (8.4)		
Yes (n = 61)	13 (21.3)	25 (41.0)	13 (21.3)	7 (11.5)	3 (4.9)	0.543	0.359
Childhood abuse							
No (n = 247)	85 (34.4)	103 (41.7)	38 (15.4)	14 (5.7)	7 (2.8)		
Yes (n = 108)	12 (11.1)	35 (32.5)	32 (29.6)	12 (11.1)	17 (15.7)		
Don`t know (n = 29)	3 (10.3)	7 (24.1)	10 (34.6)	3 (10.3)	6 (20.7)	< 0.001	< 0.001
Lack of social support							
No (n = 248)	85 (34.3)	99 (40.0)	42 (16.9)	11 (4.4)	11 (4.4)		
Yes (n = 136)	15 (11.0)	46 (33.9)	38 (27.9)	18 (13.2)	19 (14.0)	< 0.001	< 0.001
Death of near relative							
No (n = 201)	49 (24.4)	77 (38.3)	40 (19.9)	17 (8.5)	18 (9.0)		
Yes (n = 183)	51 (27.9)	68 (37.2)	40 (21.9)	12 (6.6)	12 (6.6)	0.436	0.769
Disturbed marriage							
No (n = 305)	95 (31.1)	114 (37.4)	59 (19.3)	17 (5.6)	20 (6.6)		
Yes (n = 79)	5 (6.3)	31 (39.2)	21 (26.6)	12 (15.2)	10 (12.7)	< 0.001	< 0.001
Family/neibour quarrel							
No (n = 314)	93 (29.6)	119 (37.9)	63 (20.1)	19 (6.1)	20 (6.4)		
Yes (n = 70)	7 (10.0)	26 (37.1)	17 (24.3)	10 (14.3)	10 (14.3)	0.001	0.001
Financial problems							
No (n = 262)	86 (32.8)	97 (37.0)	48 (18.3)	15 (5.7)	16 (6.1)		
Yes (n = 122)	14 (11.5)	48 (39.3)	32 (26.2)	14 (11.5)	14 (11.5)	< 0.001	< 0.001
Stressful experience							
No (n = 180)	81 (45.0)	70 (38.9)	23 (12.8)	3 (1.7)	3 (1.7)		
Yes (n = 204)	19 (9.3)	75 (36.8)	57 (27.9)	26 (12.7)	27 (13.2)	< 0.001	< 0.001

P1: Depression versus non-depression; P2: Severity of depression.

Medical risk factors

Sleep disorders and family history of depression were significantly associated with a history of depression among the patients, p < 0.001 while the history of serious illness was not significantly associated with depressions or its severity Table 7.

Table 7: Medical factors associated with depression among patients attending primary healthcare centres, Tabuk

	Depression					P1	P2
	No	Mild	Moderate	Moderately severe	Severe		
	N (%)	N (%)	N (%)	N (%)	N (%)		
Sleep disorders							
No (n = 245)	83 (33.9)	100 (40.8)	42 (17.1)	10 (4.1)	10 (4.1)		
Yes (n = 139)	17 (12.2)	45 (32.4)	38 (27.3)	19 (13.7)	20 (14.4)	< 0.001	< 0.001
History of serious illness							
No (n = 311)	86 (27.7)	120 (38.6)	59 (19.0)	23 (7.4)	23 (7.4)		
Yes (n = 73)	14 (19.2)	25 (34.2)	21 (28.8)	6 (8.2)	7 (9.6)	0.138	0.281
Family history of depression							
No (n = 289)	91 (31.5)	104 (36.0)	59 (20.4)	16 (5.5)	19 (6.6)		
Yes (n = 73)	8 (11.0)	34 (46.6)	17 (23.3)	8 (11.0)	6 (8.2)		
Don`t know (n = 22)	1 (4.5)	7 (31.8)	4 (18.2)	5 (22.7)	5 (22.7)	< 0.001	< 0.001

P1: Depression versus non-depression; P2: Severity of depression.

Suicidal thoughts

Majority of patients who had suicidal thoughts were depressed (97.4%) compared to 71.3%) of those

who hadn't suicidal thoughts, p < 0.001. Also, server depression was more reported among patients who had suicidal thoughts compared to others (43.6% versus 3.8%), p < 0.001 (Table 8).

Table 8: Association between suicidal thoughts and depression among patients attending primary healthcare centres, Tabuk

	Depression					P1	P2
	No N (%)	Mild N (%)	Moderate N (%)	Moderately severe N (%)	Severe N (%)		
Suicidal thoughts	99 (28.7)	137 (39.7)					
No (n = 345)	1 (2.6)	8 (20.5)	74 (21.4)	22 (6.4)	13 (3.8)		
Yes (n = 39)			6 (15.4)	7 (17.9)	17 (43.6)	< 0.001	< 0.001

P1: Depression versus non-depress.

Predictors of depression

Compared to single patients, married patients were at lower risk for depression (Adjusted odds ratio "AOR" was 0.36, 95% confidence interval "CI" was 0.20-0.93), p < 0.001. Patients who reported a lack of social support were more likely to be depressed than those with social support (AOR = 2.05, 95% CI = 1.03-4.07), p = 0.041. Patients who reported disturbed marriage were at almost four-folded risk of depression compared to those without disturbed marriage (AOR = 3.50, 95% CI = 1.23-9.98), p = 0.019. Patients with financial problems were at almost double risk for developing depression compared to those without financial problems (AOR = 2.37, 95% CI = 1.16-4.85), p = 0.019. Those with stressful experience were significantly more likely to have depression compared to those without stressful experience (AOR = 4.75, 95% CI = 2.58-8.71), p < 0.001. Opposed to patients without a family history of depression, those with such history were at higher significant risk for depression (AOR = 2.75, 95% CI = 1.23-6.14), p = 0.014. Also, patients who reported sleep disorders were at nearly double folds of having depression compared to those without such disorders (AOR = 2.24, 95% CI = 1.16-4.30), p = 0.016. Patients` marital status, employment, family/neighbour quarrel, childhood abuse and suicidal thoughts were not significantly associated with depression Table 9.

Table 9: Predictors of depression among patients attending primary healthcare centres, Tabuk: Results of multivariate logistic regression analysis

	B	SE	AOR	95% CI	p-value
Marital status					
Single (n = 204) [a]			1.0	---	---
Married (n = 166)	-1.021	0.288	0.36	0.20-0.63	<0.001
Divorced / widowed (n = 14)	-0.473	0.799	0.62	0.13-2.98	0.554
Lack of social support					
No (n = 248)[a]			1.0	---	---
Yes (n = 136)	0.716	0.351	2.05	1.03-4.07	0.041
Disturbed marriage					
No (n = 305) [a]			1.0	---	---
Yes (n = 79)	1.252	0.535	3.50	1.23-9.98	0.019
Financial problems					
No (n = 262) [a]			1.0	---	---
Yes (n = 122)	0.862	0.366	2.37	1.16-4.85	0.019
Stressful experience					
No (n = 180) [a]			1.0	---	---
Yes (n = 204)	1.557	0.310	4.75	2.58-8.71	<0.001
Family history of depression					
No (n = 289) [a]			1.0	---	---
Yes (n = 73)	1.012	0.410	2.75	1.23-6.14	0.014
Sleep disorders					
No (n = 245) [a]			1.0	---	---
Yes (n = 139)	0.805	0.333	2.24	1.16-4.30	0.016

[a]: Reference category; B: Slop; SE: Standard error; AOR: Adjusted odds ratio; CI: Confidence interval.

Terms of employment, family/neighbour quarrel, childhood abuse and suicidal thoughts were removed from the final logistic regression model (not significant).

Discussion

Worldwide, there is growing concern about depression among adult people [38] and Saudi Arabia is not an exception. Additionally, the integration of mental health services at primary care centres in Saudi Arabia is challenging [39]. Thus, there is a need to screen patients attending primary care centres, particularly those of middle age for depression as one of the commonest psychiatric disorders which would help in providing the real situation of psychiatric disorder burden at PHC centres in Saudi Arabia.

Prevalence of depression

In the present study, the prevalence of depression among young adult patients (20-40 years) attending primary healthcare centres in Tabuk was 74%; being mild among 37.8%, moderate among 20.8% whereas moderately severe to severe among 15.4% of them. In a study carried out previously among patients attending primary health care centres in Riyadh [39], a rate of 33.4% has been reported. In a similar study carried out in Northern Province in Sri Lanka [40], the prevalence of mild depression was 13.3% whereas that of major depression was 4.5% in adult patients attending primary care settings.

Many other studies reported various figures. However, they were carried out among the general adult population. Therefore, in comparison with the present study`s figure is not logic as this study included only young adult patients (20-40 years). A systematic review and meta-analysis carried out in Saudi Arabia by Al Ibrahim et al. in 2010 found the prevalence of depression by about 41% [22]. In the pilot study done by El Rufaie et al. in Dammam, the prevalence rate of depression was 17% [23]. A preliminary prevalence study done in the Asir region of Saudi Arabia by Al Qahtani et al. conveyed a 27% prevalence of depression in the year 2008 [24]. A screening on depression was done in a family medicine clinic by Abdul Wahid et al. in southeastern Saudi Arabia relieved 12 % prevalence rate [25]. Studies were done in Riyadh by Becker et al., in primary health, centres stated that the prevalence rate of depression was 20% [26], [27]. In Kuwait, the prevalence of depression was 20.5% [28]. In Qatar, the prevalence rate of depression was 13.5% [29]. Studies conducted in European countries confirmed the prevalence rate of depression ranged between 16.5% and 22.8% [30], [31], [32].

The observed difference in the prevalence rate in various studies, including the present one could be attributed to variation in the demographic characteristics of the studied population, particularly age, variation in the background health status of the participants (healthy or patients), and application of different tools to diagnose depression in various studies as well as cultural variation between different countries.

Predictors of depression

In the present study, patients who reported a lack of social support and those who had disturbed marriage were more likely to be depressed. Other investigators around the world documented the same finding [41], [42], [43].

Patients with financial problems were at more likely to develop depression compared to those without financial problems. Richardson T et al. have documented the same finding., (2017) [44]. Also, Richardson T et al., in 2013 [45] had performed a meta-analysis and reported that 41.7 % of persons with depression were in debt, in comparison to 17.5 % who reported having no debt.

In the current study, patients with stressful experience were significantly more likely to have depression compared to those without stressful experience. In agreement with this finding, Steel Z et al., [46] observed that the population exposed to conflict and displacements were more liable to have mental health problems, particularly depression. Also, in Sri Lanka [47], stressful events after the war were associated with depressive symptoms.

Family history of depression was a significant predictor for depression among young adults in this study. Weissman MM reported that family history of depression doubles the risk for depression [48].

Patients who reported sleep disorders were at higher risk of having depression compared to those without such disorders in the present study. The same has been observed in other studies carried out oversea [49], [50].

In the present study, patients` gender was not a predictor for depression. Other studies reported the same [51], [52]. However, in a systematic review and meta-analysis carried out previously in Saudi Arabia, women were more likely to be depressed when compared to men [22]. Also, El Rufaie et al., in Dammam, observed that depression was higher among females than males [23]. In Kuwait, depression was 20.5 % and highly prevalent among women than men [28]. In Qatar [29], depression was more reported among women than men visiting primary healthcare settings. Studies conducted in European countries confirmed that women were often having mental symptoms more than men [30], [31], [32]. In Sri Lanka [40], women reported higher rates of depression compared to men.

In the present research, most socio-economic indicators such as type of family, qualification, employment status and income were not related to the development of depression. The only significant socio-economic factor associated with depression was the marital status as married adults were less likely to develop depression. In disagreement with our findings, Abdul Wahid et al. in their study in southeastern Saudi Arabia reported that the participants who lived in a flat or an individual villa were 4.8 times less likely to suffer from depression compared to the participants who lived in a room, and working participants were more likely to be depressive than the unemployed participants [25]. In Kuwait, working, highly educated, married individuals and those with three or more children were more depressed [28].

Age was not a predictor for depression in the current study. In a study carried out in Kuwait [28], young people showed a higher rate of depression. On the other hand, in Sri Lanka [40], older individuals showed a higher rate of depression. Also, in other previous studies, older age was a predictor for depression [53], [54]. It is not practical to compare results of the present study with the studies above as we included only young adults aged between 20 and 40 years while in other studies, they included all adults, this could explain the difference between findings.

Strengths and limitations

To our knowledge, this is the first study that evaluated depression among young adult patients attending primary health care setting in Tabuk and even in the whole Kingdom of Saudi Arabia. Also, knowing the magnitude and predictors of depression in such population sought care from primary care settings rather than in the general population would be more beneficial to provide targeted services and to identify those in more need for screening. However, some limitations in the present study should be mentioned. The cross-sectional nature of the study design limits the conclusion of whether risk factors lead to depression or vice-versa. The self–reported measure utilised in this study could increase the likelihood of response bias. Depending only on the PHQ-9 in the diagnosis of depression, despite its proven sensitivity and specificity is not enough, and the final diagnosis needs to be confirmed by a clinical assessment.

In conclusion, depression among young adult patients (20-40 years) attending primary healthcare centres in Tabuk is very high. However, it is mostly mild to moderate in its severity. Suicidal thoughts were reported by one-tenth of the participants. Single marital status, lack of social support, history of disturbed marriage, financial problems, stressful

experience, family history of depression and sleep disturbance are predictors for depression among young adults in Tabuk.

Based on the results of the present study and their discussion, the following are recommended:

1. Conducting screening programs at primary healthcare settings in Tabuk to identify young adults at higher risk for depression earlier to assure better management.

2. Organizing community-based mental health programmes to increase awareness of the community regarding common psychosocial issues and psychosocial problems.

3. Training of the primary health care physicians to screen young adults for depression and manage them appropriately and refer severe cases to psychiatrists.

4. Proposing a new policy about mental disorders by policymakers.

5. A further longitudinal study is recommended in this regard

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Public Health

The Effectiveness of Online Course Intervention to Improve Knowledge of Antimicrobial Resistance among Dental Students, in Comparison to Reference Group Using a Randomized Controlled Trial

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Abstract

AIM: This study aimed to assess the effectiveness of a recognised antimicrobial resistance (AMR) online module on knowledge and perception among dental students, using a randomised controlled trial study design.

METHODS: Dental students (n = 64, aged 21-25 years) in clinical years agreed to participate in this triple-blinded, parallel, randomised controlled trial. There were 34 students in the study group and 30 students in the control group. The study group participated in an online course covering information about AMR, while students in the control group received another online course about microorganisms in dentistry. Both groups were assessed three times using online questionnaires: before the intervention (T1), after the intervention (T2), and two months later (T3). Each one of T1, T2 and T3 had 22 questions. The questions were repeated each time in T1, T2, and T3 asking about AMR but with different question format, to avoid the possibility of students to memorise the answers.

RESULTS: The mean (m) of correct answers for all students on T1 was 12.56, with standard deviation (SD) of 3.2. On T2, m = 14.03 and SD = 3.85, and on T3, m = 14.36 and SD = 3.71. Scores ranged from 0 to 22. The participants in the study and control groups showed significant score improvements from T1 to T2, immediately after the intervention, but there was no significant difference between T2 and T3. The study group students' scores did not improve significantly from T1 to T3, in contrast to the control group students' scores. More importantly, there was no significant difference in improvement from T1 to T2 when comparing the study and control groups.

CONCLUSION: Online courses might not be reliable learning methods for ensuring the optimal levels of AMR knowledge that are needed by dental practitioners.

Introduction

The World Health Organization (WHO) recognises antimicrobial resistance (AMR) as one of the greatest threats to human health [1]. According to the National Health Service (NHS) in England, antimicrobial agents are used as a treatment for bacterial infections that are characterised by widespread infection, long healing times, or having serious complications [2]. Antimicrobial drugs are classified into six main families: penicillins, cephalosporins, aminoglycosides, tetracyclines, macrolides, and fluoroquinolones [2]. In dentistry, several antimicrobial agents, including amoxicillin, metronidazole, and doxycycline, are used after dental extractions and treatment of dental abscesses, periapical discharges, and infections [3]. These antimicrobial drugs have many side effects, such as diarrhoea, nausea, vomiting, abdominal pain, loss of appetite, bloating, and indigestion [2]. In some cases, the use of antimicrobial drugs may cause mild to moderate allergies [2].

Nowadays, the NHS and health organisations all over the world are attempting to decrease the

prescribing of antimicrobials [2], especially for less serious conditions, because overuse of antimicrobial drugs (antibiotics) leads to decreasing effectiveness [2], or AMR. The WHO defines AMR as the potential for microorganisms to gain resistance to antimicrobial agents [4] when misuse and overuse of antibiotics make more sensitive bacteria die and allow non-sensitive bacteria to proliferate [5]. By 2050, according to the United Kingdom public sector information website, 10 million deaths will occur each year globally at the cost of $100 trillion for the global economy due to the failure to acknowledge and handle the threat from AMR [6].

Multiple systematic reviews and studies highlighted that dentist in an increasing trend to prescribe unnecessarily antibiotic [7], [8], [9]. A cross-sectional study conducted in Saudi Arabia found overall levels of AMR awareness are good among dentists in the Western region; however, awareness of antibiotic prescribing guidelines was insufficient [10]. Another national-scale study concluded that levels of knowledge about AMR are inadequate among dentists in Saudi Arabia on a national level [11]. Similarly, other studies in other countries such as Italy, Poland and Yemen indicating insufficient knowledge about antibiotic prescribing practices and AMR [12], [13], [14].

Some randomized controlled trials (RCTs) have been conducted among general medical practitioners, health care professionals, and the general public to assess the effects of an educational program on attitudes about antimicrobial drugs, and they have found promising results in terms of improvements in knowledge about prescribing of antibiotics [15], [16], [17], [18]. Similarly, a few interventional studies have been conducted among dental students and dental practitioners aimed at improving knowledge and attitudes about prescribing antimicrobial agents [19], [20]. One such study, conducted among dental students in three European countries, used an online interventional module and found it was a helpful and effective tool for teaching about prescribing practices for antimicrobial agents [19]. This study noted that online intervention modules are promising due to their low cost in comparison to other interventions and because they can be widely used among dental students and doctors without the necessity to physically attend classes. However, this was a preliminary study with a small sample size (n = 39), and the design lacked a control group. Another study in Germany was aimed at optimising antimicrobial prescribing behaviour among general dentists by using an interventional seminar and videotapes [20]. However, this was a methodological paper without documented results.

Two auditing studies were conducted examining interventions to improve antimicrobial prescribing practices among dentists [21], [22]. One was an audit and feedback interventional study on antimicrobial prescribing in Scotland using the Reducing Antibiotic Prescribing in Dentistry (RAPiD) program with a face-to-face educational presentation [21]. The other was an audit intervention study in England [22]. Both studies found a significant decrease in antimicrobial prescribing after auditing.

Nevertheless, more interventional studies are needed to assess improvement in AMR knowledge, attitudes, and prescribing behaviours among dental students and dentists. It is also important to assess the effectiveness of online programs, given that they are generally more convenient and cost-effective. No interventional studies have been conducted to date in Saudi Arabia that was aimed at improving dental students' or practising dentists' knowledge of AMR, despite documented low levels of such knowledge. Thus, this study aimed to evaluate the effectiveness of an AMR online module for improving knowledge about AMR among dental students in Saudi Arabia, using a randomised controlled trial study design. This interventional article aimed to assess the ability of online course as a convenient and low-cost solution to increase the level of knowledge and awareness about AMR in a dental practice. Such findings might be useful to validate the effectiveness of another similar online course in some universities, and might be a potential method to be applied by other health organisations.

Material and Methods

Design and participants

A parallel randomised controlled trial design (1:1) that was tripling blinded and used an active reference group was selected for this study. The participants were randomly allocated to either a study group or a reference group. The participants in each group received different content materials; however, both courses were online (to ensure a similar way to deliver the content material). The content material of the reference group considered to be placebo because the student received the content material with no intention to improve AMR knowledge. The intervention (in the study group) aimed to investigate the effects of an online course on levels of knowledge among dental students in Saudi Arabia. Male and female students in their fourth, fifth, or sixth academic year at the Dental Faculty of Umm Al-Qura University, Makkah, Saudi Arabia, were invited to participate in the study. The inclusion criteria were: 1. Student at Umm Al-Qura University; 2. The dental student in the fourth, fifth, or sixth year; and 3. Approve and submit an online informed consent to participate in the study.

It should be mentioned that the bachelor of dentistry in Saudi Arabia is seven years program, starting with three non-clinical years and followed by three clinical years and intern. Also, usually students

admitted to dental school immediately after graduation from high school at the age of 18 to 19.

Recruiting and randomisation

The study invitation link was disseminated to class leaders of each academic year (both male and female) along with consent forms in January 2018. Students who agreed to participate filled out an online form and were enrolled by a third party, to ensure randomisation concealment and a confirmed number of participants. After an enrollment list was created, students were randomly assigned to either the study or control group using Excel software or a random number generator. The students with odd and even numbers were allocated to either the study or the control group by a third party. The third party gave each group a special code that was revealed after data analysis finished. This was important to ensure that all participants, examiners and statistician were not aware of the students' allocation groups until data analysis was finished. Thus, the study was triple blinded.

The intervention and reference

None of the students was aware if s/he is in the study or control group to ensure participant blindness. Students in both groups received a link via mobile phone text sent by a third party to ensure examiner blindness. All the students went through a similar process when they clicked on the link, as shown in the participant's flow chart (Figure 1) and the data collection process (Figure 2).

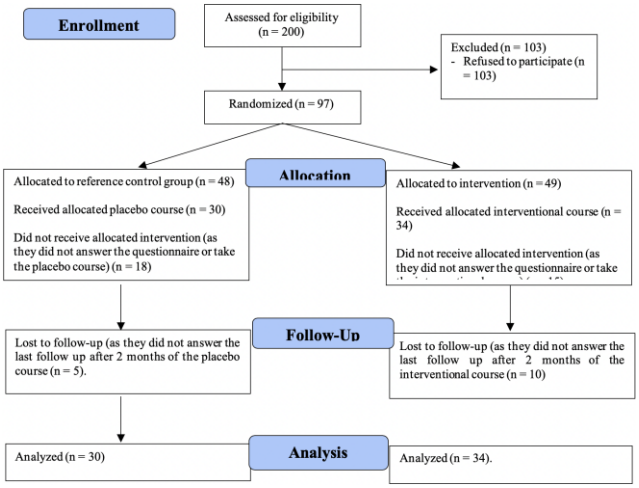

Figure 1: Flow of participants through the randomised controlled trial

First, they reviewed the consent form and signed it by clicking "next." Second, they filled out a baseline questionnaire (T1). Third, they entered an interventional online course or a reference group who had placebo online course, according to their group allocation. Fourth, immediately following completion of the online course, students answered another questionnaire (T2). These steps were done in February 2018. Fifth, about two months after the completion of the online courses (April 2018), students received another follow-up questionnaire to measure their knowledge retention (T3). In addition to the time spent taking the intervention course or the placebo course, students spent approximately 20 min to answer each of the T1 and T2 questionnaires. They spent approximately 7 min to answer the T3 questionnaire.

The study group's interventional course consisted of 25 slides with written material in English, and participants were given an option to listen to an accompanying audio recording of the written material. The interventional video was approximately 9 min and was aimed at improving participants' knowledge about AMR and the proper use of antimicrobial agents in dentistry. The content of the intervention was derived from different sources [4], [5], [6], [23], [24], [25] and was validated by a panel of eight dental consultants from a content point of view.

The content was divided into seven sections: the definition of AMR, introduction, types of actions of antibiotics, how AMR develops, causes of AMR, the dentist's role in reducing AMR, and proper prescribing of antibiotics by dentists. The link for the study group course can be accessed from here: https://goo.gl/forms/7RA079LPB5H6YE033.

The reference group was given an online course about microorganisms in dentistry (with no mention of anything related to AMR), consisting of 34 slides and written material in English. This video was about 5 min, and the content was derived from a previous resource [23]. This course was divided into eight sections: definition of normal flora, types of normal flora, common habitats of human microbial flora, changes of oral flora with lifestyle and oral habits, benefits of resident oral flora, disadvantages of resident oral flora, and factors modulating the growth of bacteria in the oral cavity. For ethical reasons, after completion of the T3 questionnaire, all students in the reference group received a link for the main interventional course to improve AMR knowledge and prescribing behaviour. The link for the control group course can be accessed from here: https://goo.gl/forms/2lMWNuG4WBuZSyEk2

Both courses' links were sent separately and individually to participants in avoid intervention contamination. Both courses were previously evaluated in two pilot studies using 12 students to ensure the validity of the intervention and the questionnaire. Also, the courses were adjusted concerning technical problems, content, structure, and course usability.

The process of recruitment of participants and the flow of the study are shown in Figure 2.

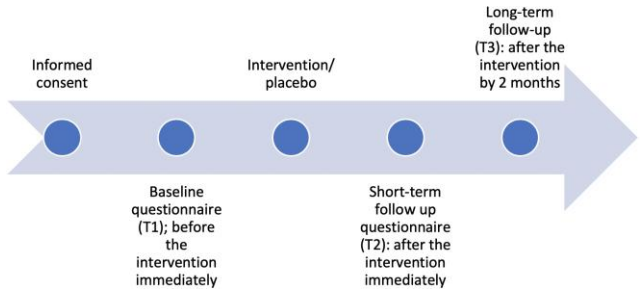

Figure 2: Data collection process

The questionnaires

Students in both groups received three questionnaires: baseline before the study (T1), immediately after the intervention/placebo (T2), and a follow-up two months later (T3). The T1 and T3 questionnaires were identical. The T2 questionnaire contained the same questions as in T1 and T2 but with minor differences in wording to eliminate the chances of participants remembering the questions. All three questionnaires (T1, T2, and T3) consisted of two sections and had a total of 28 questions. Section one was composed of 22 questions regarding knowledge of AMR that were either in the form of multiple-choice questions with only one correct answer or questions where participants needed to select all the correct answers from a list. Section two was composed of six demographic questions asking about an academic year, gender, marital status, financial status, GPA, and phone number. The phone numbers were used for follow-up at T3 and to give out randomly picked incentives; then the numbers were deleted from all documentation after data entry, making the data anonymous.

Statistical analysis

After data entry, study group and control group participant data were coded by a third party to ensure statistician blindness. SPSS was used for data analysis. Descriptive statistics were used to generate frequency tables, means, and standard deviations. Chi-square, t-test, and paired t-test were used to compare the study and control group results.

Ethical approval and incentives

This study received prior ethical approval from Umm Al-Qura University, Faculty of Dentistry, with the number 77-17 and was registered with the ISRCTN registry (ISRCTN13442659). All the students submitted an electronic consent form before participating in the study. As an incentive, students who completed all three questionnaires were entered into a random drawing for a chance to win one of six 200 Saudi Riyal coupons to a well-known bookstore in Saudi Arabia, three for male participants and three for female participants.

Results

All the students in the 4th, 5th and 6th years were invited (200 students). Only 64 completed the T1 questionnaire, took the online (study or control) course, and answered the T2 questionnaire, yielding a 32% response rate. There were 34 participants in the study group and 30 participants in the control group. All the participants answered the T1 and T2 questionnaires, with no dropouts; however, there was a dropout rate of 23.43% at T3, as shown in Figure 1. Participants' demographic data are displayed in Table 1.

Table 1: Participant demographic data (n = 64)

Variable		Total, *n* (%)	Study Group, *n* (%)	Control Group, *n* (%)	p-value
Gender	Male	27 (42.18)	10 (29.40)	17 (56.70)	0.042
	Female	37 (57.80)	24 (70.60)	13 (43.30)	
Academic year	4th year	36 (56.25)	21 (61.80)	15 (50.00)	0.702
	5th year	6 (9.30)	3 (8.80)	3 (10.00)	
	6th year	22 (34.30)	10 (29.40)	12 (40.00)	
Marital status	Single	61 (95.30)	31 (91.20)	30 (100.00)	0.241
	Married	3 (4.60)	3 (8.80)	0 (0.00)	
Family annual income	< 5,000	6 (9.30)	4 (11.80)	2 (6.70)	0.149
	5,000–15,000	28 (43.70)	11 (32.40)	17 (56.70)	
	> 15,000	30 (46.80)	19 (55.90)	11 (36.70)	
GPA	A	31 (48.40)	17 (50.00)	14 (46.70)	0.617
	B	27 (42.18)	15 (44.10)	12 (40.00)	
	C	6 (9.30)	2 (5.90)	4 (13.30)	

Comparing the demographic variables between the study and control groups using Chi-square and Fisher's exact test showed that there were no significant differences except for gender, where females were more significantly represented in the study group than in the control group ($p = 0.042$).

The mean (m) of correct answers for all students on T1 was 12.56, with SD of 3.2. On T2, $m = 14.03$ and SD = 3.85, and on T3, $m = 14.36$ and SD = 3.71. The maximum possible score was 22, and the minimum was zero.

In the study group, a paired t-test for repeated measures showed that there was a significant improvement in knowledge scores from T1 ($m = 13.5$, SD = 3.06) to T2 ($m = 14.94$, SD = 4.46); ($p = 0.040$). However, the scores from T2 to T3 ($m = 15.75$, SD = 3.47) and from T1 to T3 were not significantly different.

In the control group, a paired t-test for repeated measures showed that there was a significant improvement in knowledge scores from T1 ($m = 11.5$, SD = 3) to T2 ($m = 13.0$, SD = 2.74); ($p = 0.002$). Besides, there was a significant improvement from T1 to T3 ($m = 13.04$, SD = 3.49); ($p < 0.018$). However, the score from T2 to T3 was not significantly different.

To compare the differences in knowledge scores between the study group and the control group, we calculated the differences from T1 to T2, T2 to T3, and T1 to T3, as displayed in Table 2. Using t-test, the score differences of the study and control groups were compared, and there were no significant

differences, as shown in Table 2.

Table 2: Differences in knowledge scores on T1, T2, and T3

	Study group	Control group	
Knowledge	Mean (SD)	Mean (SD)	*p*-value
Difference between T1 and T2	1.441 (3.94)	1.500 (2.37)	0.942
Difference between T2 and T3	0.290 (2.83)	0.280 (2.44)	0.988
Difference between T1 and T3	−1.125 (3.74)	−1.320 (2.59)	0.834

Figure 3 shows the trajectory of the participants' knowledge scores for both groups. It should be noted that the paired t-test and t-test was conducted as a parametric test after meeting test assumptions including normal distribution of the variables used in these tests.

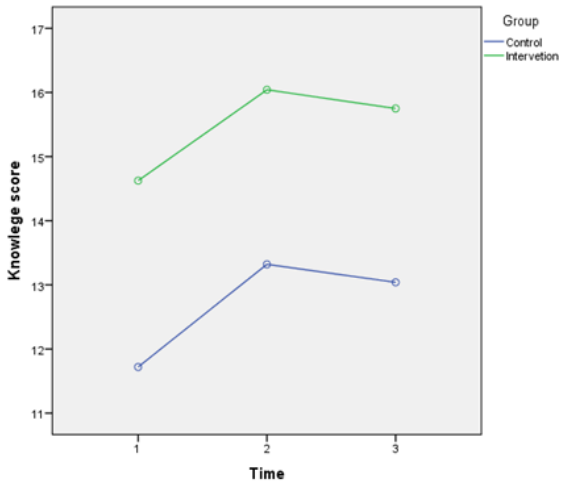

Figure 3: Knowledge scores on T1, T2, and T3 in study and control groups

Discussion

Our results suggested that there was no significant improvement in the knowledge levels of participants in the study group when compared to the control group, despite both groups showing significant improvement immediately following the intervention. Our results seem to be different from those in previous studies [19], [21], [22], which showed significant improvements in study groups after interventions. However, these previous studies did not have active placebo controls for comparison [19], [21], [22]. Our results may be more accurate due to the active control group showing that the improvement found in the study group was no different from the improvement that occurred in the control group. Nevertheless, it is hard to compare our study with the mentioned previous study in micro-level because there are many differences, bearing in mind that these studies are the only interventional studies in this research area according to our best knowledge.

A number of possibilities could explain the insignificant improvement after the intervention in the study group as compared to the control group. First, the online module might not be the ideal learning method for students because, low response to online course as mentioned in a previous article [26], not taking the course seriously, students might skip slides just to finish, which has been observed in other online courses among dental students by this study's authors. Second, the interventional course might have contained a high volume of content to be absorbed by the students, and some students might have had some technical problems [27], [28]. Third, we had a small sample size, which could have affected the ability to detect differences between the groups.

It is interesting to note that students showed an increase in scores from T1 to T2 in both groups, which were not surprising for the study group, where students had received pertinent information in their online course, but it could be a little surprising for the control group. However, this may be explained by the possibility of students in the control group searching the internet for correct answers to the questions on the T2 questionnaire, especially if they were unsure about their answers on T1.

This has important implications for mandatory online courses in the health sector in general, especially for organisations that make these online courses a prerequisite for moving forward into other job responsibilities. Online courses tend to be more feasible to ensure that participants achieve a course outcome [29] especially for the organization, and in fact, sometimes the online systems issue auto-generated certificates of completion after participants pass a course exam. Our results indicate that these mandatory online courses might be inappropriate for accomplishing student or health practitioner goals. The students in our study seemed to take a careless attitude toward the online courses, which was reflected in the results not showing the expected improvement for the study group. While the students in the pilot studies described the study group course content as straightforward and simple, that could be because the students in the pilots were personally invited to take the course. This might have made the students in the pilots approach the course more seriously and answer the questionnaires with care, while the students in the main study had no relationship with the research team and so behaved more naturally. Regardless, the main study participants represented the desired population.

Further, some online courses allow participants to take a final exam more than once until the exam is passed; this can lead to participants skipping slides and searching for answers online, or taking other actions, to pass the final exam and get their certificate [30], [31]. But this violates the main reasons for taking such courses. We believe that better specifications could make these online courses more effective. For example, courses could calculate the time spent on each slide to detect if participants are skipping quickly to the end of the course. However, such recommendations or suggestions

cannot be made without further research, investigation and innovative ideas to determine the best approaches. It should be noted that the main aim to use the placebo in the reference course in the control group is to minimise the Hawthorne effect [32] generated to the participants after answering the baseline questionnaire, as we assumed that some students would search for the correct using the internet.

In addition to future similar studies needing to use larger sample sizes, the intervention should be reviewed in terms of technical difficulties and user experiences to create a better course design. It is suggested that future interventions include a required follow-up exam that can be taken only once within a certain time period and which participants must pass with a predetermined score to receive a certificate of completion.

This study had some strengths, particularly the use of an RCT design with an active reference group and triple blindness. This is also the first study of its kind in Saudi Arabia using an online module covering this topic and examining the feasibility of online courses, given their minimal time commitments and low costs. On the other hand, this study's limitations include a small sample size, which makes it hard to generalise the study finding, short follow-up period, and a sample was taken from single-centre, which cast more doubts about generalizability for the study result. It is recommended to have a further study with a larger sample size and use multicenter for more generalizable results.

In conclusion, according to our results that showed there is no significant change in the students' AMR knowledge score after the intervention, online courses might not be an appropriate learning method for the goal of improving AMR knowledge levels because participants may not take the module seriously. Further investigations and interventional studies regarding AMR are needed to assess different modalities for improving dental students' knowledge and practices around AMR. Future studies might assess online modules with the addition of technical features to increase the levels of responsibility required so that participants are prompted to take these courses with care and attention.

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Public Health

The Effect of Noise Exposure on Cognitive Performance and Brain Activity Patterns

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Keywords: Noise; Cognitive Performance; Attention; Brain Activity; Electroencephalogram

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Abstract

BACKGROUND: It seems qualitative measurements of subjective reactions are not appropriate indicators to assess the effect of noise on cognitive performance.

AIM: In this study, quantitative and combined indicators were applied to study the effect of noise on cognitive performance.

MATERIAL AND METHODS: A total of 54 young subjects were included in this experimental study. The participants' mental workload and attention were evaluated under different levels of noise exposure including, background noise, 75, 85 and 95 dBA noise levels. The study subject's EEG signals were recorded for 10 minutes while they were performing the IVA test. The EEG signals were used to estimate the relative power of their brain frequency bands.

RESULTS: Results revealed that mental workload and visual/auditory attention is significantly reduced when the participants are exposed to noise at 95 dBA level ($P < 0.05$). Results also showed that with the rise in noise levels, the relative power of the Alpha band increases while the relative power of the Beta band decreases as compared to background noise. The most prominent change in the relative power of the Alpha and Beta bands occurs in the occipital and frontal regions of the brain respectively.

CONCLUSION: The application of new indicators, including brain signal analysis and power spectral density analysis, is strongly recommended in the assessment of cognitive performance during noise exposure. Further studies are suggested regarding the effects of other psychoacoustic parameters such as tonality, noise pitch (treble or bass) at extended exposure levels.

Introduction

The influence of noise on human cognitive performance and brain activity has been often neglected [1]. Noise has different negative effects ranging from interference with cognitive processing to damaging mental and physical health [2]. The non-auditory effects of noise exposure include perceived disturbance, annoyance, cognitive impairment, cardiovascular disorders and sleep disturbance [1]. Noise exposure is a problem in many occupational and non-occupational environments. It is estimated that 22 million workers in the United States are exposed to hazardous noise [3]. It is also reported that 100 million people are exposed to dangerous environmental noise due to traffic, personal listening devices and other sources [4]. The World Health Organization (WHO) estimates that at least 1 million healthy life-years (disability-adjusted life-years) are lost annually as a result of environmental noise in high income western European nations (with a population of around 340 million) [1]. In any vital industry, optimising human performance is a key factor in accident prevention. Noise is one aspect of the work environment that affects workplace safety. Workers in vital occupational roles require high levels of cognitive skill and they need to maintain effective performance while exposed to higher levels of noise than Threshold Limit Values (TLV). Studies show that noise causes cognitive impairment and oxidative stress in the brain [5]. According to Wang et al., with further urbanisation

and industrialisation, noise pollution has become a risk factor for depression, cognitive impairment and neurodegenerative disorders [5]. It has been observed that exposure to noise influences the central nervous system leading to emotional stress, anxiety, cognitive and memory defects [6]. Previous studies have suggested that the Limbic system in the brain is involved in emotional activities, The Amygdala and the Hippocampus are two of the main parts within the Limbic system that receives sensory information directly and indirectly from the central auditory system. Auditory stimulation itself can directly or indirectly affect these areas.

The active process of cognitive selection is called "attention" [7]. Attention plays a significant role in daily activities such as physical movements, emotional responses and perceptual and cognitive functions. When quantifiable information processing is limited, the attention system directs human behaviour based on geographic and temporal characteristics. Noise can affect performance either by impairing information processing or causing changes in strategic responses. In particular, noise increases the level of general alertness or activation and attention. Noise can also reduce performance accuracy and working memory performance, but does not seem to affect performance speed. The scope of cognitive and mental function is diverse, encompassing reaction time, attention, memory, intelligence and concentration, to name a few. Altered cognitive function leads to human error and subsequently increases accidents. This can ultimately lead to reduced performance and productivity. Some studies have shown that noise, improves performance, especially in sleep-deprived workers, mainly due to increased arousal. Certain individuals may be sensitive to noise even when it is lower than TLV. Sensitivity to noise which is referred to as environmental intolerance influence attention and recognition. There are conflicting reports regarding the effect of noise on cognitive performance in the relevant literature. The review study by Gawron regarding the effects of noise on cognitive performance revealed that among 58 studies, 29 reported a negative effect, 7 reported a positive effect and 22 reported no effect of noise on cognitive performance [8]. Noise as a sensory stimulus increases arousal which is believed to cause a reduction in the breadth of attention. In other words, loud noise causes alterations in the performance of attentional functions.

Smith believes that noise characteristics to be one of the influential parameters regarding the effect of noise on cognitive performance [9]. A study by Hockey showed that loud noise at 100 dBA (compared to 70 dBA) increased central visual stimuli processing but reduced peripheral stimulus processing [10]. Exposure to noise above 85 dBA intensity leads to many adverse auditory and non-auditory effects. The non-auditory effects of noise exposure depend on exposure duration, type of task, gender, age and sensitivity to noise. Physiological signals are comprised of: a) signals related to the peripheral nervous system, including heartbeat and Electromyogram and b) signals related to the central nervous system including electroencephalography (EEG). In recent years, interesting results have been obtained from the first group of signals, however, few studies have used EEG signals as a valuable tool for cognitive performance evaluation [11]. Cognitive theory suggests that the brain is highly involved in emotions. Basic emotions use specific cortical and subcortical systems within the brain and are different from the brain's electrical and metabolic activities. Therefore, EEG is one of the most effective and common methods of brain imaging used for Brain activity processing relating to human stress including noise [12]. EEG signals measure all fluctuations in the electrical fields resulting from nerve activity in millisecond resolutions. EEG signals are usually evaluated in multiple frequency bands to determine their relationship with stresses. These bands include the Alpha (8-12.5 Hz), Theta (4-8 Hz), Delta (1-4 Hz) and Beta (12.5-30 Hz) bands. Humphreys and Reveille suggest that fluctuations in the Alpha and Beta bands, in particular, are an indication of cognitive function. Increases in the Alpha frequency band along with decreases in the Beta frequency band causes increased cognitive function [13]. A reduction in the power of the Alpha band along with a rise in the power of the Theta and Beta bands is an indicator of neurological disorders. Marshal et al., have shown a reverse relationship in the prefrontal cortex between the Alpha power rhythm in an EEG and suffering from stressful conditions, meaning that the Alpha rhythm goes down with stress [14]. Choi demonstrated a positive relationship between the Beta power rhythm in an EEG and suffering from stressful conditions in the temporal lobe [12]. Other studies have shown a reduction in the relative power of the Alpha band when attention is reduced. Compared to other imaging techniques, Electroencephalography has certain advantages which include being non-invasive, low cost, comfortable, safe, mobile, and having high time resolution. Therefore, EEG can be a great tool not just for detecting stressors in the environment but also for predicting the negative effects of noise exposure.

Because noise level is one of the influencing factors regarding the effects of noise on cognitive function and brain signals, this study focused on 75, 85 and 95 dBA levels. Also, due to the conflicting results in other studies regarding cognitive function and its importance in many tasks and the few studies on the effects of various noise levels on brain activity patterns, this study was designed in two parts. The first part investigates the effects of various noise levels on mental workload and auditory/visual attention. The second part investigates the effects of noise on the relative power of brain frequency bands and their relationship with visual/auditory attention.

Material and Methods

Study Subjects and Selection Criteria

Study subjects were selected from university student volunteers. The including criteria was 23-33 years of age, normal hearing, no prior cardiovascular disorders, no alcohol and caffeine consumption 12 hours before testing, a BMI index of 18-28, no hypersensitivity to noise and no sleep disorders. After finalising the selection, testing procedures were trained to the study subjects. All participants had to complete ethical consent forms, General Health questionnaires (GHQ) and Weinstein's Noise Sensitivity questionnaires. The validity and reliability of the Persian version of these questionnaires had been approved in other studies [15].

Experimental Design

This experimental study was conducted in an acoustically insulated, climate-controlled room (H = 3 m, L = 3.5 m and W = 2.5 m). A total of 54 participants, including 27 males and 27 females, took part in this study. Study subjects were divided into 3 groups, each with 9 males and 9 females. All study groups were exposed to background noise (45 dBA), and three different noise levels (including 75, 85 and 95 dBA). Table 1 shows the experimental design in detail.

Table 1: Experimental Design

Study Groups	Number of subjects (Total No = 54)	Background Noise (dBA)	Exposure level (dBA)
1	18	45	75
2	18	45	85
3	18	45	95

The study protocol for each subject included a 10-minute relaxing phase before testing, followed by the Integrated Visual and Auditory Continuous Performance (IVA) test which was accompanied by background noise while EEG signals were being recorded. After a 30-minute rest, the subject was exposed to noise for 15 minutes, and at the 16th-minute mark, while the subject was being exposed to various noise levels, the IVA test was initiated, and EEG signals were once again recorded (Figure 1).

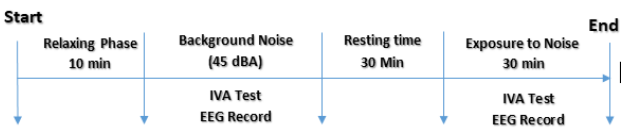

Figure 1: Study protocols timing

Noise Source and Presentation

In this study, the used noise was recorded in a household appliance factory using a B and K PULSE Multi-Analyzer System Type 3560. The recorded noise was then analysed using a B & K Sound Level Meter Type 2238. To modify the noise and obtain steady noise at 75, 85 and 95 dBA levels, the Gold Wave software version 4.26 was used. Finally, the noise was replayed using two Genius HF-2020 speakers situated on either side of the test table.

NASA-Task Load Index (NASA-TLX) Questionnaire

A NASA-TLX questionnaire is a well-known tool for evaluating subjective mental workload (as perceived by the subject). This multi-dimensional method assigns an overall score for mental load based on average weights obtained from six scales including mental demand, physical demand, temporal demand, effort, performance, and frustration. Every part of the task is assigned to a 100-point rating score. The mental load evaluation process using this indicator is comprised of three stages. In the first stage, the six scales are self-assessed by the study subject. In the second stage, after weighing the load of each scale, it is given a score by the subject. Finally, the score and the weight of the load are obtained, and the total mental load score is determined. The validity and reliability of this questionnaire have been approved by Mohammadi in Iran, and its Cronbach alpha score was 0.83 [16].

Integrated Visual and Auditory Continuous Performance Test

Integrated Visual and Auditory test, which was designed by Stanford et al., is part of the Continuous Performance Tests (CPTs) and used to evaluate auditory/visual attention [17]. It consists of a 13-minute continuous auditory and visual test that evaluates two factors of response control and attention. The task involves responding or not responding (response prevention) to 500 test stimuli. Each stimulus is presented for 1.5 seconds. The subject is asked to click once if he/she detects a 1 and not to respond if detecting a 2. This test has an appropriate sensitivity of 92% and a predictive power of 90%. The Persian version of this test has a validity index of 53% to 93% [18].

EEG Recording and Analysis

The EEG signals were recorded from 16 Ag/AgCl electrodes mounted in an elastic cap with the amplifier bandpass set to 1 – 40 Hz at a sampling rate of 250 Hz. The electrodes were placed at the frontal (Fp1, Fp2, F3, F4, F7 and F8), temporal (T3 and T4), central (Cz, C3 and C4), parietal (Pz, P3 and P4) and occipital (O1 and O2) regions. This is according to the international 10-20 system of electrode placement (Figure 2). The reference electrode was the left mastoid (A1 in Figure 2). Impedance was maintained at below 10 KΩ during the experiment. Both in the

background noise condition and during exposure to noise levels of 75, 85 and 95 dBA, while the subject was performing the IVA + Plus test, EEG signals were recorded for 10 minutes with the subject's eyes open. First, the EEG data was pre-processed using an EEGLAB 2013a toolbox [19]. Then, using Independent Component Analysis (ICA) on each electrode, artefacts about blinking, eye movements or small body movements were eliminated.

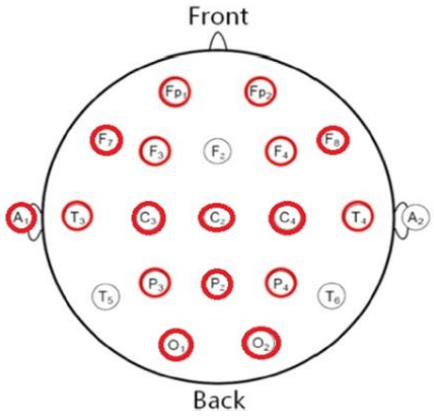

Figure 2: Electrode placement

In order to measure relative power, the filtered signals were separated into various frequency bands (Delta (1-4 Hz), Theta (4-8 Hz), Alpha (8-12.5 Hz), Beta (12.5-30 Hz) and Gamma (30 Hz upwards)) based on their power spectral density using the MATLAB software version 2017b. To calculate the relative power of the frequency bands, the following equations were used:

Let $x_{i\ (n)}$ denote the n^{th} element of i^{th} EEG channel after preprocessing and X = [x_1, x_2... x_{nc}] where NC denotes the number of EEG channel. The Power spectrum of the EEG signal was calculated using Fast Fourier Transform (FFT) which transforms the EEG signal X from the time domain to the frequency domain Z. The FFT of each EEG channel was calculated separately given by the following:

$$z_i(f) = \sum_{n=1}^{N} x_i e^{\frac{-j2\pi fn}{N}} \quad (1)$$

Where f denotes the frequency, N is the sample size; *I* is the channel number and *J* is the imaginary unit. Then absolute power spectrum (PSD) of EEG was calculated using the following:

$$PSD_i(band) = \sum_{n=k_1}^{k_2} z_i^n z_i^{n\prime} \quad (2)$$

Where k_1 and k_2 denote the frequency range of the selected band. The relative power of the selected band was then calculated by the following:

$$R_{PSD_i}(band) = \frac{PSDi(band)}{PSDi(total)} \quad (3)$$

Statistical analysis of the mental workload and attention data was carried out using the SPSS 22 software solution. Before performing t-tests, data distribution norms were checked using the Kolmogorov–Smirnov test. A *p*-value of less than 0.05 was considered statistically significant. The Generalized Estimating Equations (GEE) statistical method was applied for data analysis.

Results

Demographic Characteristics of Participants

Table 2 displays the study subjects' demographic characteristics. A total of 56 individuals, 27 males and 27 females, were enrolled in the study. Average and standard deviation of age and Body Mass Index (BMI) was 26.56 ± 2.45 and 23.81 ± 1.43, respectively.

Table 2. Study subjects' demographic characteristics (N = 54)

Characteristic	M	SD	Max	Min
Age (years)	26.56	2.45	33	23
Weight (kg)	72.65	8.24	90	55
Height (cm)	173.66	7.93	192	158
BMI (kg/m2)	23.81	1.43	27	20

Effect of Noise levels on Mental Workload

Figure 3 illustrates the effects of various noise levels on average overall mental workload compared to background noise (45 dBA) for study subjects. The results show that 75 and 85 dBA noise levels, as compared to just background noise, does not follow a particular trend and does not cause a considerable change in the average mental workload (P > 0.05). At 70 dBA level, compared to just background noise, the mental workload had decreased while at 85 dBA it had increased. At 95 dBA level, compared to just background noise, the increase in mental workload was statistically significant (P = 0.03).

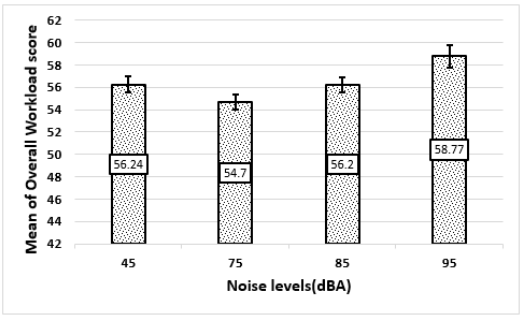

Figure 3: The effect of noise levels on mental workload. Background noise = 45dB (A)

The Effect of Noise levels on Visual and Auditory Attention

Figure 4 presents the average and standard deviation for the visual and auditory attention score at

various levels of noise compared to background noise (45 dBA). The results show that the changes in visual and auditory attention under exposure to various noise levels are very similar in pattern. At 85 dBA levels, average attention scores are reduced, as compared to just background noise, but this is not statistically significant (P > 0.05). But at 95 dBA levels, average attention scores are reduced considerably compared to background noise; this was statistically significant (P < 0.05).

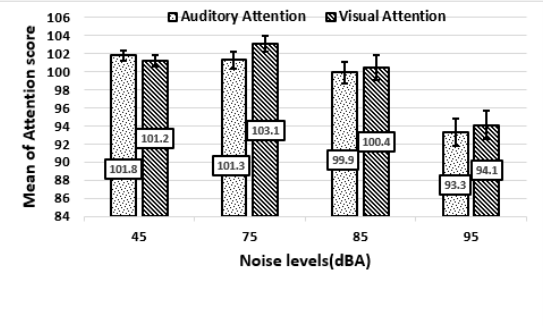

Figure 4: The effect of noise levels on visual and auditory attention

The Effect of Noise levels on EEG Fluctuations

The Kolmogorov – Smirnov test results indicated that the data were distributed normally. Therefore, the t-test was used in this part. The relative power of the intended brain frequency bands was used to analyse brain signals during exposure to various noise levels relative to background noise (45 dBA). The considered frequency bands include the Delta (1-4 Hz), Theta (4-8 Hz), Alpha (8-12.5 Hz), Beta (12.5-30 Hz) and Gamma (30 Hz upwards) bands.

Table 3: Average variation in the relative power of the Alpha band (μV^2) during exposure to noise relative to background noise (45 dBA)

Noise Level (dBA)	75		85		95	
Brain region	t-value	p-value	t-value	p-value	t-value	p-value
Fp_1	0.1273	0.9001	0.0122	0.9903	3.2470	0.0047
F_3	1.4088	0.1769	-0.9717	0.3448	-2.5478	0.0208
F_4	-0.8262	0.4201	0.0675	0.9469	2.4434	0.0257
F_7	2.4367	0.0261	2.2825	0.0356	-0.7458	0.4659
C_4	1.5379	0.1424	2.7946	0.0124	-0.6389	0.5313
P_3	0.3605	0.7229	2.0622	0.0548	2.4443	0.0257
O_1	-0.0213	0.9831	-1.3340	0.1997	5.8788	0.00001*
O_2	0.4069	0.6891	-2.8427	0.0112	2.2478	0.0381

* p < 0.05; FWE corrected.

The results show that among the mentioned frequency bands, the Alpha and Beta bands undergo considerable changes, as relative to just background noise, and are being affected by noise. Based on Table 3, going from 75 dBA to 95 dBA noise level causes a statistically significant average variation in the relative power of the Alpha band for the Fp_1, F_4, P_3, O_1 and O_2 regions of the brain (P< 0 .05). Again, based on Table 3, at 95 dBA, the largest variation in the relative power of the Alpha band is observed for the O_1 region of the brain (P < 0.001).

A significant reduction in the relative power of the Alpha band was only observed for the F_3 region (P<0.05), though a slight reduction was observed for the C_4, F_7 and F_3 regions of the brain also. The most affected areas of the brain when exposed to noise seems to be the Occipital, Prefrontal, Frontal and Parietal regions of the brain. Figure 5A shows the Scalp Topographical mapping.

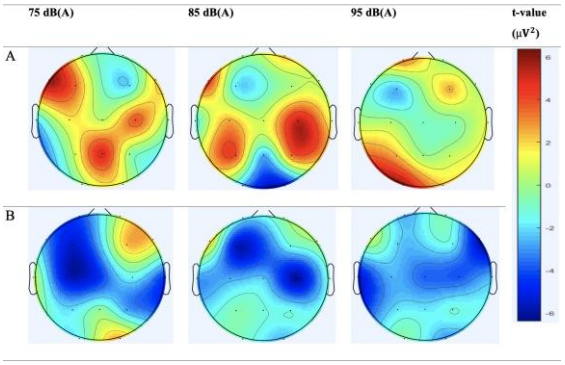

Figure 5: Topographical mapping of frequency bands' relative power during exposure to noise as relative to background noise (45 dBA)

Table 4 demonstrates average variation in the relative power of the Beta band during exposure to various noise levels relative to background noise. The results show a reduction in the relative power of the Beta band in all channels as a result of exposure to 75, 85 and 95 dBA noise, although this reduction was most prominent at 95 dBA. Based on table 4, this reduction is statistically significant (P < 0.05) and the order by which it occurs, and the affected areas are as follows: F8-T3-C4-Cz-O2-Fp1-T4-F3-C3. No significant effect was observed in the other areas of the brain under study (P > 0.05). Also, based on figure 5b, the reduction in the relative power of the beta band as a result of the increase in the level of noise occurs in the Frontal, Temporal, Occipital and Central lobes.

Table 4. Average variation in the relative power of the Beta band (μV^2) during exposure to noise as relative to background noise

Noise Levels (dBA)	75		85		95	
Brain region	t-value	p-value	t-value	p-value	t-value	p-value
Fp1	-1.4331	0.1699	-1.4425	0.1673	-2.7360	0.0140
F3	-1.8798	0.0773	-4.4633	0.0003	-2.2483	0.0381
F8	0.6888	0.5002	0.0489	0.9615	-6.0999	0.00001*
T3	0.2340	0.8177	-2.2907	0.0350	-5.6475	0.00002*
T4	-1.5475	0.1401	-0.8386	0.4133	-2.7236	0.0144
C3	-1.9134	0.0726	-2.2010	0.0418	-2.6735	0.0160
C4	-0.5552	0.5859	-4.8780	0.0001	-4.0165	0.0008
O2	0.9009	0.3802	-2.0361	0.0576	-3.1004	0.0064
Cz	-1.5521	0.1390	-1.8460	0.0823	-3.8259	0.0013
Pz	-0.1543	0.8791	-1.0180	0.3229	-1.9732	0.0649

* p < 0.05; FWE corrected.

Discussion

The results of this study showed that as a stressor, noise affects cognitive performance and brain signals. Also, noise pressure level is an important factor regarding impairment of cognitive function and power spectral density of the brain, meaning that low levels noise is not as effective compared to high levels of noise. It can be said that the results of this study are in agreement with the proposal that a relationship exists between low performance and high levels noise [20]. Previous studies have neglected to investigate cognitive performance during exposure to noise [21], [22]. Some studies have used qualitative measurements including subjective responses for the evaluation of the effects of noise exposure on cognitive function. In this study, however, quantitative indicators were used in combination, including the evaluation of mental workload, evaluation of auditory/visual attention and brain signals (power spectral density) analysis.

In a study by Yoorim Choi, EEG signals were used as a new method for environmental stressor analysis. This method is suggested to overcome the limitations in physiological evaluation techniques [12]. Share et al., also suggest that to improve cognitive and mental stress evaluation, a combination of these tools should be used [23]. Sabine et al. revealed that Stroop and mental arithmetic performance increased when exposed to 50 dBA levels noise compared to 70 dBA levels noise. Melamed et al. stated exposure to higher than 85 dBA intensity noise causes irritability, fatigue and stress which is consistent with the present study [24]. In previous studies, the effects of noise exposure on heartbeat and blood pressure at 95 dBA were compared to 75 and 85 dBA [25]. Elmenhorst et al. demonstrated that noise exposure causes increased reaction times and errors in field and laboratories study [26]. The result obtained by Patricia Tassi et al. indicated that noise exposure reduces attention in subjects which is also consistent with the present study [27]. The effects of high levels of noise exposure on cognitive performance can be amended to the Poulton arousal model which states that noise exposure increases cognitive performance at first. The reason for this is an increase in arousal to reduce the effect of noise on cognitive function. But gradually, the effect of arousal wears off, and the negative effects of noise exposure on cognitive function begin to show [28]. The results in the present study can be explainable using arousal theory. This theory states that the level of central nervous system activity (which alternates between being asleep and awake) regulates human response to stimuli. There is no overall consensus on the validity of this theory at present, and some have suggested that it cannot be used to describe the relationship between noise exposure and cognitive performance. In any case, considering this theory, it can be said that when arousal is high or low, or in other words, in both low stress and high-stress situations, performance is reduced [29].

There were conflicting results regarding the effects of noise on cognitive function in previous studies. Some studies determined that noise had improved cognitive function [30]. While others had concluded that noise had reduced cognitive function [31]. This is part of the reason why, in this study, quantitative measurements were used in combination. The results of the present study reveal that the reduction of cognitive function and brain signals was only significant when exposed to noise at 95 dB level and not at 75 or 85 dBA. This could be due to other psychoacoustic factors such as noise pitch, tonality, exposure duration, and noise type. The importance of noise pitch and its effects on cognitive function and brain activity has been emphasised in other studies. The results of the study by Kazempour et al., showed that “base” noise (low frequency) reduces computational accuracy and performance [32]. Pawlaczyk et al. observed a higher sensitivity to “base” noise that caused reduced cognitive function as compared to reference noise [33]. Naserpour et al., also exhibited that “base” noise at 500 Hz caused longer reaction times as compared to “treble” noise at 800 Hz [34]. The study by Allahverdy and Jafari showed the complexity of brain activity increases at midrange frequencies, showing the effects of the change in frequency on brain activity [35].

Another effective parameter regarding noise and performance is noise tonality. In the study by Joonhee et al., it was observed that performance was reduced with increasing noise tone strengths [36]. Type of noise is also important when evaluating the effects of noise on cognitive function. Studies have shown that the effect of fluctuating noise on cognitive function is higher than steady noise [37]. Steady noise was the only type used in our study. Also, exposure times used were rather short, which may result in a reduced effect of noise on performance and brain signals when exposed to lower than TLV noise. The lesser effect of lower than TLV noise (45, 75 and 85 dBA) on performance and brain activity may also be due to non-psychoacoustic parameters as well. For instance, scope and diversity are influential in the methods used for cognitive function evaluation [38]. Simplicity or complexity of the task is another example as a complex task cause a greater cognitive dysfunction when compared to simple tasks. Personal characteristics may also be a factor when subjects are exposed to noise. As some may experience reduced cognitive function while others may not, and some may even show increased cognitive function [38]. These factors may not be as influential in the present study as the subjects were prescreened for mental disorders, cardiovascular disorders and behavioural abnormalities before selection. Many aspects of brain function and behaviour can only be discussed in terms of neurons communicating with each other. All cognitive processes in the brain are carried out

through neuronal activity such as synapses and spikes. Orientation and executive function which are involved in the processing of attention are specifically undermined to enable information processing. The disruption of attention likely occurs in subjects whenever there is a need for sustained attention.

Here, Brain signal analysis disclosed that the Alpha and Beta frequency bands were affected by noise. With an increase in noise levels, the relative power of the Alpha and increased while the relative power of the Beta band decreased. Topographical mapping of the scalp shows that all four lobes of the brain are usually affected by noise, but this is more pronounced in the frontal and occipital lobes, which is consistent with the results of other studies [39]. Other conclusions can be made from this study regarding the relationship between visual / auditory attention and the relative power of the Alpha and Beta bands. In this regard, it can be said that with increasing noise levels, participants' auditory / visual attention score went down while the relative power of the Alpha and Beta bands increased and decreased respectively. Topographical mapping of the scalp indicates that the area responsible for attention processing is located in the frontal, temporal and occipital regions of the brain which is consistent with the results of Liz et al., [40]. Therefore, the results of this study suggest that when one is exposed to various noise levels, mental workload, visual / auditory attention and the relative power of the frequency bands follow a similar trend. In studies that pertain to brain signals and cognitive performance, attention to artifacts such as eye and body movement, electrical interference, impedance fluctuations, sleep disorders, personality characteristics, age, sex and race are all important, and this has been reiterated in various studies [41]. The benefits of using the NASA TLX and IVA +Plus tests along with EEG signal recording in the psychological and neurophysiological evaluation include the ease of administration, non-invasiveness, short evaluation times and low cost. It is suggested that in future studies on the evaluation of the effects of noise, other psychoacoustic parameters such as noise pitch, tonality and also extended periods of exposure be considered. It is also suggested that more than 16 channels be used for the EEG recordings for better and more detailed evaluations of the various brain regions.

In conclusion, noise levels seem not to have the appropriate sensitivity at levels below 85 dBA on cognitive performance. Therefore, other psychoacoustic parameters that influence cognitive function, including noise pitch and tonality are suggested as candidates for future research. Scalp topographic mapping indicates that the frontal and occipital regions along with the Alpha and Beta frequency bands are most affected by exposure to noise considering the influence of task complexity, personality characteristics, the effects of other psychoacoustic parameters on cognitive and neuro-physiological functions, applying new methods such as the use of brain biosignals along with power spectral density in the evaluation of environmental and occupational stress, especially in the case of noise exposure is suggested. It can thus be concluded that the evaluation of mental workload, auditory / visual attention and brain signals (power spectral density) in combination can be considered as a useful indicator for the assessment of the effects of noise exposure on cognitive performance.

Acknowledgements

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Ethics Approval

The Research and Ethics Committee approved the study proposal of Shahid Beheshti University of Medical Sciences (Ethical code. IR. SBMU. PHNS.1396, 63). Written consent was obtained from the participants after the explanation of the purpose and benefits of research.

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The Awareness of Patients' Bill of Rights among Medical Interns and Medical Students at Tabuk University

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Abstract

BACKGROUND: Incorporating patient priorities and preferences into their healthcare can improve desirable proximal outcomes related to communication such as the patient feeling heard, understood, respected and engaged in their care, which can soften the negative effects of the illness and can help clinicians in decision-making.

AIM: To determine the level of awareness and knowledge of Patients' Bill of Rights and factors affecting it among undergraduate students and medical interns.

METHODS: This is a cross-sectional study carried out in Tabuk city among all medical interns doing their clinical rotations at Tabuk city (n = 70) as well as the 4th, 5th and 6th-year medical students, Tabuk University (n = 219). An English self-administered valid and reliable questionnaire, based on patients` Bill of Rights (PBR) document published by the Ministry of Health (MOH), Kingdom of Saudi Arabia (KSA) was utilised for data collection.

RESULTS: The study included 205 medical students and interns out of invited 289, giving a response rate of 70.9%. All were Saudis. Almost two-thirds (69.3%) reported hearing about patients` bill of rights. Among those who have heard about these rights, 40.2% gained their information from lectures whereas 16.2% gained the information from hospital posters. The total knowledge score about Patients` Bill of Rights ranged between 0 and 32 (out of a possible maximum of 34) with a mean ± SD of 24.6 ± 4.6 and median (IQR) of 25 (23-27). There was a significant positive correlation between student`s age and total score of knowledge of patients` bill of rights, Spearman`s correlation coefficient (r) = 0.18, p = 0.014. The mean rank of the total knowledge score was 83.98 among 4th-grade medical students and reached to 125.07 among medical interns, p = 0.003.

CONCLUSION: Overall awareness and Knowledge of the senior medical students and interns in the College of Medicine, Tabuk University regarding patients` rights age acceptable. However, some deficient issue needs to be improved.

Introduction

Human rights are rights inherent to all human beings, regardless of race, sex, nationality, ethnicity, language, religion, or any other status [1].

The term “human rights” refers to those rights that have been recognised by the global community in the Universal Declaration of Human Rights (UDHR), adopted by the United Nations (UN) the Member States in 1948, and in other international legal instruments binding on States [2]. Human rights are not only a generic term representing a symbol of our contemporary society but are also the reflection of a common perception of human values [3]. Health is a major part of our human rights and our understanding of a life in dignity [4]. The World Health Organization (WHO) defined health as “a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity”.

WHO also states that “the enjoyment of the highest attainable standard of health is one of the fundamental rights of every human being without distinction of race, religion, and political belief, economic or social condition [5]. Patients' rights differ from country to another and from different authorities, also depending upon prevailing cultural and social norms [6]. Saudi Ministry of Health issued a Patient’s

Bill of Rights (PBR) in 2006 and defined it as "Patient's rights are policies and rules that must be preserved and protected by the Health facility toward patients and their families."

In recent years, the concerns about the patients' values and preferences of treatment have raised, to participate in the decision-making process. Incorporating patient priorities and preferences into their healthcare can improve desirable proximal outcomes related to communication such as the patient feeling heard, understood, respected and engaged in their care, which can soften the negative effects of the illness and can help clinicians in decision-making. This would enhance the medical and physiological outcomes, as well as result in decreased anxiety, greater confidence in and adherence to doctor's treatment plans, increased satisfaction with care and higher levels of trust in healthcare providers [7]. Human rights principles that apply to patient's care include the right to get the highest attainable standard of health, which covers both positive and negative guarantees in respect of health, as well as political rights ranging from the patient's right to be free from torture and cruel treatment to liberty and security of person. They also brought to the attention the right of socially excluded groups to be free from discrimination when providing health care. Critical rights that relevant to health care providers involve the freedom of association and the enjoyment of decent work conditions [8]. Patients have the right to accept standards of quality care, to treatment within the available resources and with a high level of personal dignity. They also have the right to receive all the necessary information regarding the individual(s) responsible for their care, treatment and services.Patients have the right to receive complete details regarding their diagnosis, treatment, procedures and prognosis of illness in a way and language that is easily understood, and the same should be considered while drafting the informed consent form [9].To make sure the rights of patients are protected requires more than educating policy measures and health providers; it requires educating people about what they should expect from their health care providers, about the kind of treatment they should receive [10]. Patients' bills of rights are derived from the values and ethics of the medical profession. Like the right of informed consent, confidentiality, privacy, autonomy, safety, respect, treatment choice, refuse the treatment and participating in the treatment plan [11]. Patients must be competent to understand the relevant information and the decision choices and must not be enforced into accepting treatment against their wishes.

Many studies have been conducted internationally to assess the awareness and implementation of Patients' Bill of Rights among undergraduate students, medical interns and physicians but only a few studies done locally and no single study has been done in Tabuk area. In a study done in Saudi Arabia in 2012 by Saad Abdullah Alghanim titled "Assessing knowledge of the patient bill of rights in central Saudi Arabia: a survey of primary health care providers and recipients" explore the implementation of the PBR that was introduced recently in the Saudi health care system and showed that more than three quarters of patients and one third of PHC providers did not know about the existence of the bill. Among those who knew about its existence, about three-quarters of patients and almost half of PHC providers had little (or very little) knowledge about the bill contents. In general, patients scored lower means of perception than PHC staff about the implementation of the bill's aspects. PHC staff reported several obstacles that may hinder the implementation of the PBR in Saudi Arabia [12].

Another study done in Riyadh, Saudi Arabia in 2014 by Salwa B. El-Sobkey and her colleagues with a title "Knowledge and attitude of Saudi health professions' students regarding patient's bill of rights" was aimed to investigate the knowledge of health professions' students at College of Applied Medical Sciences (CAMS) Riyadh Saudi Arabia regarding the existence and content of Saudi PBR as well as their attitude toward its ineffectiveness. The results showed that half (52.3%) of the students had perceptual knowledge regarding the existence of Saudi PBR and only 7.9% of them were knowledgeable about some items (1-4 items) of the bill. Privacy and confidentiality of the patient were the most common known patient's rights. Students' academic level was not correlated to neither their knowledge regarding the bill existence or its content nor to their attitude toward the bill. The majority of the students (93%) reported that only one course within their curriculum was patient's rights-course related. About one quarter (23.4%) of the students reported that teaching staff used to mention the patient's rights in their teaching sessions [6].

In a recent and local study done in 2017 in the Eastern Province of Saudi Arabia by Sarah A. Al-Muammar and her colleague to determine the doctors' knowledge of patients' rights at King Fahd Hospital of the University. The researchers found that about 44% of physicians had adequate knowledge about PBR and 55.56% had inadequate knowledge. Regarding physician's response to each item of PBR, the majority (98.1%) gave the correct answer to Item 2: "Patients should know the identity and professional status of the healthcare providers responsible for their treatment" (98.1%). Item 25: "Doctors are entitled to withhold any procedures related to a patient's condition if the patient refuses their choice of treatment" was the item with the least correct response (15.5%) and suggested that the institution should provide training and motivate physicians, especially younger doctors regarding PBR to ensure good health for all and safeguard the integrity of both the physician and the hospital [13].

Another study done in Mecca city, Saudi Arabia by Hager A. Saleh and her colleague titled

"Physicians' Perception towards Patients' Rights in Two Governmental Hospitals in Mecca, KSA", In this research paper the perception of physicians concerning patients' rights and their fulfillment in two governmental hospitals in Mecca, Saudi Arabia is compared, using a self-administered questionnaire which examined the physicians' knowledge, attitude and perception towards these rights. Results of this study demonstrated the physicians' opinion about patients' rights. Regarding hospital (A), the agreement of physicians on investigated rights to be a patient right in their working hospital ranged from 85.7% up to 100% for 6 rights investigated. Regarding hospital (B), the agreement ranged from 73.1% up to 100% for 4 rights investigated.

All physicians in both study hospitals indicated that the rights to know the name of attending physician, the right to be treated with caring and respect and the right to know treatment alternatives is considered an actual patient right. They concluded the study by saying that there is a similar discrepancy between physicians in both hospitals, most physicians are aware of patients' rights and in particular of the basic human rights as respect, privacy and confidentiality and most of the physicians agreed on the importance of the patients' rights in both hospitals while only a few percentages of them reported that patients' rights were maintained in both hospitals [26].

A study done in 2012 in Iran aimed to assess the knowledge of students about patient Rights and its relationship with some factors. A survey was conducted on 270 medical and paramedical students of Hamedan in simple randomised sampling. Data collecting instruments were a questionnaire form that contains demographic information and educational questions regarding patient rights which its reliability and validity were made through the same measurement by two researchers.

Based on survey results mean of awareness were 10.3 with a standard division of 1.5%. Forty-seven percent of the students mentioned who are not familiar with the Bill of Rights. Low awareness was 31%, medium 53%, and high awareness was only 16%, in total. There was not any statistically significant relationship between awareness and any demographic variables. According to this study, awareness of most students about patient rights was low. So, promote awareness in the field of educational planning should be done [27].

Another study was done in 2011 in South Africa to elicit South African medical students' experiences of witnessing patient rights abuses and professional lapses during their clinical training. Of 223 students surveyed, 183 (82%) responded, 130 (71%) of whom reported witnessing patient rights abuses and professional lapses, including physical abuse (38%), verbal abuse (37%), disrespect for patients' dignity (25%), and inadequately informing patients about their treatment (25%). Students attributed abuse to stressed health workers, overburdened facilities, and disempowered patients. Most students who witnessed abuse (59%) did not actively respond, and 64% of survey respondents felt unprepared or uncertain about challenging abuses in the future. Interviews with 28 students yielded detailed accounts of the abuses witnessed and of students' emotional reactions, coping strategies, and responses.

Most students did not report abuses; they feared reprisal or doubted; it would make a difference. These results highlight the need to align medical ethics and human rights with medico-legal protocols in theory and clinical practice [28].

Material and Methods

This is a cross-sectional study; it was carried out in Tabuk city, which is the capital city of the Tabuk Region in northwestern Saudi Arabia. This study concentrated on two subjects: Medical interns doing their clinical rotations at Tabuk city and the 4th, 5th and 6th-year medical students at Tabuk University. The total number of participants is 289 participants.

An English self-administered questionnaire was given to all participants. It was used previously in a Saudi study and proved that it was valid and reliable [13]. It is based on PBR document published in 2007 by the Ministry of Health (MOH), Kingdom of Saudi Arabia (KSA) [14]. It consists of three sections. Section 1 includes the demographics of the participants (age, gender and professional status). Section 2 inquiries about the experience with patients` rights (History of hearing about patients' bill of rights, Source of hearing about patients' bill of rights and history of reading the bill and knowing its contents). The third section includes 34 statements to explore participant`s knowledge regarding PBR. All items have three possible responses; "Agree", "Disagree", and "Do not know". Each correct answer was assigned a score of 1 and for every incorrect or "don`t know" responses, a score of "0" was assigned. The total score was computed for each participant, tested for normality of distribution and utilised for comparisons

Approval of the research proposal was obtained from the Regional Research and Ethics Committee. Administrative approvals from the Dean of College of Medicine, Tabuk University, was obtained. Verbal consents were taken from all participants before data collection. Confidentiality of information was assured.

The collected data were analysed with the help of a biostatistician using Statistical Package for the Social Sciences (SPSS) program version 25

developed by International Business Machines (IBM®) Corporation.

Descriptive analysis like frequencies, percentages, mean, range and standard deviation were used. Since the total PBRs knowledge score was abnormally distributed as seen by significant Shapiro-Wilk test, non-parametric statistical tests were applied; Mann-Whitney test to compare two groups and Kruskal-Wallis test to compare more than two groups. Spearman`s correlation test was utilised to correlate between two continuous variables. P-values of less than 0.05 were considered significant.

Results

The study included 205 medical students and interns out of invited 289, giving a response rate of 70.9%. The age was available for 193 participants and ranged between 21 and 29 years with a mean±SD of 23.3±1.4 years. All were Saudis. Table 1 shows their gender and professional status distribution. About two-thirds (68.3%) were females, and 30.2% were recruited from the 4th year medical students, whereas 22.4% were interns.

Table 1: Gender and professional status of the participants (n = 205)

	Frequency	Percentage
Gender		
Male	65	31.7
Female	140	68.3
Professional status		
4th-year medical student	62	30.2
5th-year medical student	45	22.0
6th-year medical student	52	25.4
Medical Intern	46	22.4

Experience with patients' bill of rights

A group of 69.3% of the participants reported hearing about patients` bill of rights. Among those who have heard about these rights, 40.2% gained their information from lectures, whereas 16.2% gained the information from hospital posters, Figure 1.

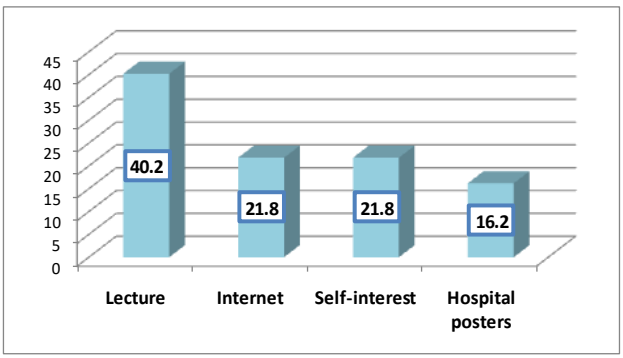

Figure 1: Source of hearing about patients' bill of rights among the participants (n = 142)

Among those who have heard about PBRs, 54.2% have read the bill and knowing its contents.

Knowledge about patients` bill of rights

Majority of the participants could recognize that patient should be notified about the diagnosis and all treatments updates in an understandable language (98.5%), consent must be written in a language understandable by the patient (97.1%), the medical team should report any violence against children to the concerned authority (95.1%), patient has the right to know in advance about his treatment cost and insurance coverage (95.1%), patients should be examined in a private examination room (94.8%), in order to get the patient participation in a research he must be provided with clear and comprehensive information (91.2%), patient should be aware of both common and rare complications (91.2%), patients have the right to complain to the administration (90.7%) and patients are required to be treated with courtesy and respect during times of emergency (90.2%).

Table 2: Knowledge of the participants about different elements of the Patients` Bill of Rights

	Right response	
	No.	%
1. Patients are not required to be treated with courtesy and respect during times of emergency (Disagree)	185	90.2
2. The patient should know the identity and professional status of the health care providers responsible for his treatment (Agree)	168	82.0
3. A patient is entitled to know the name of the physician performing the procedure except in emergency case (Agree)	131	63.9
4. Patients are entitled to know a method of contacting his treating physician (Agree)	121	59.0
5. The patient should be notified about the diagnosis and all treatments updates in an understandable language (Agree)	202	98.5
6. patient's culture & beliefs should be respected even if it was against medical advice (Agree)	151	73.7
7. A patient may have the possibility of obtaining a second opinion within the same hospital or another (Agree)	171	83.4
8. Patients should be examined in a private examination room (Agree)	194	94.8
9. When examining a patient, a third party should be present (Agree)	156	76.1
10. Treatment options should be discussed within the health team; patients are only entitled to know the treatment plan (Disagree)	93	45.4
11. The patient's medical record can be accessed by the health care team – Researchers – other hospital Staff (Disagree)	101	49.3
12. A doctor can disclose an adult patient information to anyone upon his permission (Agree)	134	65.4
13. A doctor can disclose patients information to a research team without his permission (Disagree)	158	77.1
14. A doctor can disclose adult patients information to a specific family member (Father-Husband-Wife) without his permission (Disagree)	179	87.3
15. A doctor can disclose patients information to the judicial department only with his permission (Disagree)	56	27.3
16. A doctor can disclose patients information in case of communicable diseases (Agree)	156	76.1
17. Visitors have the right to know about the patient's condition (Disagree)	171	83.4
18. Procedures or interventions should be briefly discussed with the patient (Agree)	116	56.6
19. A consent form is required for both routine and emergent lifesaving procedures (Agree)	131	63.9
20. Written consent is required in all procedures even if a verbal consent was acquired (Agree)	171	83.4
21. Consent must be written in a language understandable by the patient (Agree)	199	97.1
22. The patient should be provided by one consent for different interventions like surgery, anaesthesia, radiology (Disagree)	96	46.8
23. The patient should be aware of both common and rare complications (Agree)	187	91.2
24. Treatment procedure should be done even if refused by the patient (Disagree)	175	85.4
25. Doctors are entitled to withhold any procedures related to a patient condition if the patient refuses their choice of treatment (Disagree)	55	26.8
26. To get the patient participation in research, he must be provided with clear and comprehensive information (Agree)	187	91.2
27. Patient in governmental hospitals doesn't have the right to refuse participation in any research done by the hospital (Disagree)	160	78.0
28. The patient doesn't have the right to quit after agreeing to participate in research (Disagree)	135	65.9
29. The patient has the right to know in advance about his treatment cost and insurance coverage (Agree)	195	95.1
30. The patient doesn't need to know about treatment cost if he was covered by insurance (Disagree)	124	60.5
31. The patient has the right to request a medical report at any time (Agree)	144	70.2
32. The patient has the right to choose his statements to be written in the medical report (Agree)	49	23.9
33. Patients have the right to complain to the administration (Agree)	186	90.7
34. The medical team should report any violence against children to the concerned authority (Agree)	195	95.1

On the other hand, less than half of them could recognize that the patient's medical record cannot be accessed by health care team, researchers or other hospital staff (49.3%), patient should not be provided by one consent for different interventions like surgery, anaesthesia, radiology (46.8%), treatment options should not be discussed within the health team, patients are only entitled to know the treatment plan (45.4%), a doctor cannot disclose a patients information to judicial department only with his permission (27.3%), doctors are entitled not to withhold any procedures related to a patient condition if patient refuses their choice of treatment (26.8%) and patient have the right to choose his statements to be written in the medical report (23.9%).

The total knowledge score about Patients` Bill of Rights was abnormally distributed as shown by significant Shapiro-Wilk test ($p < 0.001$). It ranged between 0 and 32 (out of a possible maximum of 34) with a mean ± SD of 24.6 ± 4.6 and median (IQR) of 25 (23-27), Figure 2.

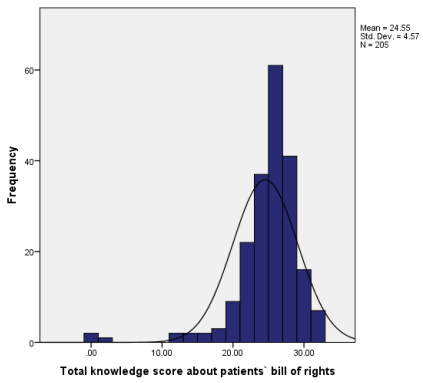

Figure 2: Knowledge score of the medical students and interns in Tabuk University regarding Patients` Bill of Rights

Factors associated with knowledge about the Patients` Bill of Rights

- Participants` age

There was a significant positive correlation between student`s age and total score of knowledge of patients` bill of rights as shown in Figure 3, Spearman`s correlation coefficient (r) = 0.18, p = 0.014.

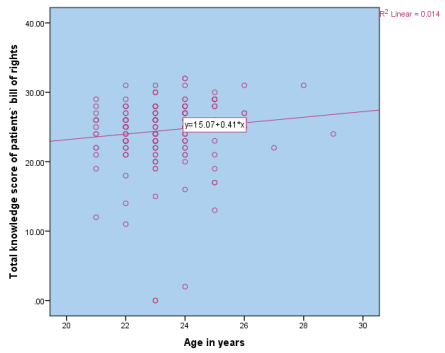

Figure 3: Correlation between student`s age and total knowledge score of Patients` Bill of Rights

- Participant`s gender

As clear from Table 3, there was no statistically significant association between participants` gender and total knowledge score about patients` bill of rights.

Table 3: Association between participant`s gender and total knowledge score about patients` bill of rights.

Gender	Total knowledge score about patients` bill of rights		
	Median	IQR	Mean rank
Male (n = 65)	25	22.5-27	99.05
Female (n = 140)	25	23-27	104.84

IQR: Inter-quartile range; P value of Mann-Whitney test = 0.513.

- Professional status

It is evident from table 4 that there was a statistically significant increase in the level of knowledge regarding PBR with advancing in the professional status as the mean rank of the total knowledge score was 83.98 among 4th-grade medical students and reached to 125.07 among medical interns, p = 0.003

Table 4: Association between participant`s professional status and total knowledge score about patients` bill of rights.

Professional status	Total knowledge score about patients` bill of rights		
	Median	IQR	Mean rank
4th year medical student (n = 62)	24	22-26	83.98
5th year medical student (n = 45)	26	23-26	97.46
6th year medical student (n = 52)	25.5	23.25-27.75	110.95
Medical Intern (n = 46)	26	25-29	125.07

IQR: Inter-quartile range; P value of Kruskal-Wallis test = 0.003.

- Source of hearing about Patients` Bill of Rights

Although the highest level of knowledge was observed among those who had their information about PBR from hospital posters (mean rank was 85.59), compared to other sources (lecture, internet and self-interest), the association between source of hearing about PBR and knowledge about it was not statistically significant.

Table 5: Association between the source of hearing about Patients` Bill of Rights and total knowledge score about it among the participants.

Source of hearing about patients' bill of rights	Total knowledge score about patients` bill of rights		
	Median	IQR	Mean rank
Lecture (n=57)	25	23-28	69.98
Internet (n=31)	24	21-26	60.42
Self-interest (n=31)	26	23-28	74.92
Hospital poster (n=23)	26	25-27	85.59

IQR: Inter-quartile range; P value of Kruskal-Wallis test = 0.152.

- History of reading the bill and knowing its contents

There was no statistically significant association between history of reading the bill and knowing its contents and total knowledge score about it among the participants, as shown in Table 6.

Table 6: Association between the history of reading the bill and knowing its contents and total knowledge score about it among the participants.

History of reading the bill and knowing its contents	Total knowledge score about patients` bill of rights		
	Median	IQR	Mean rank
No (n=65)	25	23-27	68.08
Yes (n=77)	26	23-27.5	74.38

IQR: Inter-quartile range; P value of Mann-Whitney test = 0.361.

Discussion

The patients' bill of rights (PBR) has been introduced in the Saudi health care system several years ago, despite that, awareness about it is not adequate as evidenced by previous studies carried out among different categories and different places such as primary health care providers and recipients in central Saudi Arabia [12], physicians working at a university hospital in the Eastern Province of Saudi Arabia [13], patients admitted to hospitals in Al-Madinah Al-Munawarah [15], patients attending outpatients `clinics in Taif [16], and students of College of Applied Medical Sciences in Riyadh [6].

During clinical training, medical students and interns are in direct contact with patients; therefore, they should be aware of patients' rights and also should respect patients and keep their information confidential [17]. The awareness and knowledge of patient right is the initial step for doing work in the right way, so it is impossible to implement it without having sound knowledge about it [18]. Since the awareness and knowledge of patients' rights in medical practice service is important for future physicians, it is important to investigate clinical years` medical students and interns` knowledge about patient`s bill of rights. Therefore, this study aimed to explore their knowledge about PBRs in Tabuk.

The overall knowledge score about Patients` Bill of Rights in the present study ranged between 0 and 32 (out of a possible maximum of 34) with a median (IQR) of 25 [23], [24], [25], [26], [27], which indicates an intermediate level of knowledge. In a similar study conducted by Saeede et al. (2016) [19], the knowledge score of medical students regarding patients` rights in operation room was 20.06 ± 3.41, keeping in mind that different tools were utilised in both studies. In Iran [20], about 53% of the medical students had an inadequate awareness about patient's bill of rights with a mean of awareness of 10.3% with a standard division of 1.5%. In another Iranian study [21], 35.6% of the students had poor knowledge, and 27.7% and 36.7% had moderate and good knowledge, respectively. Khodamorad et al. [22] reported that 68.4% of students were satisfactorily knowledgeable of the patients' right to have access to medical services. Also observed that 71.5% of them had a sufficient knowledge of a patient's right to accept or refuse treatment and 69.8% were aware of the confidentiality of a patient's information. However, Rangrazjedi and Rabee reported that only 23% of the students had a satisfactory awareness of patients' rights in the area of access to medical services [23] Yaghoubi reported in his study that the majority of the medical and nursing students had sufficient knowledge regarding patients' rights [24]. Almost half (52.3%) of students of College of Applied Medical Sciences in Riyadh were knowledgeable about the existence of Saudi PBR, and only 7.9% were able to recognise some items (1–3) of the bill [6]. Comparison of various studies in this regard is impossible due to using different tools and methods to assess the knowledge regarding PBRs.

In the present study, almost two-thirds of the students and interns were aware of the patients' rights, and the majority of them were knowledgeable concerning patients` rights in different aspects regarding diagnosis, treatment, privacy, respect and confidentiality. The same has been observed in other studies carried out among physicians and students. [6], [12], [13]. However, low rate of knowledge was observed regarding some important issues such as accessing of patient's medical record cannot be done by health care team, researchers or other hospital staff, providing consent for each of different interventions, discussion of treatment options within the health team, a doctor cannot disclose a patients information to judicial department only with his permission and are entitled not to withhold any procedures related to a patient condition if patient refuses their choice of treatment and patient have the right to choose his statements to be written in the medical report. In a similar study carried out in Iran, most of the medical students were aware of freedom of the individual patient while the lowest level of awareness was observed regarding the right of access to health care [20].

The main source of knowledge about patients` rights in the present study was lectured. This finding necessitates organising an educational session to senior medical students and interns regarding patients` rights.

In the present study, no gender difference was observed regarding the knowledge of patients` rights. In another study carried out by Saeede et al. in Iran [19], female students` knowledge was higher than male students`. In another Iranian study, Rangrazjedi et al. observed the same [23].

A significant relationship was found between age and academic level of the students and interns and their knowledge regarding patients' rights as the highest level was observed among interns and those with advancing age. In another Saudi study carried out among students of the College of Applied Medical Sciences in Riyadh [6], students' academic level was not correlated to their knowledge regarding patients` rights. However, in the present study, we included

interns besides students. In Iran, awareness of patients` rights was not significantly associated with any studied socio-demographic factor [20]. In another study carried out in Iran, knowledge of patients` rights was significantly associated with age, gender, educational level and health education [21].

It has been documented that awareness of patient right among medical students is essential, but its application in the future is more essential [25].

In conclusion, overall awareness and Knowledge of the senior medical students and interns in the College of Medicine, Tabuk University regarding patients` rights age acceptable. However, some deficient issue needs to be improved such as accessing of patient's medical record by health care team, researchers or other hospital staff, providing consent for each of different interventions, discussion of treatment options within the health team, a doctor cannot disclose a patients information to judicial department only with his permission and are entitled not to withhold any procedures related to a patient condition if patient refuses their choice of treatment and patient have the right to choose his statements to be written in the medical report. There was age, and the academic level difference between the participants in this regard as older students and interns were more knowledgeable of patients` rights. The main source of information regarding the Patients` Bill of Rights was lectured.

Acknowledgement

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Quantitative Estimation of Anti Hypertension Combination by Ratio Subtraction Spectrophotometry Method

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Keywords: Irbesartan; Hydrochlorothiazide; Spectrophotometry ultraviolet; Ratio subtraction

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Abstract

BACKGROUND: Irbesartan and hydrochlorothiazide are a group of anti-hypertensive drugs that are very effective and safe to use to reduce blood pressure and oedema. The combination has a small active ingredient content so that if the treatment didn't meet the requirements for therapeutic doses, it not achieved to the maximum therapy.

AIM: The research aims to the simultaneous determination of irbesartan and hydrochlorothiazide in tablets by Ratio subtraction spectrophotometry method.

METHODS: The absorption spectra and sample measurement in the Ratio subtraction method performed on Irbesartan at a wavelength of 247.6 nm and 273.6 nm for the Hydrochlorothiazide (HCT) using 0.1 N NaOH as a solution. This method is validated with linearity, accuracy, and precision in intraday and interday, LOD and LOQ and applied in the determination of a mixture of irbesartan and hydrochlorothiazide in the dosage tablet.

RESULTS: The validation test for IRB is 101.03 for accuracy, with a precision of 0.57; with precision testing at intraday 0.34 and interday 1.34, and LOD is 0.70 and LOQ is 2.12. Meanwhile, validation for HCT that the accuracy 100.34%; precision 0.89 and precision on intraday 1.20 and interday 1.18, and LOD 0.78 and LOQ 2.37 with IRB levels are 101.03 ± 0.63% and HCT is 100.59 ± 0.91%.

CONCLUSION: The ultraviolet spectrophotometric method in subtraction ratio method was validated a method of linearity, accuracy, precision in intraday and interday, LOD, and LOQ and according to ICH guidelines and successfully applied for the determination simultaneous of irbesartan and hydrochlorothiazide in the tablet's dosage form.

Introduction

Clinically the problem-related side effects may be solved by introducing low-dose combination drugs for first-line antihypertensive therapy. The combination of Irbesartan and Hydrochlorothiazide is one of the right combinations for antihypertensive and effective to reduce blood pressure and oedema in hypertensive patients who are usually accompanied by complications and minimal side effects, namely hyperkalemia [1]. The combined use of angiotensin receptor (AR) blockers and diuretics is better tolerated, but more costly, than generic ACE inhibitors and diuretics, mostly because of the absence of cough and much lower incidence of angioedema [2].

Irbesartan (IRB), with chemically names 2-Butyl-3 - [[20- (1H-tetrazole-5-yr) [1, 10-biphenyl] -4-yl] methyl] 1, 3-diazaspiro [4.4] non-1 -en-4-one is angiotensin II receptor (ARB) inhibitors are used to treat hypertension and diabetic nephropathy. Because blood vessels can narrow due to the influence of angiotensin II and serves to inhibit these effects, thus dilating blood vessels and reducing pressure on blood vessels [3]. Irbesartan is an angiotensin II antagonist (AT1 receptor subtype) which is the primary vasoactive hormone involved in RAAS and a strong vasoconstrictor, and is formed from angiotensin I through a reaction catalysed by angiotensin-converting enzyme (ACE, kininase II). Irbesartan works against angiotensin II by blocking the effects of vasoconstrictor and aldosterone secretion from angiotensin II by binding selectively to the angiotensin II AT1 receptor [4].

Hydrochlorothiazide (HCT), with chemical

names 6-Chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide 1, 1 dioxide is a group of diuretics and first-line drug therapy for the treatment of hypertension. Thiazide diuretics class of highly effective in preventing stroke and heart failure in patients with hypertension [3]. Hydrochlorothiazide is a treatment for mild to moderate hypertension. Often in more severe cases combined with other drugs to strengthen the effect, especially beta-blockers. Combination of ACE inhibitors, reducing resistance to activated renin-angiotensin-aldosterone system (RAAS) [2].

The combination of IRB with HCT significantly reduces BP in patients not controlled by IRB or HCT alone. The addition of IRB had positive effects on HCT induced biochemical abnormalities. In matrix studies, IRB appeared to blunt hypokalemia associated with HCT, and uric acid and total cholesterol levels were lower with the combination than with HCT monotherapy. IRB did not increase the HCT associated increases in serum triglycerides. Finally, IRB-HCT combination brings positive effects of IRB on undesired effects of diuretic, provides advantages of AR blockade, decreases resistance because of activated RAAS, simplifies treatment regimen, besides providing better BP control. Moreover, HCT may increase protective organ benefits of the AR antagonist by providing better BP control in this combination [1], [2], [4], [5], [6], [7].

The Irbesartan level was determined by ultraviolet spectrophotometry with acid solvents at wavelengths 224 and 246 nm, and hydrochlorothiazide assayed by ultraviolet spectrophotometry in an alkaline solvent at a wavelength of 274 nm and high-performance liquid chromatography method with buffer-acetonitrile mobile phase P (6: 4) and a mixture of monobasic sodium phosphate P 0.1 P M-acetonitrile (9: 1)) [3], [8]. Several articles have been published regarding the determination of the mixture of irbesartan and hydrochlorothiazide levels, among others, by using high-performance liquid chromatography, by capillary electrophoresis [8], [9], the zero-crossing method with spectrophotometry [10].

Spectrophotometry is a simple, fast and relatively easier method compared to other methods but the main problem with a mixture of binary or ternary spectrophotometry analysis is the determination of compounds simultaneously in the same mixture of drugs without prior separation [3.7]. Several studies of simultaneous spectrophotometric determination of the combinations of IRBs and HCTs have been published, among others, methods the zero-crossing method with spectrophotometry, Simultaneous equation and absorbance ratio, ratio subtraction coupled with constant multiplication, ratio difference and constant centre [9], [10], [11].

Ratio subtraction method (RSM) is one of the spectrophotometric methods that can be used to analyse two or more mixtures of drugs simultaneously without having to do a separation, easily applied to the routine analysis and without the need for derivatisation first. Several articles on the establishment of a mixture by using RSM has published, among others, the determination of omeprazole, tinidazole, and clarithromycin with ethanol, Benazepril and amlodipine with methanol and Timolol and dorzolamide [12], [13], [14], [15]. The ratio subtraction method shows that this method is very simple, accurate, and does not require complicated mathematical calculations using only constants in the spectrum of ratios that can be applied to the IRB and HCT levels. [16], [17], [18].

The purpose of this study is to prove the subtraction ratio method can be used to determine IRB and HCT levels in tablet dosage forms.

Material and Methods

Material

Pharmaceutical grades of IRB were from the National Agency of Drug and Food Control of the Republic of Indonesia, HCT was from PT Kimia Farma. Tablet C (PT. Sanofi) contained 300 mg IRB and 12.5 mg HCT, NaOH, Methanol.

Apparatus and conditions

UV-Visible Spectrophotometer (Shimadzu 1800) with a computer equipped with UV probe 2.43 software (UV-1800 Shimadzu), the absorption was recorded at a wavelength of 200-400 nm using UV-probe software, Analytical balance (Sartorius), sonicator (Branson 1510).

Preparation of standard stock solution

Carefully weighed of 50 mg IRB and HCT, then transferred to a 50 mL volumetric flask dissolved it in 0.1 N NaOH by adding it to the line. Stock solution concentration was 1000 µg/ml. Pipetted 5 mL of stock solution transferred to a 50 mL volumetric flask diluted it using 0,1 N NaOH by adding it to the line, and the concentration would be 100 µg/mL that is working solution.

Validation test

The solution standard for IRB and HCT for absorption spectrum was made by the selected wavelength points 247.6 nm for IRB, while the HCT used wavelengths of 273.4 nm. Determined zero orders from series C are used after manipulate to get a regression equation for each component [19], [20].

Precision

Reparability of the methods was studied by repeating the methods six times. To study intra-day precision, a method was repeated three times in a day. Similarly, the method was repeated on three different days to determine inter-day precision.

The determination of precision is based on the relative standard deviation (RSD) value 2% [19], [20].

Intraday and interday precision

Intraday and interday are precision measurements with simultaneous samples. Intraday is a repetition that is done every day in one day, during every day at certain hours in a few days. Intraday and repetition at each concentration. Intraday is three repetitions on the same day (morning, afternoon, evening) and three repetitions with 3 different days (days 1, 2, 3). Determination of interday and intraday precision was seen from its relative standard deviation < 2.5% [19].

Accuracy

Accuracy test was calculated by measured recovery percentage in three specific points which were: 80%, 100%, and 120%. In each of the specific points, the test used 70% from the sample and 30% from the pure active substances (standard addition method) [19], [20].

Construction of Absorption Maximum Spectrum and Ratio Absorption Spectrum

The working solution was pipetted each containing 10 µg/mL IRB and 8 µg/mL HCT and a mixed solution of 10 µg/mL IRB and 8 µg/mL HCT then each transferred in a 25 mL volumetric flask Diluted using 0.1 N NaOH, and measured the absorption spectrum. Furthermore, are used 5-15 µg/mL IRB solution and 4-12 µg/mL for HCT and a mixture of the two drugs in the same range and prepared for made an absorption spectrum of RSM then scanned in the range of 200-400 nm and overlapped the third spectrum. Useful for the ratio subtraction method (RSM) extended ratio subtraction method (EXRSM) method, where the mixture of IRB and HCT shows overlapped spectra, IRB represents unextended spectrum and HCT representing an extended-spectrum [3], [9], [10].

Determination IRB and HCT mixture by RSM

The IRB and HCT can determined by using the ratio subtraction method by a standard spectrum of 8 µg/mL HCT´ as a divisor producing a new curve that represents IRB/HCT´ + HCT/HCT´ (constant) and 10 µg/mL IRB as a divisor producing a new curve that represents HCT/IRB´ + IRB /IRB´ (constant). Then the curve is subtracted with constant values (HCT/HCT´) and (IRB /IRB´), then multiplication curve by the standard spectrum of 8 µg/mL HCT´ for IRB and 10 µg/mL IRB´ for HCT which is the same divisor used, therefore the obtained spectrum is zero-order absorption spectrum of IRB and HC. Then can be summarised as the following [8], [9]:

$$\frac{X+Y}{Y^o} = \frac{X}{Y^o} + \frac{Y}{Y^o} + Constant \dots\dots\dots\dots\dots\dots\dots\dots .1$$

$$\frac{X}{Y^o} + Constant - Constant = \frac{X}{Y^o} \dots\dots\dots\dots\dots\dots\dots .2$$

$$\frac{X}{Y^o} \; x \; Y = X \dots\dots\dots\dots\dots\dots\dots\dots .3$$

$$Y = \frac{X+Y}{X^o} + \frac{X}{X^o} - \frac{Y}{X^o} \dots\dots\dots\dots\dots\dots .4$$

Determination of the constants directly derived from the curve with a straight line parallel to the X-axis is the wavelength in the region where y expanded

Note: X = IRB; Y = HCT; Y° = HCT is Divisor; and X° = IRB as Divisor.

The concentrations of IRB and HCT are calculated using the linear relationship between the absorbance at its λ max versus the corresponding concentration [8], [9].

Preparation of sample solution

Twenty tablets are weighed and crushed homogeneous. Furthermore, weighed the amount of powder equivalent to 300 mg of IRB and 12.5 mg of HCT and the equality of TRI contained in there is calculated. It should be weighed up to six repetitions. Subsequently incorporated into the flask 50 mL and diluted with 0.1N NaOH (with sonicator for 15 minutes), and then paid back with 0.1 N NaOH until the line mark, shaken until homogeneous. The solution is then filtered, approximately 10 mL of the first filtrate discarded. Taken 0.5 mL, put in a 25 mL flask and paid back with diluted with 0.1 N NaOH until the line marks to obtained solution in which there are IRB and HCT concentration of 10 µg/mL and 8 µg/mL, respectively. Measured absorption was at a wavelength of 200-400 nm.

Results

Spectrum overlapping studies were obtained from the maximal absorption spectrum of IRB and HCT as well as a mixture of IRB and HCT. In the maximal absorption spectrum of IRB the concentration

of 10 µg/ml was used and for HCT concentrations of 8 µg/ml, a ratio of 5: 4 was obtained for the IRB and HCT respectively and can be seen at Figure 1.

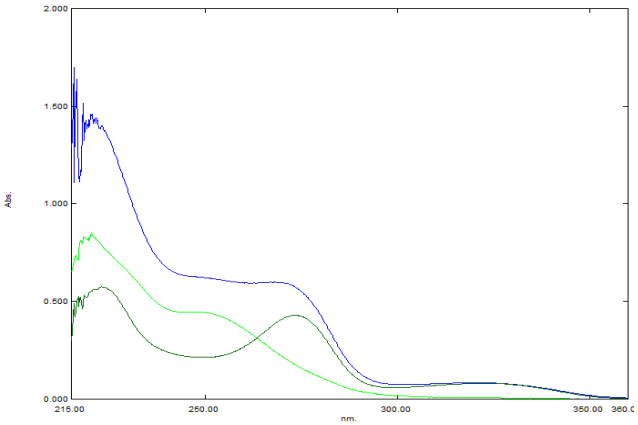

Figure 1: Overlain spectrum of IRB (10 µg/mL), HCT (8 µg/mL) and mixture (IRB 10 and HCT 8 µg/mL)

Based on Figure 1, the overlapping spectrum of IRB and HCT shows that HCT is more extended than IRB so that HCT is used as the initial divisor for the subtraction method ratio. According to Kamal et al. (2016), the determination of overlapping spectrum studies is the starting point for determining the levels using the ratio subtraction method. This analysis of the use of length used for analysis, which shows that the initial divisor is used for the ratio subtraction method, where the initial divisor is used the next step [8].

Method validation

The developed method is validated for linearity, precision, and accuracy. The validation results are shown in Table 1.

Table 1: Validation Methods of IRB and HCT in Ratio subtraction method

No.	Parameter	IRB	HCT
1	Analytical wavelengths	247.6 nm	273.6 nm
2	Concentration (µ/ml	5-15	4-12
3	Regression equation	Y_{irb} = 0.0482X - 0.0083	Y_{hct}= 0.0585X – 0.0068
4	Correlation coefficient	0.9994	0.9988
5	Accuracy (%)	101.03	100.59
6	Precision (RSD) (%)	0.57	0.89
7	Interday (% RSD)	1.34	1.18
8	Intraday (% RSD)	0.34	1.20
9	LOD (µg/mL)	0.70	0.78
10	LOQ (µg/mL)	2.12	2.37

Based on Table 1, It can be seen that the calibration curves of IRB and HCT were linear in the range of 5-15 µg/mL and 4-12 µg/mL, respectively.The regression equations of calibration curves were Y_{irb} = 0.0482X - 0.0083, *r* = 0.9994 for IRB and Y_{hct}= 0.0585X – 0.0068, *r* = 0.9988 for HCT. Relative standard deviations (% R.S.D.) for interday were found to be 1.34 and 1.18 for IRB and HCT, respectively. The intraday precision showed % R.S.D 0.34 and 1.20 for IRB and HCT, respectively. The LOD for IRB and HCT was found to be 0.7005 µg/mL and 0.7834 µg/mL respectively. The LOQ for IRB and HCT was found to be 2.1227 µg/mL and 2.3741 µg/mL respectively. Based on the table above shows all the interday and intraday results meet the requirements < 2% RSD, this means that the RSM method can be stated to have good precision because after testing on intra-day and intra-day it gives insignificant results in statistical calculations and meets ICH requirements 2015 [19].

Accuracy

The percentage recoveries of a drug from marketed formulation were determined by standard addition of pure drugs at three (80%, 100%, and 120%) known concentrations and excellent recoveries were obtained at each level. The accuracy studies are shown in Table 2

Table 2: Accuracy study

No.	Drug	Concentration (%)	Mean % recovery
1	IRB	80 %	100.97
2	IRB	100%	101.08
3	IRB	120%	101.03
4	HCT	80 %	99.80
5	HCT	100%	100.37
6	HCT	120%	100.37

The percentage of recovery for IRBs is three levels, 80%, 100%, and 120% respectively 100.97, 101.08 and 101.03, while for HCT is 99.80, 100.37 and 100.37, that is mean the validation of RSM is a requirement with ICH 2015 [19].

Ratio Subtraction Method

This method is operated using the UV probe 2.43 application by doing a manipulate from the data set spectrum, when the mixtures of IRB and HCT, where the spectrum of HCT is more extended, the determination of IRB in the mixture can be done by scanning the zero-order spectra of IRB and HCT, dividing them by a carefully chosen concentration of HCT´ standard (8 µg/mL) as a divisor. The choice of this divisor is very crucial because it can affect the results of the spectrum obtained. After dividing the new ratio, the spectrum is generated which represents IRB/HCT´ + constant as in Figure 2 (step 1).

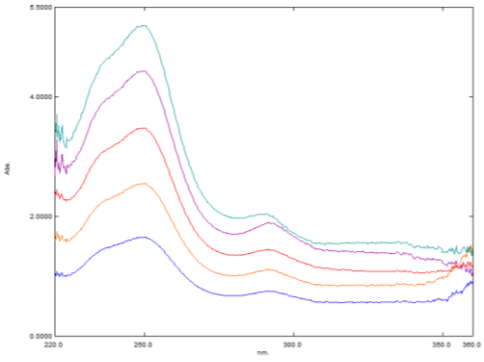

Figure 2: Spectra of mixtures IRB and HCT using 8 µg/mL of HCT´ as a divisor

The results of the spectrum are subtracted constant, namely HCT/HCT´ to produce the spectrum shown in Figure 3 (step 2).

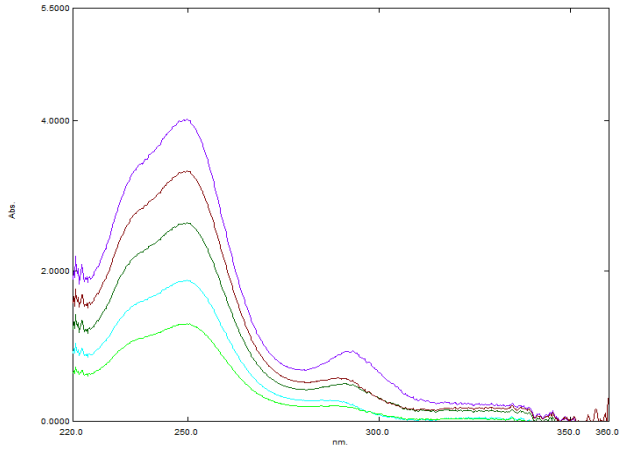

Figure 3: Spectra of mixtures IRB and HCT using 8 µg/mL of HCT´ as a divisor and after subtraction with constants (HCT/HCT´)

Then proceed with the multiplication of the obtained spectra by the divisor HCT´ (8 µg/mL) as shown in Figure 4.

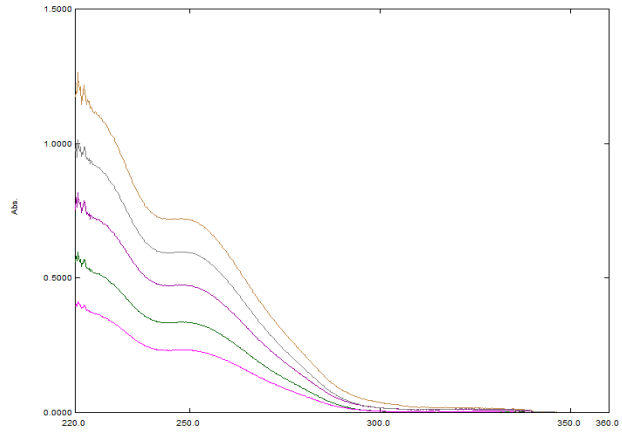

Figure 4: The zero-order absorption spectra of IRB by the proposed RSM after multiplication by the divisor

Finally, the original spectra of IRB can be obtained, Figure 4, which was used for direct estimation of IRB at 247.6 and calculation. The concentration of zero-order curves of IRB at 247.6 against the corresponding concentrations.

The determination of HCT can be obtained by the spectra of IRB by a carefully chosen concentration of the standard IRB´ (10 µg/mL) producing ratio spectra representing the constants IRB/IRB´. The scanned zero-order IRB and HCT were divided by the IRB´ (10 µg/mL) standard as a product of the new ratio spectra which represent HCT/IRB´ + constant as shown in Figure 5.

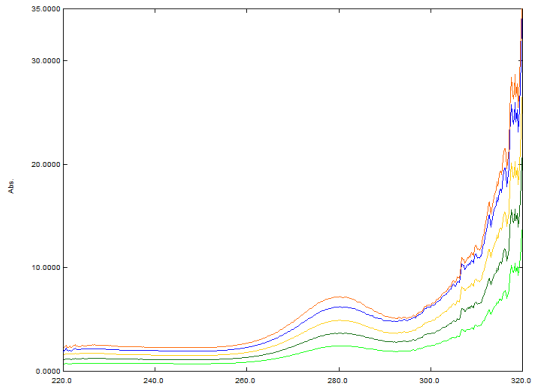

Figure 5: Spectra of Mixtures IRB and HCT using 10 µg/mL of IRB´ as a divisor

The spectra are subtracted by the constant so that the new ratio spectra, which represent HCT/IRB´ are shown in Figure 6.

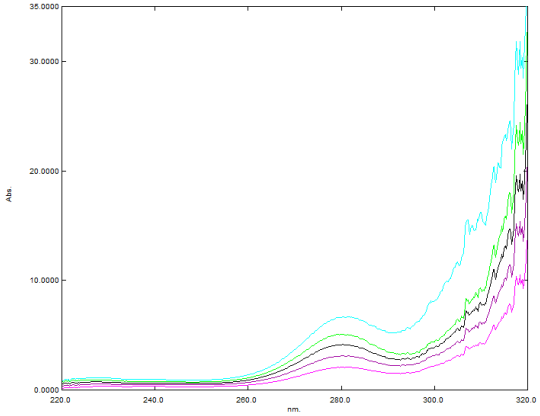

Figure 6: Spectra of Mixtures IRB and HCT using 10 µg/Ml Of IRB´ as a divisor after Subtraction with Constants (IRB/IRB´)

Then the spectra are multiplication by the IRB´ divisor (10 µg/mL) which is the same as before. Finally, the original spectra of HCT, Figure 7, which can be used for determination of HCT at 273.4 nm in the form of corresponding regression equation curves of HCT at 273.4 nm against the corresponding).

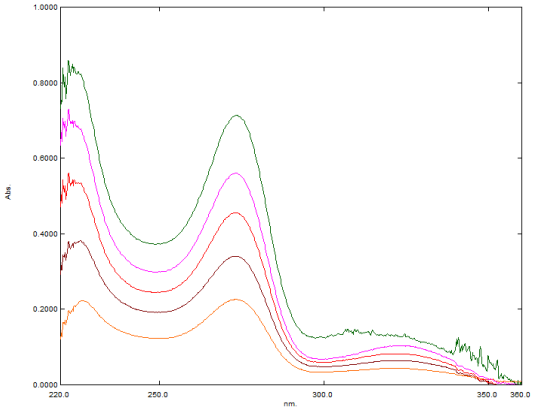

Figure 7: The Zero Order Absorption Spectra of HCT By The Proposed RSM

The methods are considered to be complementary to each other as the two components of interest in the mixture can be determined. The results obtained upon using the suggested methods for the analysis of IRB and HCT in marked tablets.

Table 3: Results of simultaneous estimation of IRB and HCT by RSM

Component	Claim on the label (mg)	The content (mg)
IRB	300	313.60 ± 1.88
HCT	12.5	12.66 ± 0.20

Based on Table 3 can be seen that the proposed ratio subtraction method gives accurate and precise have been developed and validated for irbesartan and hydrochlorothiazide in the marketed formulation (tablets) without prior separation and is easily applied for routine analysis. The most interesting feature of the ratio subtraction method is its simplicity and rapidity. The validation method has been demonstrated by a variety of tests for linearity, accuracy, and precision. The proposed methods were successfully applied to the determination of these drugs in commercial tablets.

Discussion

Based on the result above, it can be seen that there are irbesartan and hydrochlorothiazide mixture in the tablet dosage form is a requirement for Indonesian pharmacopoeia. This method means that pharmaceutical preparations containing the composition of the two substances can be used as a mixture of anti-hypertensive drugs. Its combination is primarily indicated for moderate-to-severe hypertension, but it could be useful for other irbesartan indications, as well. The combination significantly reduces BP in patients not controlled by IRB or HCT alone. Therefore, it is possible to evaluate the clinical efficacy under 2 main titles: BP-lowering efficacy, and the end-organ protection. The combination are fixed-dose combinations achieved BP targets in 77% of patients with systolic, 83% for diastolic and 69% for both BP levels [1], [2], [4], [5], [6], [7]

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Public Health

Improvement of Healthy Diet Related Knowledge among a Sample of Egyptian Women in Three Upper Egypt Governorates Using a Community Based Intervention

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Abstract

BACKGROUND: Fostering a community-based approach is one of the United Nations Children's Fund (UNICEF) strategies to empower the public with the knowledge and tools required for improving the nutritional status.

AIM: The current study was conducted to assess the knowledge of mothers/caregivers towards a healthy, safe, and affordable diet and to cover the detected knowledge gap using a community-based approach.

METHODS: A pre-posttest experimental design was carried out at a community level at three Upper Egypt governorates: Assiut, Qena, and Sohag over six months from September 2017 till February 2018. In the preparatory phase, 22 non-governmental organisations (NGOs) were selected per governorate, and 15 trainers were prepared at the central level to train 40 trainees from each governorate. In the implementation phase, 11,000 women were approached, 6548 of them agreed to participate in the baseline knowledge assessment: 1774 women from Assiut, 2337 from Qena, and 2437 from Sohag.

RESULTS: A significant improvement in the participants' subtotal and total knowledge scores in all dimensions of nutrition education which are: food economics, food safety, and a healthy diet. The highest percent change was in Assiut 77.1 (69.3: 109.9), followed by Qena 54.9 (27.2: 93.3), and then Sohag 43.7 (31.6: 61.4) which was noticed among the participants from the 3 governorates.

CONCLUSION: This community-based approach was a successful intervention to deliver effective health education messages; thus, improving participants' knowledge regarding food safety, healthy diet, and food economics. It represented the success of NGOs to enhance health and nutrition literacy among the participating women living in underprivileged areas. It is recommended to encourage collaboration with NGOs to move the community towards healthy behaviours.

Introduction

The global burden of malnutrition remains unacceptably high, and the progress made for solutions is unacceptably slow. Children under five years of age face multiple burdens; 150.8 million are stunted, 50.5 million are wasted, and 38.3 million are overweight [1]. Adolescent malnutrition rates are on the rise, representing a major public health problem [2]. Obesity rates among adults are at record levels (38.9%). Also, millions of women are still underweight. Africa and Asia bear the greatest share of all malnutrition forms [1]. Despite the efforts adopted by Egypt to reach the second goal of sustainable development goals (SDGs), which is to end hunger, achieve food security, and improve nutrition, malnutrition rates in Egypt are still high. Among children under the age of five years, one in every five children is stunted. Also, wasting and underweight rates are 8% and 6%, respectively, and the incidence of anaemia is 27% [3]. The percentage of obesity and overweight among females (5-19 years of age), and even married women (15-49 years) are 36% and 85%, respectively. Also, the rate of anaemia among women in the reproductive age is 25% [4].

School-age children, adolescents, and adults all over the world, regardless of wealth, are eating too many refined grains and sugary foods and drinks, and not enough food that promotes health such as fruits,

vegetables, legumes, and whole grains. About a third (30.3%) of school-aged children do not eat any fruit daily, yet 43.7% of them consume soda every day [1]. Previous research assessing knowledge, attitude and practice of healthy habits among Egyptian families revealed that there is a lack of health literacy among caregivers, contributing to unhealthy decisions [5].

At the family level, improvement of malnutrition cannot be achieved without exploring the existing level of knowledge among mothers regarding healthy diet, then delivering nutrition-related messages. Also, understanding households' food safety practices are of great help to reduce food-borne diseases at home [6]. Fostering a community-based approach is one of the UNICEF strategies to empower the public with the knowledge and tools required to improve the nutritional status [1]. Therefore, the current study was conducted to assess the knowledge of mothers/caregivers towards a healthy, safe, and affordable diet and to cover the detected knowledge gap using a community-based approach.

Methods

Study design, period, and setting

This study adopted a pre-posttest experimental design and was carried out at a community level in three of Upper Egypt governorates: Assiut, Qena, and Sohag. The study spanned over six months from September 2017 till February 2018.

Preparatory phase (at central level)

- Selection of governorates: Three of Upper Egypt governorates: Assiut, Qena, and Sohag were purposefully selected according to the Central Agency for Population Mobilization and Statistics (CAPMAS) definition of poverty [7].

- Creating a Core training team:

Three Public Health staff members participated in the following:

- Preparation of a training manual for a healthy diet, food safety, and food economics according to the international standards adopted from My Plate [8], [9], [10].

- Conducting training for 40 members at the central level (Cairo governorate):

- Holding a three-day training of trainers (TOT) workshop by the research team for forty trainees. The selection was open to women with a minimum of 12 years of education and who are willing to spend 20 hours in the week for the project.

- The first day was an orientation about the project objectives, principles of TOT, and basic communication skills.

- The second day was an orientation about the healthy diet.

- The third day was an orientation about food economics and food safety.

- Finally, a Core Training Team was formed at the central level, composed of 15 members out of 40 trainees after post-intervention assessment. The assessment included a passing score of 80% and a health education presentation to evaluate the knowledge gained and soft skills acquired after attending the workshops.

Preparatory phase (At the governorate level)

- Orienting the governors and governorate leaders of the three purposefully selected governorates by sending them faxes about the project objectives and asking them to nominate the most actively participating NGOs in their governorates. The governors and their deputies were oriented about the importance of promoting inter-sectoral cooperation and coordination with the NGOs to promote healthy nutrition.

- After nomination, the project management team visited the nominated NGOs to assess their capabilities and willingness to participate in the training activities and define places for implementing the proposed training in addition to delivering women HE classes. Accordingly, 22 NGOs were selected per governorate.

- Contacting the selected non-governmental organisations in Assiut, Qena, and Sohag to nominate staff to participate as trainers after receiving a TOT. Forty trainers were selected for each governorate. The selection was open to men or women with a minimum of 12 years of education and willingness to spend 20 hours in the week for the project.

- In each of the selected governorates, the Central Core Team implemented a two-day workshop attended by 40 participants from the governorate. The training covered communication skills and healthy nutrition. The trainee's completed pre- and post-tests, and, accordingly, 15 trainees were selected and added to the final central core team.

- Women aged17-69 years were recruited from different parts of each governorate using the NGOs registries; then they were personally approached and invited to participate in disseminating the messages received through the whole village. Out of 11,000 women approached, 6548 agreed to participate: 1774 women from Assiut, 2337 from Qena, and 2437 from Sohag.

Baseline assessment

A pre-tested structured interview questionnaire was used to collect data from the study participants. It included two sections:

i) Socio-demographic characteristics: age, family size, education, and occupation.

ii) Nutrition knowledge of study participants: knowledge questions (13 questions) were classified into three categories: healthy diet (6 questions), food economics (4 questions), and food safety (3 questions). The questions were coded, so that true answers were given a score of 1, while wrong answers or answering with I don't know were given a score of 0. The total raw score (if all answers are correct) was 13. Per cent score was calculated by dividing the raw score over 13 (maximum achievable score) and then multiplying the result by 100. Questions used in this section were adopted from the available literature [8], [9], [10].

The same tool was used in the post-intervention phase to assess the change in the participants' knowledge.

Content of the questionnaire was validated by four faculty members who are experts in nutrition, and the required modifications were done. Reliability was tested using internal consistency, and a Cronbach's Alpha ranging from 0.82 to 0.92 was found for the 13 knowledge questions in the 3 subtotal and total scores.

A pilot test was performed to test the clarity of the questions by interviewing 25 women (not included in the study). The required modifications were applied.

Intervention phase

The health education sessions (Table 1) were in the form of PowerPoint presentations, posters, and flashcards covering the knowledge gaps evolved from the baseline assessment in the pre-intervention phase. Regarding healthy diet, food safety, and food economics, the content was adopted from the available literature [8], [9], [10].

Two sessions, in the form of group meetings, were conducted over one day. Each session lasted for 60 minutes with a 15-minute break in-between. After the second session, participants were encouraged to ask any questions in case they needed to. The average number of participants in each session was 25; with one instructor for each group and using the same educational materials for all groups. Health education materials were simplified, modified, and designed in the Arabic language to be suitable for the Egyptian culture [8], [9], [10].

Table 1: Summary of the standardised health education intervention about healthy diet, food safety, and food economics for women at NGOs

	Contents
Overview	Pre-test (baseline assessment) Introduction to the session Orientation about the objectives and possible impact of the research
Session I	Food Economics Food Safety
Session II	Healthy diet
Recap	*Recap and take-home messages*

Post-intervention assessment

Participants (n = 6548) who attended the sessions and responded to the pre-test questionnaire before the educational intervention were contacted after 3 months (using their phone numbers) and invited for another interview at the NGOs for a post-test. Out of the 6548 women included in the pre-test and educational intervention, 750 were lost and did not attend the interview, making a total of 5798 participants in the post-test (11% non-response rate).

Data Management and Statistical Analysis

Pre-coded revised data were entered into the Statistical Package of Social Science (SPSS) version 21.0 (SPSS Inc. IBM, U.S.A.). For categorical data, frequencies and percentages were used for expression. For numerical data, mean and standard deviation were used for normally distributed data, while the median and interquartile ranges were used for data that were not normally distributed. Comparison between groups was made using the chi-square test for qualitative variables and Analysis of Variance (ANOVA) test for quantitative variables which were normally distributed. Non-parametrical Kruskal-Wallis test was used for quantitative variables which were not normally distributed. Comparison between pre- and post-intervention scores was performed using the McNemar's test for qualitative data and Wilcoxon's signed test rank test for quantitative data that were not normally distributed. The 3 subtotal and total knowledge scores were computed for each group of questions where correct answers received one point, while incorrect or did not know answers received nil. P-value ≤ 0.05 was considered statistically significant.

Ethical considerations

The Ethical Review Committee at the Faculty of Medicine, Cairo University revised and approved the study protocol. All participants were treated according to the Helsinki Declaration of biomedical ethics. Informed consent forms were obtained from the study participants after proper orientation regarding the study objectives and data confidentiality. Women were informed of their right to withdraw from the study at any stage.

Results

Table 2 shows the socio-demographic characteristics of the study participants. The mean age was 35 ± 8. The median (Q1: Q3) family size was 5 (4: 6). The majority of participants were housewives. About two-fifths of them were illiterate; about one quarter could read and write, more than a tenth had primary and preparatory education, and one quarter had secondary education or higher.

Table 2: Socio-demographic characteristics of the enrolled participants

	Assuit N = 1774	Qena N = 2337	Souhag N = 2437	Total 6548	p
Age					
(mean ± SD	34.53 ± 7.83	35.41 ± 8.71	34.96 ± 7.29	35.02 ± 8.2	0.008
Range	(17-69)	(17-69)	(18-67)	(17-69)	
Family Size					
Median (Q1: Q3)	5 (4:6)	5 (4:6)	5 (4:6)	5 (4:6)	0.041
Occupation					
Housewife	1704 (96)	2104 (90)	2311 (94.83)	6119 (93.4)	< 0.001
Works	70 (4)	233 (10)	126 (5.17)	429(6.6)	
Education					
Illiterate	746 (42.1)	1026 (43.9)	608 (25.1)	2380 (36.3)	
Reads and writes	459 (25.9)	382 (16.3)	867 (35.6)	1708 (26.1)	< 0.001
Primary & preparatory	169 (9.5)	404 (17.3)	224 (9.2)	797 (12.2)	
Secondary and Higher	400 (22.5)	525 (22.5)	738 (30.1)	1663 (25.4)	

Table 3 shows that there was a significant improvement in the percentage of correct answers to all questions among the study participants in the 3 governorates after the intervention. The improvement in the percentage of correct answers to the question about the adequate amount of water (question 3) among participants in Qena was not statistically significant.

Table 3: Percent of correct answers among the study participants

Question	Assuit N = 1576			Qena N = 2064			Souhag N = 2158			Total N = 5798		
	Pre	Post	P	Pre	Post	P	Pre	post	P	Pre	Post	P
Beans with chickpeas are better than beans with rice	399 25.32 %	1257 79.76%	< 0.001	747 36.1 9%	1231 59.6 4%	< 0.0 01	731 33.8 %	2002 92.7 %	< 0.0 01	1877 32.37 %	4490 77.44 %	< 0.001
Measuring portions by hand and is inaccurate and is wasting	465 29.5 %	1371 86.99%	< 0.001	789 38.2 3%	1260 61.0 5%	< 0.0 01	828 38.3 7%	1778 82.3 9%	< 0.0 01	2082 35.91 %	4409 76.04 %	< 0.001
3 cups of water are adequate	1626 80.08 %	1515 96.13%	< 0.001	1688 81.7 8%	1706 82.6 6%	0.2 22	1437 66.5 9%	1933 89.5 7%	< 0.0 01	4387 75.66 %	5154 88.89 %	0.001
Frequent hand washing is a waste of time	1290 81.85 %	1515 96.13%	< 0.001	1570 76.0 7%	1676 81.2 0%	0.0 02	1712 78.3 3%	1948 90.2 7%	< 0.0 01	4572 78.85 %	5139 88.63 %	< 0.001
Leafy vegetables should be washed with running water alone	348 22.08 %	1485 94.23%	< 0.001	553 26.7 9%	1689 81.8 3%	< 0.0 01	532 24.6 5%	1891 87.6 3%	< 0.0 01	1433 24.72 %	5065 87.36 %	< 0.001
Potato and rice meal are a balanced meal since potato is a vegetable and rice is carbohydrate	726 46.07 %	1476 93.65%	< 0.001	911 44.1 4%	1745 84.5 4%	< 0.0 01	628 29.1 0%	1888 87.4 9%	< 0.0 01	2265 39.07 %	5109 88.12 %	< 0.001
Natural ghee is the best type of fat for cooking because it tastes good	348 22.08 %	1433 90.93%	< 0.001	475 23.0 1%	1802 87.3 1%	< 0.0 01	1286 59.5 9%	1439 66.6 8%	< 0.0 01	2109 36.37 %	4674 80.61 %	< 0.001
It is essential to separate raw food from cooked food	1127 71.51 %	1325 84.07%	< 0.001	1421 68.8 5%	1592 77.1 3%	< 0.0 01	1597 74.0 %	1999 92.6 3%	< 0.0 01	4145 71.49 %	4916 84.79 %	< 0.001
Healthy clean food should be expensive	901 57.17 %	1376 87.31%	< 0.001	1206 58.4 3%	1725 83.5 8%	< 0.0 01	1148 53.2 0%	1833 84.9 4%	< 0.0 01	3255 56.14 %	4934 85.10 %	< 0.001
Food helps in building tissues, provides the body with energy, protects from diseases	1272 80.71 %	1367 86.74%	< 0.001	1632 79.0 7%	1829 88.6 1%	< 0.0 01	1629 75.4 9%	2089 96.8 0%	< 0.0 01	4533 78.18 %	5285 91.15 %	< 0.001
Can a serving of cottage cheese substitute a serving of meat?	337 21.38 %	1335 48.71%	< 0.001	456 22.0 9%	1772 85.8 5%	< 0.0 01	567 26.2 7%	1990 92.2 2%	< 0.0 01	1360 23.46 %	5097 87.91 %	< 0.001
Iodized salt is just the same like the non-iodized salt	1133 71.89 %	1401 88.90%	< 0.001	1164 56.4 0%	1283 62.1 6%	< 0.0 01	1471 68.1 6%	1745 80.8 6%	< 0.0 01	3768 64.99 %	4429 76.39 %	< 0.001
Can leftover food be used to prepare a new meal the next day?	427 27.09 %	1378 87.44%	< 0.001	862 41.7 6%	1647 79.8 0%	< 0.0 01	692 32.1 0%	1993 92.3 5%	< 0.0 01	1981 34.17 %	5018 86.55 %	< 0.001

Table 4 depicts the significant improvement in participants' subtotal and total knowledge scores in the three dimensions of nutrition education: food economics, food safety, and a healthy diet. This was noticed among participants from the three governorates. The lowest baseline subtotal and total knowledge score were that of food economics.

Table 4: Percent of subtotal and total knowledge scores before and after nutrition education among the study participants

	Assuit		Qena		Souhag		Total	
	Pre Median (IQR)	Post Median (IQR)	Pre Median (IQR)	Post Median (IQR)	Pre Median (IQR)	Post Median (IQR)	Pre Median (IQR)	Post Median (IQR)
Food Economics	31.9 (26.3:37.8)	91.9 (80.9:99.5)*	36.1 (31.2:45.9)	72.9 (66.6:83.8) *	30.9 (26.4:49.9)	86.9 (81.5:93) *	33.2 (27.7:45.9)	84.8 (74.8:93.9) *
Food Safety	61.3 (55.4:66.3)	92.7 (88.9:100) *	52.8 (48.9:66.3)	83.3 (66.7:91.7) *	59.5 (48.3:66.8)	91.1 (81.9:98.2) *	57.6 (50.4:66.7)	91.3 (77.8:99.4) *
Healthy diet	59.7 (53.5:66.3)	93.5 (89.3:99) *	58.8 (48.6:65.8)	81.2 (69.5:93.9) *	58.7 (46.3:70.9)	85.3 (82.1:89.9) *	59.4 (50.3:67)	88.6 (79.7:93.9) *
Total	49.9 (47.0:55.8)	94.3 (87.3:97.7) *	48.4 (42.1:56.7)	78.6 (65.3:88.5) *	61.5 (52.5:69.6)	87.5 (83.5:93) *	53.4 (47.1:61.5)	87.5 (81.1:93.6) *

IQR: interquartile range; *indicates a statistically significant difference between pre scores and post scores.

Table 5 shows percent change in participants' subtotal and total knowledge scores. The highest percent change in all governorates was that in food economics, followed by the percent change in a healthy diet, then in food safety in Assiut and Qena. Comparison among the 3 governorates revealed that the highest total knowledge percent change occurred in Assiut, followed by Qena, then Souhag.

Table 5: Percent change of subtotal and total nutrition knowledge scores among the study participants

scores	Assuit	Qena	Souhag	Total
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)
Food Economics	166.7 (125.5:287.2)	95.4 (27.8:166.8)	188.6 (75.4:242.1)	144.3 (79.7:225.5)
Food Safety	44.7 (32.1:63.6)	33.4 (13.5:93.4)	61.0 (36.5:79.8)	48.4 (29.0:71.7)
Healthy Diet	48.4 (39.4:69.7)	42.0 (15.1:63.9)	48.5 (23.2:88.9)	47.3 (30.8:68.5)
Total	77.1 (69.3:109.9)	54.9 (27.2:93.3)	43.7 (31.6:61.4)	58.8 (36.3:84.4)

The lowest percent change was the change in food safety in Qena. By asking the women participated in the study about the impact of the workshop on their family feeding practice, about 90% reported that their family feeding practice was improved, while 10% reported little or no improvement (untabulated data). The best-improved dimension in the families' feeding practices was reported to be food safety in more than two-thirds of the women who reported improvement, healthy diet in about one fifth, and food economics in one-tenth of these women (Figure 1).

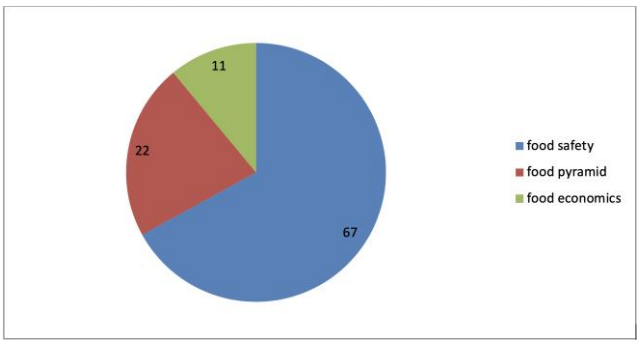

Figure 1: Percent of best improvement as reported by the study participants

Discussions

In the current study, a community-based approach was implemented to deliver nutrition education messages to participating women living in underprivileged areas in three Upper Egypt governorates (Assiut, Qena, and Sohag). People living in these areas suffer more in terms of poverty and malnutrition. A baseline assessment of their knowledge gap in nutrition was performed, followed by nutrition education intervention. A significant improvement in the participants' knowledge was noticed after the intervention. The current study focused on improving women's knowledge about healthy food selection, food safety, and food security which are the main factors of malnutrition [11]. During the last years, food prices and insecurity have been steadily increasing in Egypt, leading to the complexity of malnutrition [12], [13]. Therefore, it was essential to educate the participants about the elements of an affordable diet through the current intervention.

Community-based health promotion program is considered as a low-budget, feasible, and sustainable method to change health knowledge and practice in regions where the health system has restricted resources [14], [15]. Similar to the current study findings, other studies from developing countries proved that the community-based integrated approach targeting maternal education is one of the most important strategies to improve maternal and child survival [16], [17]. Investing in malnutrition is one of the core investment strategies recommended by the World Health Assembly Resolution [18], [19].

The current study showed a significant improvement in the knowledge of participating women after the intervention in areas of food economics, healthy diet, and food safety. This coincides with the findings of other studies [20], [21], [22] where health education interventions had improved the participants' knowledge significantly.

Despite the increasing public concern about food safety, food-related risks and diseases are increasing. This shows that domestic food handlers still lack adequate food safety knowledge, leading to incorrect food-handling practices [23]. In this study, food safety knowledge among Upper Egypt females was 57.9%. A similar study in Saudi Arabia showed that Saudi Arabian females experienced poor knowledge of food-handling practices (passing rate of 30.4%). Another study in six faculties and institutions of Alexandria University assessed food safety knowledge and practices among 270 working women, showing that the mean score percentage of the total safety knowledge of the participants was 67.4 [24].

In conclusion, this community-based approach was a successful intervention to deliver effective health education messages, leading to the improvement of participants' knowledge regarding food safety, healthy diet, and food economics. It represented the success of non-governmental organisations to enhance health and nutrition literacy among the participating women living in underprivileged areas. It is recommended to encourage the collaboration of non-governmental organisations to move the community towards healthy behaviour.

Limitations

Governmental support would have a better effect on improving the nutritional habits of the communities. Greater opportunities would be available to reach the served communities, e.g. mothers coming to primary health care centres to receive family planning or vaccination services.

Further planning is required to ensure the sustainability of the provision of health and nutrition literacy, and, accordingly, moving the community's attitude and behaviour towards healthy nutrition.

Significance of public health

Recent estimates show that malnutrition is a major public health problem in Egypt. Improving nutrition awareness among women is essential for understanding and meeting their families' nutritional needs. Very serious areas of concern are the deprived and vulnerable places where the resources are limited, and food prices are steadily increasing. The current study utilised a community-based approach to assessing nutrition knowledge among women in three deprived Upper Egypt governorates. The detected knowledge gap was covered by a nutrition education intervention which focused mainly on educating the participating women how to plan healthy, safe, and affordable diets for their families. This intervention resulted in a significant improvement in nutrition knowledge among these women. Adopting a similar approach on a larger scale will strengthen the capacity of the community to meet their nutritional needs. Cooperation between the governmental and non-governmental organisations will have a tremendous effect on fighting malnutrition.

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Review Article

Risk of Lower Eyelids Malposition in Subciliary Compared to Transconjunctival Approach in Maxillofacial Fractures Management: A Systematic Review and Meta-Analysis

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Keywords: Lower eyelids malposition; Subciliary; Transconjunctival; Maxillofacial fractures

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Abbreviations: PICO = Patients, Intervention, Comparator, Outcome; ORIF = Open Reduction Internal Fixation; DOAJ = Directory of Open Access Journal; RR = Risk Ratio; JBI = Joanna Briggs Institute; PRISMA = Preferred Reporting Items for Systematic Review and Meta-Analyses; RCT = Randomized Control Trial; Ec = Ectropion; En = Entropion; ZMC = Zygomaticomaxillary Complex; CI = Confidence Interval

Abstract

BACKGROUND: Both subciliary and transconjunctival approaches have been used for decades to visualise the site of the maxillofacial fracture. The most common complication following those procedures is lower eyelids malposition.

AIM: This meta-analysis will analyse which approach (subciliary and transconjunctival approaches) is more favourable to decrease lower eyelids malposition incidence.

METHOD: This meta-analysis was conducted based on PRISMA guidelines. The electronic search was conducted using keywords ("Lower Eyelids Malposition" OR "Complications" OR "Ectropion" OR "Entropion") AND (Transconjunctival) AND (Subciliary) AND (Maxillofacial Fractures) in PubMed, The Cochrane Library, and Directory of Open Access Journal (DOAJ). This review included full-text studies (observational and randomised controlled trials) in English comparing subciliary and transconjunctival approach in patients with maxillofacial fractures in the last 10 years. The data collected were the type of fractures and approaches, ectropion and entropion incidence as well as follow-up duration. The risk of bias was assessed using Joanna Briggs Institute critical appraisal checklist. Statistical analysis was done using Review Manager 5.3 (Cochrane, Denmark).

RESULT: This study included 3 cohort studies and 2 Randomized Controlled Trial (RCT) studies from 2012 to 2017 with a total of 574 samples. Subciliary approach had a significant higher ectropion incidence when compared to transconjunctival approach (RR = 4.64, 95% CI: 1.68-12.81, p = 0.003). There was also a significant reduction of entropion incidence in patients with subciliary approach compared to transconjunctival approach (RR = 0.16, 95% CI: 0.04 – 0.69, p = 0.01).

CONCLUSION: There was no superiority between one procedure toward another since each procedure related to different lower eyelids malpositions.

Introduction

Maxillofacial fractures is a common entity in the urban setting that rarely life-threatening. Despite the impact on physiologic function, maxillofacial fractures may also have an unfavourable effect on facial aesthetics of the trauma victim. Challenges in the comprehensive management of facial bone fractures are not only how to achieve a physiologic union of the bones, but also to manage all of the effects caused by the broken bones [1], [2].

Different approaches have been developed especially to access the infraorbital rim and the orbital floor to fix the fractures [3]. The conventional approaches including cutaneous infraciliary or subciliary incisions, mid-lower eyelid or subtarsal and infraorbital incisions. These conventional techniques produce a scar which may be cosmetically unfavourable. Transconjunctival incisions is an alternative technique that provides adequate exposure of the bone and avoids the visible scar at the same time because the incision that made through the conjunctiva [4].

Both subciliary and transconjunctival approaches have been used for decades. The most common complications following those procedures are lower eyelids malposition comprises of ectropion, entropion and scleral show. Ectropion is the most frequent problem results in aesthetically and functionally disturbances of the eyes such as outdoor runny eyes. Entropion can also result in pain as the cilia can irritate the cornea of the patients [1], [5].

Given the frequency and associated morbidity of the lower eyelids malposition, identifying approaches to minimise the unwanted complication may be an important contribution to enhance the treatment outcome. This study presented a systematic review and meta-analysis using the available evidence to understand further which one of two surgical approaches (subciliary and transconjunctival) is more favourable focusing in terms of lower eyelids malposition risk (ectropion, entropion).

Methods

Eligibility criteria

Eligibility criteria were created based on the PICO framework. PICO criteria can be seen in Table 1.

Table 1: PICO criteria of the study

Patient	Maxillofacial Fractures
Intervention	Subciliary Incision
Comparator	Transconjunctival Incision
Outcome	Lower Eyelids Malposition

Type of studies

This review included full-text studies in English comparing subciliary and transconjunctival approach in patients with maxillofacial fractures in the last 10 years. We exclude case report, review, animal, anatomic, cadaveric, qualitative and economic studies. Studies that do not report the information required for performing a meta-analysis were excluded. Articles made by the same author in the same institution were performed sample evaluation to prevent sample duplication.

Type of participants

This review included studies with patients of all ages and gender who underwent Open Reduction Internal Fixation (ORIF) for maxillofacial fractures management with subciliary and transconjunctival approach. Maxillofacial fractures refer to any injury that results in a broken bone or bones of the face that required subciliary or transconjunctival incision to reach the site of the fracture.

Type of interventions

The reviewed surgical interventions were subciliary approach in comparison to the transconjunctival approach for maxillofacial fractures management. In this context, the preseptal or septal transconjunctival incision with or without lateral canthotomy was included. All of the subciliary incision techniques were also included in this study (the skin only type, skin-muscle type and stepped technique).

Type of outcomes

The results investigated in this review was lower eyelids malposition. Lower eyelid malposition was defined as abnormal positioning of lower eyelids that were observed post-operatively before any treatment or correction is given to reduce the complications. The types of abnormalities included were ectropion and entropion.

Information sources

We extracted the eligibility criteria (PICO) into keywords using Boolean operator. In this study, we used keywords (“Lower Eyelids Malposition” OR “Complications” OR “Ectropion” OR “Entropion”) AND (Transconjunctival) AND (Subciliary) AND (Maxillofacial Fractures) in PubMed database, The Cochrane Library and Directory of Open Access Journal (DOAJ) as search engine to find eligible journals.

Study selection

The study selection process was conducted by three authors (PP, PM and EM) to reduce the possibility of disposing of relevant studies. The decision of the first, second and third author was considered when disagreement occurred. The study selection began with the removal of duplicate records. The irrelevant studies then excluded by screening the titles and abstracts. Studies that passed the first screening were further evaluated for the compliance of the inclusion and exclusion criteria of this review. Finally, the studies were further evaluated for their quality before eventually included.

Data collection process

Electronic data collection form was used to collect data from each author. The collected data by each author was merged and managed with software Review Manager 5.3.

Data items

The data items were the author’s name, year of publication, type of study, sample size, type of

fractures, surgical approaches, lower eyelids malposition incidence, and follow – up duration. Lower eyelids malposition incidence was calculated for risk ratio (RR) and were performed the meta-analysis.

Assessment of quality of study

Studies that complied with inclusion and exclusion criteria are assessed for their quality to ensure the validity and reliability of the studies. This process was done independently by two authors (PP and PM) using a standardised critical appraisal tool to minimise the possibility of bias in study selection. The critical appraisal tool in this study was the Joanna Briggs Institute (JBI) critical appraisal tool based on study design. The decision of the first, second and third author was used when disagreement occurred. Cut off point was defined to determine the quality of the study. Cut off point in this review was half of the total score in each JBI critical appraisal checklist. The low-quality study was defined as a score below the cut-off point while otherwise referred to as high-quality study.

Synthesis of result

The RR of lower eyelid malposition were pooled and analysed. Meta-analysis was conducted using software Review Manager 5.3.

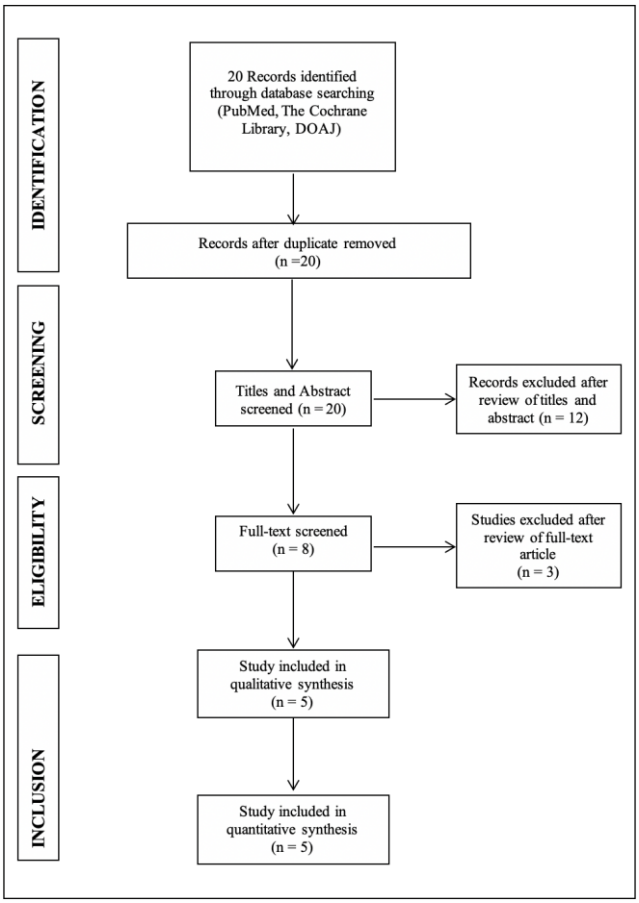

Figure 1: PRISMA Flow Diagram

Results

Study Selection

Using the initial search strategy, we found a total of 20 studies. Based on the title and abstract screening, we excluded 12 articles. That left us 8 relevant studies. Studies that didn't provide all the information needed in this meta-analysis were excluded. After screening and qualitative evaluation were done, we finally have 5 articles that further used in this study. PRISMA study flow diagram is described in Figure 1.

Study Characteristics

We included 5 full-text articles which are 2 retrospective cohort studies, 1 prospective cohort studies and 2 Randomized Control Trial (RCT). The publication year of these articles varied between 2012 to 2017 with a total of 574 samples included. The summary of finding and studies characteristics can be seen in Table 2.

Table 2: Summary of Findings and Studies Characteristics

Author	Type of Study	Level of Evidence	Fractures Type	Intervention (n)	Outcome (n)	Control (n)	Outcome (n)	Follow Up
Giraddi *et al.* (2012) [4]	Prospective Cohort	2a	Orbital Floor and Rim Fractures	Subciliary Skin Muscle Flap (10)	Ec: 3 En: 0	Perceptual Transconjunctival with Lateral Canthotomy (10)	Ec: 1 En: 3	3 Months
Pausch *et al.* (2015) [5]	Retrospective Cohort	2b	Orbital Floor Fractures	Subciliary Skin Muscle Flap (225)	Ec:12 En: 0	Transconjunctival (121)	Ec:1 En:5	6 Months
Vaibhav *et al.* (2015) [6]	RCT	1b	Infraorbital Rim Fractures	Subciliary (20)	Ec:2 En:0	"Sutureless" Preseptal Transconjuntival (20)	Ec:0 En:1	3 Months
Neovius *et al.* (2016) [3]	Retrospective Cohort	2b	Facial Fractures	Subciliary (37)	Ec:3 En:0	Transconjunctival (91)	Ec:2 En:0	6 Months
El-Anwar *et al.* (2017) [7]	RCT	1b	ZMC Fractures	Subciliary (20)	Ec:2 En:0	Transconjunctival with Lateral Canthotomy (20)	Ec:0 En:4	6 Weeks

Ec: Ectropion; En: Entropion.

Risk of bias within studies

The risk of bias was analysed using JBI critical appraisal tool for cohort and RCT studies. All 5 articles included in this study were passed the quality evaluation. Complete result of bias risk was described in Table 3.

Table 3: Risk of Bias Summary

Study (Year)	Question no.													
	1	2	3	4	5	6	7	8	9	10	11	12	13	Total
Reviewer: PP														
Giraddi *et al.* (2012)	Y	Y	Y	Y	N	Y	Y	Y	Y	N	Y			9/11
Pausch *et al.* (2015)	Y	Y	Y	Y	Y	Y	N	Y	Y	N	Y			9/11
Vaibhav *et al.* (2015)	Y	Y	Y	Y	N	N	Y	Y	Y	Y	Y	Y	Y	11/13
Neovius *et al.* (2016)	Y	Y	Y	N	N	Y	Y	Y	Y	N	Y			8/11
El-Anwar *et al.*, (2017)	Y	Y	Y	Y	N	N	Y	N	Y	Y	Y	Y	Y	10/13
Reviewer: PM														
Giraddi *et al.*, (2012)	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y			10/11
Pausch *et al.* (2015)	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y			10/11
Vaibhav *et al.* (2015)	Y	Y	Y	Y	N	N	Y	Y	Y	Y	Y	Y	Y	11/13
Neovius *et al.* (2016)	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y			10/11
El-Anwar *et al.*, (2017)	Y	Y	Y	Y	N	N	Y	Y	Y	Y	Y	Y	Y	11/13

Ectropion

As shown in Figure 2, incidence of ectropion between subciliary and transconjunctival approach were 7.0% and 1.5%, respectively. Based on fixed effect model with low heterogeneity (I^2 = 0%; χ^2 = 0.34; p = 0.99), pooled risk ratio between subciliary and transconjunctival approach on ectropion incidence was 4.64 (p = 0.003; 95% CI: 1.68-12.81).

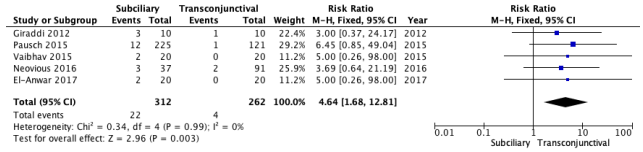

Figure 2: Forest plot comparing subciliary and transconjunctival approach on ectropion incidence

Entropion

As shown in Figure 3, the incidence of entropion in the transconjunctival group was 4.7% while there was no event in subciliary group. We excluded study by Neovious *et al.* in the analysis due to ectropion incidence absence in both subciliary and transconjunctival group. Based on fixed effect model with low heterogeneity (I^2 = 0%; χ^2 = 0.28; p = 0.96), pooled risk ratio between subciliary and transconjunctival approach on entropion incidence was 0.16 (p = 0.01; 95% CI: 0.04 – 0.69).

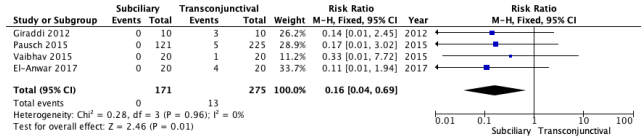

Figure 3: Forest plot comparing subciliary and transconjunctival approach on entropion incidence

Discussion

This study aimed to compare the frequencies of lower eyelids malposition including ectropion and entropion after the use of subciliary or transconjunctival approach in maxillofacial fractures management. Subciliary approach is usually made by a few millimetres' parallel incision below the ciliary line from medial punctum to the lateral canthus. There are three types of subciliary incision comprise of the skin only type, skin-muscle type and stepped technique. The transconjunctival approach is made by eversion of the inferior lid while conjunctiva is incised below the tarsus. The incision then continued to the orbital rim in a preseptal or retroseptal technique [8]. Using transconjunctival incision with a lateral canthotomy for infraorbital rim and floor fractures give wider exposure of the entire lower orbital rim and zygoma [4].

In this study, the incidence of ectropion between subciliary and transconjunctival approach were 7.0% and 1.5%, respectively. This result is consistent with all of the studies in this review that stated the subciliary approach was significantly associated with the higher rates of ectropion and the lower rates of entropion. Most of the studies included in this study concluded that there was no superior technique between approaches, except one study that favoured the transconjunctival approach due to its overall minimum complications. This study also showed that each approach is associated with different complication where pooled risk ratio of the ectropion in subciliary group was 4.64 (p < 0.05) and the pooled risk ratio of the entropion in subciliary group was 0.16 (p < 0.05). In the other hand, the incidence of ectropion in the transconjunctival group was 3.5% while there was no event in subciliary group. These findings are also by a meta-analysis by Ridgway *et al.*, showing that the risk of ectropion was highest in subciliary incisions (14%) compared with subtarsal (3.8%) and transconjunctival incisions (1.5%) (P < 0.001) [9]. The possible underlying mechanism of postoperative ectropion and entropion is scarring at the anterior and middle lamella of the eyelid after a subciliary incision while post-operative scar of the posterior lamella can occur after the transconjunctival incision. By the time the scar becomes mature, it can create the outward retraction in the former and the inward retraction in the latter [1], [2], [5].

Pausch *et al.,* used a skin muscle flap for the subciliary approach instead of skin-only technique because the later technique is more susceptible to soft tissue complication [5]. The skin – only type involves the skin dissection from the orbicularis oculi muscle, is often linked with a higher risk of several complications such as cutaneous necrosis, ecchymosis, and ectropion [8]. Subciliary incision that is made too close to the lid margin can increase the risk of ectropion and epiphora while a visible scar and massive oedema caused by lymphatic drainage impairment can happen if the incision is placed too far from the lid margin [10], [11].

In contrast to the subciliary approach, the conjunctival incision results in less conspicuous scar except in the skin lateral to the lateral canthus [10], [12], [13]. The transconjunctival approach doesn't emphasise in the involvement of the lower eyelid skin and orbicularis oculi muscle so that reduced the risk for postoperative lower eyelid retraction, scleral show, and ectropion. In this review, most studies performed transconjunctival incision with lateral canthotomy to get enough exposure and visibility [2]. Despite its cosmetic advantages, the main disadvantages of this approach are its technique sensitivity, a relatively limited exposure when used alone but relatively higher rates of lower eyelid malposition when combined with a lateral canthotomy [3]. A study by Neovius *et al.* described that when combined with lateral

canthotomy, a transconjunctival approach often result in canthal malposition that needs surgery correction (2.2%). Therefore, they performed a transconjunctival approach to reduce the risk of ectropion and scleral show without lateral canthotomy as much as possible to eliminate Chantal malposition risk on their practice [4].

Because of its every advantage and disadvantages, most studies recommend using both approaches interchangeably depends on the surgeon as well as an individual patient basis. The subciliary approach gives wider exposure of the infraorbital rim and is better used to repair extensively displaced fractures. The transconjunctival approach gives better aesthetic results and less overall postoperative complications but requires an additional lateral canthotomy in cases extension of exposure needed [6].

The limitation of this review is the limited number of randomised controlled trial study (2 studies). Cohort study might be susceptible to some biases including selection, confounding, and information biases. Some studies also acknowledge the limited number of samples in their studies and the possibility of bias due to different surgical operators and outcome evaluators. Also, the search strategy of this review possibly missed other relevant articles to be included in the analysis.

In conclusion, there was no superiority between one procedure toward another since each procedure related to different complication. More prospective studies should be done to determine the best approaches with their modification in preventing lower eyelid malposition in maxillofacial fractures management.

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Review Article

Indications for Surgery in Non-Traumatic Spleen Disease

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Abstract

The spleen is the largest lymphatic organ that acts as a site for filtration of foreign particles from the blood, erythropoiesis and hematopoiesis. Splenectomy represents the first line of treatment for spontaneous splenic rupture, abscesses, cysts, tumours. It is also used to control hereditary, autoimmune, and myeloproliferative disorders alternatively. Numerous diseases have been indicated for surgery in non-traumatic spleen diseases such as non-traumatic spleen rupture, immune thrombocytopenic purpura (ITP), haemolytic anaemias, Felty's syndrome, Hodgkin's and non-Hodgkin's lymphoma among others. This result because the spleen is the most affected lymphoid organ following its overactivity that occurs during sequestration of dead or disrupted RBCs and lymphocytes. Abdominal pain is one of the major manifestations of splenomegaly, and can also designate other associated complications such as liver cirrhosis or bacterial endocarditis. As a secondary lymphoid organ, the spleen is more often an organ for lymphomas. Although splenectomy is a curative alternative in a few diseases, it is a complementary means of treating several other diseases. Splenectomy is a salvage therapy used when other therapeutic alternatives fail. Despite its indication in numerous diseases, controversies are still inbound of its use.

Introduction

As the largest of the lymphatic organs, the spleen also helps in the filtration of foreign matter from the blood and serves as a major site of erythropoiesis and hematopoiesis [1]. Weighing between 75 and 250g in healthy adults, its size decreases with age [2], [3]. The spleen also acts as a storage site for iron, erythrocytes and platelets; and produces antibodies that remove bacteria [4], [5], [6]. Non-traumatic spleen rupture is a rare condition and can occur in a pathological spleen due to a variety of diseases [7].

Indications for Surgery in Non-traumatic Spleen Disease

The most widespread indications for surgery in non-traumatic spleen disease includes conditions such as; immune thrombocytopenic purpura (ITP), haemolytic anaemias, malaria, thalassemia, splenic abscesses, congestive splenomegaly, splenic cysts, Felty's syndrome, Hodgkin's and non-Hodgkin's lymphoma, leukaemias, myelofibrosis e.t.c. [5], [6], [8]. Sreekar *et al.,* and Schlittler and Dallagasperina, in their study, reports that splenic abscess is a rare and potentially fatal disease found especially in men, with *Escherichia coli*, *Klebsiella pneumonia, Staphylococcus aureus* and *Salmonella typhi* as causative factors [9], [10]. They also noted neoplasia, splenic infarcts, diabetes mellitus and immunosuppressive conditions as possible risk factors associated with the disease. Another condition that has drawn attention for surgery is a splenic cyst. Splenic cysts, which is asymptomatic with greater incidence in women are benign and without solid components [11]. The aetiologies of splenic cysts are numerous and include congenital, post-traumatic pseudocysts, peliosis and cystic neoplasias such as lymphangioma, hemangioma and lymphoma [11], [12].

Surgery has also been indicated in spontaneous splenic rupture (SSR) or non-traumatic rupture of the spleen, a rare, lethal, but potentially

treatable condition [13]. With predisposing factors such as leukaemias, malaria, lymphomas, liver cirrhosis, rheumatoid arthritis, pancreatitis, e.t.c, SSR accounts for more than 20 percent mortality rate [14], [15]. SSR diagnosis is important in subjects with haematological malignancies accompanied by unexpected abdominal pain, hypotension and shock [13]. The autosomal recessive disorders (Thalassemias) where one or more globin chains are reduced, results in defective erythropoiesis, haemolysis, and consequent hypersplenism following overactivity of the spleen. The disease courses with a hypercoagulable state, thrombosis with the risk of thromboembolic complications [16]. Haemangioma is the most common, asymptomatic benign neoplasm of the spleen that is also indicated for surgery [17]. Splenic metastases which are rare, have 0.9-1.86 percent prevalence in breast cancer cases [18], [19]. Malaria is an important disease especially in tropical regions of the world caused by protozoa of the genus *Plasmodium spp.* (*falciparum, vivax, malariae, ovale* and *knowlesi*). This disease affects the fundamental function of the spleen, as the spleen removes dead cells or cells infested by parasites and returning intact erythrocytes to the blood. It has also been observed that asplenic and hypoplastic individuals are more susceptible to fatal progress of the disease. This disease can cause spontaneous splenic rupture and in rare cases, splenic infarction, which are indications for splenectomy [20], [21]. Another close relative in terms of anaemia, is sickle cell disease (SCD), a genetic disease of haemoglobin leading to tissue damage and anaemia.

One important complication of sickle cell disease is splenic sequestration, where red blood cells become entrapped in the spleen, causing the spleen to enlarge, pooling and resulting in the final destruction of red blood cells [22]. The spleen is also implicated in hereditary spherocytosis in that it is the site of sequestration and phagocytosis of non-deformable red cell, leading to anaemia [23]. This disease is characterised by pallor resulting from the anaemia, jaundice from the hyperbilirubinemia and splenomegaly. While autoimmune haemolytic anaemia is a disorder caused by autoantibodies directed against red blood cells, idiopathic thrombocytopenic purpura (ITP) with no specific cause is characterised by thrombocytopenia and microangiopathic haemolytic anaemia, diagnosed by the definite presence of schizocytes in the peripheral blood smear film. Majority of these conditions may cause spleen enlargement. Spleen enlargement (splenomegaly) can also be caused by cirrhosis of the liver, lymphoma, and acquired immunodeficiency syndrome (AIDS) and venous thrombosis. Splenomegaly is characterised by pain in the upper left quadrant referred to the shoulder and sensation of early satiety [24]. Surgery is indicated in cases of severe thrombocytopenia associated with spontaneous bleeding, post-transplant splenic sequestration, or abdominal pain due to repeated splenic infarction [25]. Chronic venous congestion of the spleen, most often caused by sinusoidal intra-hepatic cirrhosis or splenic artery aneurysm, which can produce chronic venous obstruction by direct compression of the splenic vein can also cause congestive splenomegaly (CS) [5], [26]. Splenectomy is indicated as the treatment of choice for patients with severe residual thrombocytopenia, with venous thrombosis as the underlying cause of CS. However, in splenic artery aneurysm, a satisfactory result has been obtained with a patient treated with aneurysm resection and/or complementary splenectomy [5], [26].

As a secondary lymphoid organ, the spleen is generally involved by lymphomas [27], [28]. While the primary splenic lymphomas (PSL) originate inside the organ, the splenic tissue is compromised by diffuse dissemination of Hodgkin's Lymphoma and Non-Hodgkin's Lymphoma in the secondary splenic lymphomas (Silva and Gunasekera, 2015). Among the diverse haematological malignancies, lymphoid and myeloid leukaemias are well-known. Persons with these diseases may develop considerable splenomegaly and then splenic sequestration, which is responsible for the worsening of anaemia and pre-existing thrombocytopenia in bone marrow failure, observed especially in leukaemias [29], [30]. Felty's syndrome (FS) is a severe form of rheumatoid arthritis (RA) with longstanding, severe and erosive arthropathy. FS causes splenomegaly and neutropenia, which result from increased neutrophil sequestration, peripheral neutrophil destruction, and bone marrow failure to produce neutrophils. Splenomegaly which is manifested by abdominal pain from splenic infarcts, can indicate other complications such as liver cirrhosis or other infections, like bacterial endocarditis [9]. Surgery is indicated in the majority of these non-traumatic diseases affecting the spleen. This is because; the spleen is an important lymphoid organ, whose role in erythropoiesis, immunity cannot be denied, as it is often enlarged (splenomegaly) following excessive activity from sequestration of old and damaged RBCs and lymphocytes. This overactivity affects the blood supply to the spleen, increasing its risk of infection.

Complications/Laparoscopy

The major complications of surgery which may involve removing the spleen (splenectomy) include haemorrhage, thromboembolism, subphrenic abscess, thoracic infection and fulminate sepsis. The greater mortality rates are majorly due to haemorrhage, bacterial infections and myelofibrosis [4]. The risk of devastating post-splenectomy infection (OPSI) is both more common and is characterised by hypotension, altered consciousness or cardio-circulatory shock.

Conclusion

Splenectomy, despite being indicated in several conditions, is still controversial and remains open to further studies. Although splenectomy is a curative alternative in a small number of diseases, it is a complementary treatment in numerous other clinical disorders. While its purpose and its effects on the host's homeostasis are not fully understood. Unlike its indication in trauma, it is compromised by chronic disease and with the use of corticosteroids; immunity is reduced in these patients. Clotting disorders, changes in platelet function and associated diseases are common. This exposes these individuals to serious risks and complications, greater morbidity and mortality rates compared to other intra-abdominal surgical procedures. Therefore, as a serious surgical procedure, splenectomy should be undertaken only after the depletion of the clinic and non-invasive therapeutics.

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