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Basic Science

Determination of Highest Dose of Ammonia without Effect at Work Environment through the Expression of Interleukin-2 Cell in *Rattus Novergicus*

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Keywords: Ammonia; Interleukin-2; NOAEL; White Mice; Remmele Scale Index

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Abstract

BACKGROUND: For determining the threshold limit value firstly determined the highest dose of ammonia without effect (NOAEL). However, research on the determination of NOAEL ammonia didn't conduct in Indonesia.

AIM: The aim of this study to determine the value of the highest dose of ammonia without effect (No Observed Adverse Effect Level/NOAEL) through interleukin-2 (IL-2) expression on white mice.

METHODS: This study used experimental laboratory research with post-test only control group design using white mice as experimental subjects. The treatment group divided into 6 groups (a group of controls and five groups with different ammonia exposure through inhalation). The trend curve of Remmele Scale Index (IRS) and histopathologic analysis could be used for determining NOAEL.

RESULT: The location of the highest dose of ammonia without any effect (NOAEL) of white mice was in the second group, with 0.0103 mg/kg body weight dose. Analysis of statistical tests Kruskal Wallis stated there was no significant difference in interleukin-2 expression between the control with ammonia exposed group with a significance of p (0.747) > α (0.05).

CONCLUSION: There is no difference between some interleukin-2 expression in the lymphocyte cell lung white mice group exposed to ammonia and control group. The highest dose of ammonia without any effect (NOAEL) on white mice was 0.0103 mg/kg body weight.

Introduction

Indonesia threshold limit value of chemicals was adopted from other countries or institutions in the world, such as the ACGIH and OSHA. Some overseas institutions also had different threshold limit value. OSHA, for example, has an ammonia TLV ppm, NIOSH (50 ppm/5 minutes CEIL), ACGIH (25 ppm). Unfortunately, the threshold limits value of chemicals set by the result of economic and technological considerations [1].

If a country didn't have a chemical threshold limit value, the country could use other research data of the highest dose of ammonia without effect or *No Observed Adverse Effect Level*/NOAEL in the workplace. This also applies to the determination of the highest dose of ammonia gas in Indonesia; the research results can be used as the standard highest dose of ammonia in the working environment. However, until now there has been no research on the standard of the highest dose of ammonia gas in the work environment in Indonesia [2].

The requirement of ammonia in Indonesia is increasing every year. For example, ammonia gas consumption for ZA fertiliser industry averages about 400,000 tonnes per year. Unfortunately, this increase production and distribution of ammonia have an

impact on increasing risk accidents for the worker. To assess ammonia standards for workers must be preceded by the experimental animals. During this time, the experimental animals were often used are white mice with Rattus sp. [3].

Effects of ammonia in the mice include anatomical changes observed by hepatologist, but it is also observed by biomolecular immune response aspects including interleukin-2 (IL-2). IL-2 expression of the cells was observed from the number of immunoreactive cell and intensity of colour. It is said to have an effect when there is a decrease in the immune response of lymphocyte cells i.e. IL-2, and it is said to be without effect if there isn't decline in the immune response to cells immunoreactive IL-2. This study never was done in Indonesia.

Therefore, the purpose of this study is to determine the highest dose of ammonia without effect (NOAEL) through the expression of IL-2-2 in white mice.

Material and Methods

This study was experimental laboratory research with post-test only control group design using experimental animals (white mice with Rattus novergicus) as experimental subjects. The treatment in research was giving ammonia with varying doses on white mice. The basic principle as the requirements of experimental studies was two (2) terms. The first requirement was replication or repetition of the conditions given equal treatment with all samples in all treatment groups. The second requirement is a random system or random element in the distribution of sample number 2 (two) groups of the study, the group that is exposed to ammonia through the inhalation system, and the group without being given exposure to ammonia or a control group. The control group was used to increase the validity of research results [4]. Group exposure ammonia divided into five sub-groups. Group 1 with concentration of ammonia 0.0872 mg/m^3, group 2 with concentration 0.1309 mg/m^3, group 3 with concentration 0.1963 mg/m^3, group 4 with concentration 0.2944 mg/m^3, and the last in group 5 with concentration 0.4416 mg/m^3.

Experiment unit

The study used species *Rattus norvegicus* rat or white mice from an animal laboratory of Pharmacy Faculty, University of Airlangga. White mice were selected with male sex, weight 138-142 gram and aged 2-3 months (white mice breeding) [5]. The white mice were kept by following several requirements such as control, environmental and health status monitoring, surveillance on researchers and workers then food and beverages supervision.

Determination of the number of replication follows the formula of determining the replication performed by Federer obtained the number of samples of 24 white mice [6]. This research already approved by the Ethical Committee of Veterinary Faculty, Airlangga University with ethic number 212-KEK in 2012. Tools for experimentation such as 5 sets of Metabolite Kit, measuring cup, 5 pieces of 1 l chemical, Scales, simple Respirometer, 3 ml dropper. For materials was NH_4OH 25%, BJ 0,91 kg/l, aqua shades, *Rattus novergicus*, and KOH powder.

Dose determination of animal toxins in the body

To determine the dose of toxin in the form of gas that enters the living body, including animals used formula of [2]:

$$\text{Dose} = \frac{(\alpha)(\text{BR})(\text{C})(\text{t})}{(\text{W})}\ (\text{mg/kg})$$

Note:

α = % Absorbed substances lungs, = 100% when the unknown.

BR = *breathing Rate* (Respiratory rate of experimental animals, the unit m^3 / h

T = *time* (Long working time, hours)

C = c*oncentration* (Concentration of toxin in the air, mg / m^3)

W = w*eight* (Animal weight, kg)

The highest dose of toxin without effect (NOAEL) in the experimental animals

NOAEL determination was performed on a test sub-acute [7], [8], [9], [10]. General protocol sub-acute toxicity test method is tested on 14 days, using 3 doses of test and control. The dose is causing toxic effects but not cause death; smallest dose did not leave a toxic effect, the middle dose is between the two doses of the above and Control = all ingredients used unless the active substances generally are solvent.

This calculation also conducted chemical analyses of blood or urine, and histopathology and others analyse if possible. NOAEL, along with LOAEL was the main indicator of environmental quality criteria. The range between the concentration NOAEL and LOAEL a concentration range of acceptable substances or match (Maximum Acceptable Toxicant Concentration) [1].

Analysis of Statistics

Analysis statistics uses a Kruskal Wallis test

with Alfa 0,05 to know the difference in interleukin-2 expression between control and ammonia exposed group (group 1, 2, 3, 4).

Histopathology

IL-2 expression in lymphocyte cells was a semiquantitative scale of IRS IL-2 (Remmele Scale Index) which is the result of multiplication percentage of the immunoreactive cell (A) with colour intensity score on the immunoreactive cell (B) according to the modified Remmele method (IHC staining, 1000 x magnification) [12].

IRS CD8 = (A x B)

A representation of the percentage of immunoreactive with score 0: no immunoreactive cell, score 1 for immunoreactive cell less than 10 %, score 2 for the immunoreactive cell was 11-50%, score 3 immunoreactive cell was 51-80% and score 4 if the immunoreactive cell has percentage more than 80%.

B was represented for the colour intensity of the cell. Score 0 for no colour, score 1 for moderate colour intensity, score 2 for moderate colour intensity and score 3 for strong colour intensity [12]. This picture was
obtained by staining the HE; enlargement of the 400x; Olympus BX-50. Camera Digital Pentax Optio 230; 2.0 megapixels) with scale 600 µm.

Results

Observation

Dosage of ammonia in the control group was 0.0000 mg/kg, in the group 1 is 0.0068 mg/kg, group 2 is 0.0103 mg/kg, group 3 is 0.0154 mg/kg, group 4 is 0.0231 mg/kg and group 5 is 0.0346 mg/kg body weight of mice on Table 1.

Table 1: Results of Ammonia Dose in White Mice with Time Exposure 8 hours/day

NH_3 Concentration (mg/m^3)	The percentage of ammonia absorbed (α)	The average rate of respiration (BR) (L/h)	W (average weight) (kg)	NH_3 Dose (Mg / kg)
0.0000	100%	1.3750	0.1405	0.0000
0.0872	100%	1.3755	0.1405	0.0068
0.1309	100%	1.3809	0.1410	0.0103
0.1963	100%	1.3809	0.1410	0.0154
0.2944	100%	1.3657	0.1395	0.0231
0.4416	100%	1.3754	0.1405	0.0346

Source: Primary Data.

The Observation of IL-2 (Interleukin-2) Expression in Lymphocytes

On slide K (control) in Figure 2 is still has a low IL-2 expression by the percentage shown immunoreactive cell number is still less than 10% and moderate colour intensity. In group 1, immunoreactive cells had increased in number between 11-50% and moderate colour intensity. In the group 2 with a dose of 0.0103 mg/kg weight, immunoreactive cell count between 51-80% greater than the number of cells group 1, but the intensity of the colour was same as the intensity of colours in the group 1. In group 3 immunoreactive cell count between 11-50%, but the colour intensity is lower than the intensity of the colour in the group 2. Thus, expression cell score in the group 3 lower than the score of the IRS group 2. In group 4 has immunoreactive cell less than 10% with moderate colour intensity. In group 5 has immunoreactive cell less than 10% and colour intensity was almost the same as group 4 but from expression is still lower than group 4. IRS score tends to fall because of continuous exposure to ammonia.

Table 2: Observations Expression of Interleukin-2 Lymphocyte Cell Lung in White Mice

Group of white mice	NH_3 dose (mg/kg)	Interleukin-2 expression (IRS)
Control	0.0000	2.75
Group I	0.0068	4.00
Group II	0.0103	4.50
Group III	0.0154	4.25
Group IV	0.0231	3.75
Group V	0.0346	3.25

p = 0.747; α = 0.05; Source: Primary Data.

Expression of Interleukin-2 and Color Intensity

From IRS score shown in Table 2, the highest IRS score contained in group 2 (4.50) at a dose of 0.0103. From that number then fell to 3.25 at the highest dose in group 5. From these data, it can be concluded location of the highest dose of ammonia without any effect on the lungs of white mice is in the second group, i.e. 0.0103 mg/kg body weight of white mice.

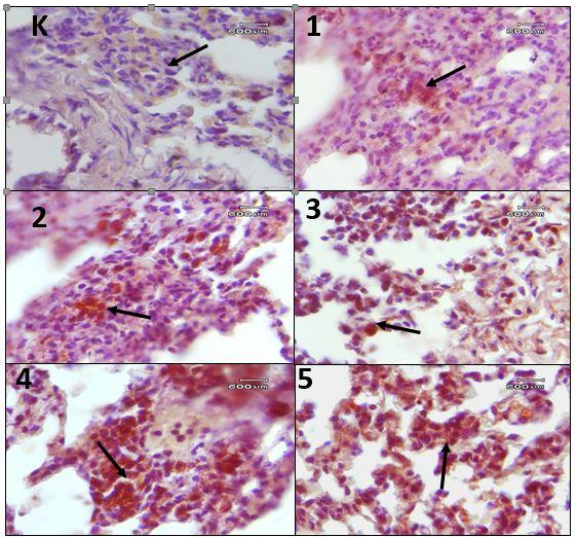

Figure 1: Comparison of Expression of IL-2 on Lung white mice after exposure to ammonia with different doses. K represents a control group with no exposure to ammonia, and 1, 2, 3, 4 and 5 respectively represent the group exposed to ammonia in a row with a dose of ammonia 0.0068; 0.0103; 0.0154; 0.0231; 0.0346 (mg/kg)

From the results of the Kruskal Wallis test, it can be seen that there is no significant difference in the expression of IL-2 (interleukin-2) between the

control group and the ammonia exposed group with p significance (0.747) > α (0.05). No significant difference can be seen from the number of IRS score in each group were almost the same as that shown by using immunohistochemical staining (Figure 2).

In Figure 3, the IRS score of IL-2 (interleukin-2) at a dose group 2 is higher than the other, which rose after a dose group 1 and then fell after a dose of group 2. Thus, one can say that the immune response or IRS score of IL-2 (interleukin-2) is group 2 (with a dose of 0.0103 mg/kg) is the highest and could be called as the location of the highest dose without effect or NOAEL.

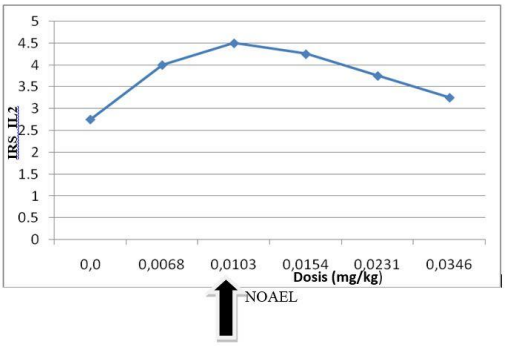

Figure 2: Relationship NH_3 Dose in the White Mice Body Exposed Ammonia Group and Control with IRS score of Interleukin-2 Immune Response

Discussion

Expression of Interleukin-2 in White Mice Exposure to Ammonia and Control Group

Of the four leading causes of death: injuries, infections, degenerative diseases, and cancer - only the first two causes usually cause the patient's death before reproductive age, which means potentially eliminating genes. Therefore, any mechanism that reduces the impact of highly valuable in sustaining life, it can be observed in a sequential process, recovery, and immunity [13].

Ammonia is polar thus can be absorbed into the cell by passive diffusion with facilities/carrier protein. Ammonia as a hapten binds to carrier protein will be immunogenic and entry into T cells, including IL-2 (interleukin-2). With continuous exposure to ammonia at IL-2 (interleukin-2) caused a reduction in lymphocyte cells IL-2 (interleukin-2) immune response.

Recipient T cell or TCR (T Cell Receptor) has the typical domain structure consisting of protein molecules containing amine groups. Thus, structurally T cells that contain amine groups will easily bind with ammonia [13]. As the immune system containing immunoglobulin superfamily, IL-2 (interleukin-2) group containing ammonia. Hapten ammonia has the chemical formula analogous to the amine group on IL-2 (interleukin-2). With the similarity of chemical compounds, IL-2 (interleukin-2) can absorb ammonia [13], [14]. Thus, the number of issued immune response is also almost the same.

IRS score is also increased to 4.50 in group 2 at a dose of 0.0103 mg/kg. This means that the immune response of white mice at group 2 is still good and mice have not shown the effects resulting from exposure to ammonia. This is due to the immune system of white mice were able to function well to recognise and dispose of ammonia hapten [13], [14], [15].

From the description above, it can be concluded a dose of ammonia in group 2 didn't give effect for ammonia caused the white mice body is still able to recognise and dispose of ammonia.

Meanwhile, ammonia groups 3, 4 and 5 affects the body of white mice because the immune response decreases. From these data, it can be concluded that the location of the highest dose of ammonia without effect (NOAEL) in the mice of group 2, at a dose of 0.0103 mg/kg body weight of mice. With ammonia NOAEL found in the mice can be used to determine the highest dose of ammonia without any effect on the worker's body.

Ammonia Highest Dose without Effect (NOAEL) in White Mice

In this histopathologic analysis of the dose in group 2, provides tissue lesion score higher than the first group and the control group, but not statistically significantly different. Thus, the dose group 2 i.e. 0.0103 mg/kg body weight of white mice is the highest dose of ammonia without any effect on mice. This is accordance with the opinion of Vermeire and Leeuwen that there is no significant relationship between dose in the exposed group and the control, and the highest doses of the exposed group were the highest dose without effect called NOAEL [16].

The results of Kruskal Wallis test produces a value above alpha, so it can be seen that there are no significant differences in the expression of IL-2 (interleukin-2) between the control group and the ammonia exposed group 1, 2, 3, 4 and 5 with p (0.747) > α (0.05). However, descriptively, IRS score in dose group 2 is higher than the others which rose after the dose in group 1 and then fell after the dose in group 2, it can be stated that the immune response or IRS IL-2 (interleukin-2) in the group 2 highs and could be called as IRS lies at the highest dose of ammonia without effect or NOAEL in white mice.

According to the EPA, NOAEL is experimentally determined the dose at which no statistically significant indication of the toxic effect or biological concern. Thus, although Kruskal Wallis test

immunoreactive expression of IL-2 (interleukin-2) IRS score did not differ significantly can be used as a reference in determining the NOAEL [17].

Moreover, from the aspect of molecular weight (Mw) were also influential. If compare with another lymphocyte cell such as CD4 and CD8, molecular weight IL-2 (interleukin-2) is 15,000, CD4 has a molecular weight of 60,000 and CD8 was 75,000. Because, CD8 has molecular weight greater doses of the same ammonia smaller mole, so its concentration is too small, and the reaction rate is also smaller. This is by the law of the greater speed of reaction that more greater concentration, it can also increase the reaction rate. Thus, the reaction rate is greater on CD4 and IL-2 (interleukin-2) than the CD8. Therefore, when group 3 began contained ammonia effect on mice. From these findings, IL-2 (interleukin-2) can be used as a marker for determination of ammonia in the highest dose of ammonia without any effect on mice.

From Figure 3 it can be stated the higher ammonia dose, the higher damage include congestion, oedema, infiltration, degeneration, necrosis, fibrosis, and hyperplasia. It is different with the immune response to IL-2 which begins with a rising curve that shows the immune response is still good (have not had a negative effect) but after it decreased immune response that show already negative effect. Thus, the pattern of immune response curve makes it easy to determine the highest dose without effect or NOAEL compared with the curve patterns of tissue damage. This is confirmed by a conception that immune as a gateway to the occurrence of diseases. Meanwhile, the effect occurs because there is a decrease in the immune response [18].

When the immune response decreases, this indicates that there has been an effect or impact of ammonia in the body [19], [20]. Similarly, at the time when the results of this study showed that there would be lymphocyte IL-2 (interleukin-2) decrease, it can be meant there was an impact of ammonia in the body. At the time of the ammonia dose, 0.0103 mg/kg in white mice body is the peak immune response seen from the rise in IRS score of IL-2 (interleukin-2), and after that IRS score, each lymphocyte cells was decreased. Thus, the dose of ammonia 0.0103 mg/kg can be referred to as the highest dose without effect on the body (NOAEL).

The integrity of the immune system is needed to defend themselves against microorganisms and toxic products they produce, and it is said to be a decline in immune response when a T-cell count is low, it can be seen using fluorescence microscopy. From the observation using fluorescence microscopy was found decreased IRS score on the third dose group whereas in dose group II had the highest immune response compared with the first dose group in IL-2 (interleukin-2). Thus, it can be stated that the highest dose of ammonia without any effect on the body lying on a white mouse to the second dose is 0.0103 mg/kg body weight of mice [21].

We can conclude that:

1. The level of Interleukin-2 expression ammonia exposed group did not differ significantly with the number of IL-2 expression on control.

2. The highest dose of ammonia without any effect (NOAEL) was 0.0103 mg/kg body weight of mice.

Recommendation

Based on the findings in this study will also provide a solution for the certification bodies such as the Center for Drug and food (BBPOM) in determining the safety certification of a food or a product. This is due to encountered a product has been certified but later turned out to be harmful to health, such as DDT, sodium benzoate on instant noodles. With the ease of determining the NOAEL will provide facilities that also determine a safe dose for workers.

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Ethical Clearance

Ethical Clearance took from the Faculty of Public Health, Airlangga University, Indonesia Committee.

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Comparison of the Microwave-Heated Ziehl-Neelsen Stain and Conventional Ziehl-Neelsen Method in the Detection of Acid-Fast Bacilli in Lymph Node Biopsies

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Abstract

BACKGROUND: Tuberculosis is a chronic inflammatory disease with lymphadenopathy being the most common extra-pulmonary manifestation. The conventional Ziehl–Neelsen method plays an essential role in the diagnosis of tuberculosis; however, it has a low sensitivity in detecting acid-fast bacilli.

AIM: The present study emphasises the role of the microwave-heated method (modified Ziehl–Neelsen) over conventional Ziehl-Neelsen stain and to set at the best condition for irradiation.

MATERIAL AND METHODS: The study included 90 patients with clinically suspected tuberculous lymphadenopathy who were referred to the Department of Pathology at Omdurman Military Hospital, Sudan. Demographic data such as age, sex, and site of swelling were documented for each patient. Specimens were stained with conventional Ziehl-Neelsen, fluoresce and the modified methods.

RESULTS: Patient's age ranged from 20 to 70 year. Of the total 90 cases with clinically suspected tuberculous lymphadenopathy, 18 cases were positive for AFB in conventional Ziehl-Neelsen method giving a sensitivity of 13.3%, while in microwave-heated method 82 cases of TB were detected positive for AFB yielded sensitivity and specificity of 97.6% and 85.7%, respectively, and positive and negative predictive values of 98.8% and 75.0% respectively compared to fluorescence methods.

CONCLUSION: In the present study, the microwave-heated Ziehl-Neelsen method, was found to have sensitivity and specificity of 97.6% and 85.7%, respectively which matches the fluorescence technique. It has specificity in detecting lymph node tuberculosis that makes it superior over all other modified methods. However, the availability and cost-effectiveness might limit the use of fluorescence in routine practice. Furthermore, the study set the best staining temperature is provided at power 1 level (60 w) for 1.5 minutes.

Introduction

Tuberculous lymphadenitis is a chronic, specific granulomatous infection of the lymph node with cassation necrosis, caused by Mycobacterium tuberculosis and less frequently by *Mycobacterium bovis*. Tuberculosis remains a major global public health problem. The average incidence of new cases worldwide is 26,000 and the global treatment success rate of 83%, similar to recent years. Globally, the incidence of tuberculosis is falling at about 2% per year [1].

Lymphadenopathy is the most common form of extra-pulmonary TB. Tuberculous lymphadenitis accounts for nearly 35% of extra-pulmonary TB. The diagnosis of tuberculous lymphadenitis can be morphologically by fine needle aspiration cytology (FNAC) of the lymph node [2].

The diagnosis of tuberculous lymphadenitis posses great challenge because it mimics other pathologic processes. The diagnosis of TB often requires a tissue biopsy and staining for acid-fast

bacilli (AFB). Newer advance and expensive tests such as polymerase chain reaction (PCR) help obtain an early diagnosis but, it is costly to be routinely used in developing countries [3]. *Conventional Ziehl-Neelsen* (*ZN*) stain is a rapid, useful and practical *method* for detecting *acid-fast bacilli* (*AFB*). However, the major disadvantage of *Conventional Ziehl-Neelsen* is its low sensitivity, ranging from 20% to 43 % [4], [5].

The cell wall of Mycobacterium possesses mycolic acid that makes it impervious to staining by aqueous staining solutions. Heating the slide helps to soften the mycolic acid on the bacterial cell wall as in conventional ZN stain. Many previous studies focused on improving the sensitivity of *conventional Ziehl-Neelsenusing different techniques*. Controlled heating can be provided with a microwave oven. There is scant literature on microwave method.

The few published literature on the microwave- heated methods concentrated on sputum smear. The diagnosis of tuberculous lymphadenitis posed great challenged for the practitioner. Thus, the present study emphasized the role of the microwave-heated method (modified Ziehl-Neelsen) over conventional Ziehl-Neelsen stain and to set at the best condition for irradiation in diagnosing tuberculous lymphadenitis.

Methods and Materials

The studies comprised 90 cervical lymph node specimens obtained from patients with clinically diagnosed tuberculous lymphadenitis. The specimens were obtained by excision biopsy. Tissues specimens were referred to the Department of Pathology in the Military Hospital Omdurman, Sudan between 2016 and 2017. The cases comprised both gender and all age group. The study was approved by the Ethics committee board of University of Gazira and Ministry of Health Sudan. Demographic data were collected from patients' case notes.

Inclusion criteria: All patients with lymph node blocks diagnosed as tuberculosis, suggestive or highly suggestive tuberculosis (T.B) and not on antituberculous treatment before specimen result.

Exclusion criteria: Patients diagnosed as tuberculosis and on antitubercular therapy.

Sample preparations

Two hundred seventy paraffin wax sections from the ninety lymph nodes tissues (3 specimens from each lymph node tissues) each with a thickness of 3-5 microns by using rotary microtome were prepared.

All sections of lymph nodes paraffin were mounted on clean glass slides (Cat NO.: 7.105, Size: 25.4X76.2 mm, Thick: 1-1.2 mm) and stained as follows:

Group (A): control cases which include 90 paraffin wax sections thickness 3-5 mm applied fluorescence dyes Auramine-O Rhodamine.

Group (B): 90 paraffin wax sections stained routinely by classical Ziehl-Neelsen stain heat carbol fuchsin and differentiated by 1% acid alcohol.

Group (C): which included 90 slides stained by modified Ziehl-Neelsen stain under microwave oven level 1 (60 w) 1.5 min.

Microwave Oven

A domestic oven (Sharp model-20AS (W)) with a maximum output of 600 watts, four power settings, and a digital timer was used.

Steps for conventional Ziehl–Neelsen Preparations [6]

1. Deparaffinized and rehydrated through graded alcohol to distilled water.

2. Flood the sections with freshly carbol fuchsin and heated to steaming with intermediate flaming 15 minutes 56-60^{0}C.

3. Wash in tap water 2 minutes.

4. Differentiation with 1% acid alcohol 3 minutes.

5. Wash in tap water to remove the excess of the acid counter.

6. Stain in methylene blue 15 seconds.

7. Dehydrate clear and mounted with DPX.

Steps for fluorescence techniques Preparations [6]

1. Deparaffinize and rehydrated through graded alcohol to D.W.

2. Stain, preheated (60^{0}C) fluorescence dyes for 10 minutes (Auramine-rhodamine-B).

3. Wash in tap water.

4. Differentiation with 0.5% acid alcohol (0.5 ml coc.HCL +99.5 70% alcohol).

5. Wash in tap water 2 minutes.

6. Eliminate the background fluorescent by 5% potassium permanganate for 2 minutes.

7. Wash in tap water.

8. Mount in aqueous mounting media.

Steps for microwave technique preparations

1. Deparaffinize and hydrated the slide to filter water.

2. Placed slide in carbon fuchsin in glass Coplin jar vol. 25 ml and microwave at power 1 level (60 w) for 1.5 minutes dipped the slide up & down several times and remained in warm solutions 15 minutes.

3. Washed in running tap water to removes the excess of stain.

4. Differentiation with 3% acid alcohol until sections became pale pink.

5. Washed in running tap water 1 minute 2 changes in D.W to remove excess of acid.

6. Counterstain the background with methylene blue 15 seconds.

7. Washed in water rinsed in 95% absolute alcohol 2 changes.

8. Cleared in 3 to 4 change in xylene.

9. Mounted media DPX.

Results

A total of 90 excised cervical lymph nodes diagnosed as tuberculous lymphadenitis were included in the present study.

Patient's ages ranged from 20 to 80 years. The majority (37.8%) of subjects' age ranged from 20-40 years, followed by age group of 61-80 years (34.4%), while middle age represented the minority of cases (27.8%). Females gender preponderance was noted accounting for 66.7% (60/90) with a male to female ratio of 2:1, Table1.

Table 1: Frequency of age and gender distribution

Age group	Frequency	Per cent
20-40	34	37.8
41-60	25	27.8
61-80	31	34.4
Sex		
Females	60	66.
Males	30	733.3

Data presents as number (%).

Of the 90 specimens, tissue positivity for AFB on the conventional ZN method was 20% (18/90), the positivity increased to 91.1% (83/90) on the modified ZN method. The correlation between the conventional ZN method and the modified ZN method (Table 2) showed statistical significance ($\chi 2 = 108.542$, df = 1, $P < 0.00$.

Table2: Comparison of detection rate between classical Z.N, Modified Z.N with microwave and fluorescence technique

Tests	Result		Total
	Positive (%)	Negative (%)	
Classical	18 (20)	72 (80)	90
Modification	82 (91.1)	8 (8.9)	90
Fluorescence	83 (92.2)	7 (7.8)	90

P > 0.0001; Data presents as number (%).

Figures 1 showed the rod shape acid-fast bacilli (arrow) in lymph node paraffin wax section by ZN classical method (x 100).

Figure 1: Rod shape Acid alcohol fast bacilli (arrow) in lymph node paraffin wax section by conventional Z.N method (x 100)

Figure 2, showed acid-fast bacilli were easily visualised as fragmented or beaded red rods when it was set at the best condition of 1 W and 1.5 min. irradiation (x 1000).

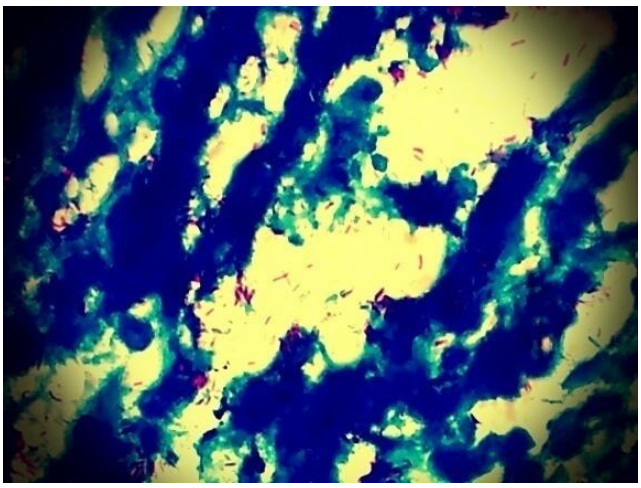

Figure 2: Fragmented or beaded Red rods (A.F.B) arrow seen inside and outside macrophages in modified ZN staining (x1000)

Figure 3 showing acid-fast bacilli in lymph node paraffin wax performed using the fluorescence technique (x1000).

Histopathology diagnosis of the tuberculosis lymph node was based on the criteria proposed by Jain 1999(7) (i) purulent with caseation; caseation with epithelioid cells (ii); only caseation (iii); and (iv) noncaseating with epithelioid cells. All cases in classical ZN method showed granuloma and giant cells no other chronic inflammation cells were demonstrated.

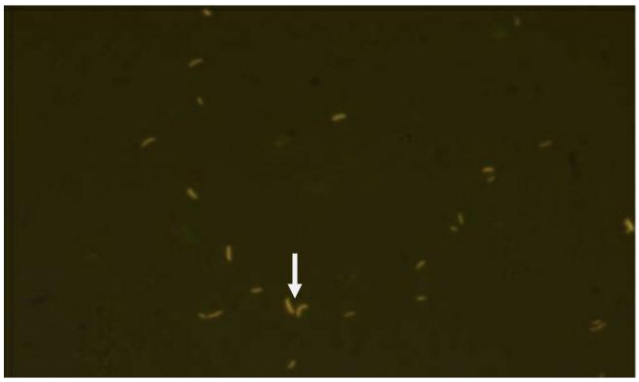

Figure 3: Acid-fast bacilli (Arrow) byAuramine – Rhodamine Fluorescence stain in lymph node paraffin section (x1000)

In the modified Z.N method the majority of histological features were 61(75.3%) granuloma with giant cells, while there were few epitheloid cells 6(7.4%) and gaseous necrosis similar to the finding obtained by fluorescence method Table 3.

Table 3: Comparison of the histopathological features, by classical Z.N, Modified Z.N with microwave and fluorescence technique

Histopathological feature	Classical ZN method (n = 90)	Fluorescence method (n = 90)	Modified ZN method (n = 90)
Epitheloid cells	0	7 (8.4)	6 (7.4)
Granuloma	0	10 (12.1)	10 (12.3)
Granuloma & giant cells	18 (100)	61 (73.5)	61 (75.3)
Gaseous necrosis	0	5 (6)	5 (6.2)
Total positive	18	83	81

Data presents as number (%).

Discussion

This study aimed to evaluate the efficacy of microwave-heated method as an example of an effective method for the diagnosis of acid-fast bacilli in comparison with conventional Ziehl-Neelsen stain. The validation of microwave-heated method based on revealing high sensitivity (97.6% specificity (85.7%) and positive and negative predictive values (98.8%), (75.0%) respectively in comparison with fluorescence techniques as a standard gold method. While the conventional method showed a sensitivity of 13%, this result highlighted the ability of microwave-heated method over the conventional methods that have a poor selectivity in detecting AFB ranging from 20% to 43% [3], [4]. The fluorescence method for the microscopic identification of tubercle bacilli in the present study as a standard gold test showed that 92.2% (83/90) of cases were positive AFB, this result is comparable to a previous study done [8].

It is known that the success of the Mycobacteria staining depends on the degree and stability of temperature at which dye uniformly penetrate the waxy cell wall barrier of the organism without cell distortion [9]. We attributed the higher detection rate of *acid-fast bacilli* by the microwave-heated method in the present study to the degree of temperature used to facilitate staining (power 1 level (60 w) for 1.5 minutes). This level of temperature and time frame in this study provided the optimal and the best condition for irradiation compared to heating beneath the slide as in conventional ZN STAIN.

Furthermore, excessive use of phenol in all preparations steps could negatively influence the identification of Mycobacterium leading to removing much of the dye and hence decreasing microscopic efficacy. In this study, phenol is used in only the last step. A previous study was done by Funashima Y [10] and his colleagues exploring the potential use of a microwave to improve microscopic efficacy in identifying AFB in sputum they proposed 600 W and 10-sec irradiation as the best condition.

The present study showed that the higher incidence of TBLA in age group (20-40) and (61-80) than Age (41-60), this finding is similar to the result obtained by Eshete and his colleagues 2011 [11]. Moreover, studies from Somalia and Asia showed that the age group between 25-44 and 45-64 years of age were more likely to have EPTB than young age group 15-24 years. In contrast, in Greenland, the oldest age groups were relatively less likely to be infected compared to the younger age group [12]. The occurrence of tuberculosis at the extreme of age can be explained by a diminution in immune function in these age groups. Globally, it was reported that age greater 65 years is associated with a higher risk of morbidity and mortality due to atypical clinical manifestations which result in a delay in diagnosis and initiation of anti-tuberculous therapy [13].

Tuberculous lymphadenitis was reported to be more common among females than males. Previous studies showed that Asian women and black women are at a higher risk than males for developing tuberculous lymphadenitis [14], [15]. In the present study we reported that female to males ratio was 2:1; this result is similar to study from India that yielded a ration of 2:1 [16]. The present study cannot explain gender preponderance, but some authors suggest socio-dynamics factors as risk for increase incidence among females [17].

In the current study, all subjects were previously diagnosed as having tuberculous lymphadenitis depending on variable histological evidence. According to these histological evidence we calcified the histopathologic patterns into epithelioid histiocytes only, granuloma, granuloma with giant cells, and caseation necrosis. Compared to other histopathology variables, granuloma with giant cells in the present study are considered as strong evidence for establishing the diagnosis of tuberculous lymphadenitis. This study showed that all cases in classical ZN method demonstrated granuloma with giant cells while this was demonstrated in 73.5% and

75.3% in fluorescence and the modified method respectively, Such findings have been previously reported by some authors (Ahmed, Vincent) [18], [19]. However, the absence of a characteristic histopathological picture of TB does not imply the absence of the disease [20].

We conclude that the modified ZN method is more sensitive and specific than the conventional ZN method. Furthermore, it is simple and easy. The modified ZN method greatly improves the diagnostic value of microscopic examination, and it is comparable to fluorescence technique in identifying patients with low-density bacilli. Furthermore, the study set the best staining temperature is provided at power 1 level (60 w) for 1.5 minutes. Further studies are warranted to investigate the diagnostics accuracy of this method in tuberculous lymphadenitis.

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Basic Science

The Study of the Wound Healing Activity of the Gel with a Comprehensive Therapeutic Effect

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Keywords: Chitosan-miramistin-hemopexin complex; Development of wound healing drugs; Models of linear and planar wounds; Wound treatment gel

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Abstract

AIM: The study was aimed at researching the specific wound healing activity of the drug with a comprehensive therapeutic effect based on derivatives of glucosamine and acrylic polymers to treat the infected wounds of various origins on a model of a planar infected wound.

METHODS: The model of septic wounds in rats as per the method of P.I. Tolstykh was used during the study of the specific activity of the drug with a comprehensive therapeutic effect based on derivatives of glucosamine and acrylic polymers for the treatment of infected wounds. The infection was performed with the *S. aureus* and *E. coli* strains. The study lasted 18 days, and during this period no full scarring occurred. The wound diameter was chosen as the effectiveness criterion. The planimetric method was used to assess the course of the wound process in experimental animals.

RESULTS: The obtained data prove the specific action of the drug with a comprehensive therapeutic action based on derivatives of glucosamine and acrylic polymers to treat the infected wounds of various origins. The study has shown that bacterially infected wounds healed worse than noninfected ones. Both types of wounds — infected and uninfected ones — healed faster when applying the test drug or Levomekol ointment.

CONCLUSION: On the model of a planar infected wound, the developed drug with a comprehensive therapeutic action has shown better wound healing effect compared with the Levomekol reference drug.

Introduction

The drug with a comprehensive therapeutic action based on derivatives of glucosamine and acrylic polymers to treat the infected wounds of various origin is a translucent gel based on hydroxypropyl cellulose and polyacrylamide, with the inclusion of chitosan, hemopexin, miramistin and lidocaine hydrochloride as pharmaceutical agents (Brkich, Pyatigorskaya, 2017) [1].

The drug for the treatment of infected wounds has four types of pharmacological effects, namely necrolytic, antimicrobial, wound healing and anaesthetic ones.

The necrolytic effect is ensured by hemopexin, providing a lysing effect on septic substrates and cleansing the wound surface from purple-necrotic masses. When applied to tissue, hemopexin splits fibrinous formations and necrotic tissue structures, and blood clots. It also thins exudate and vicious secret. Himopsin restores microcirculation in the wound walls, improves metabolic processes, which is clinically manifested by a decrease in local inflammation.

Chitosan is a carrier of the enzyme complex. It provides a prolonged therapeutic effect of himopsin and has a wound healing effect.

The antimicrobial action is ensured by the presence of Miramistin antiseptic (benzyl dimethyl [3-(myristoylamino) propyl] ammonium chloride monohydrate). The compound has a pronounced bactericidal action against aerobic and anaerobic bacteria, gram-positive (Staphylococcus, Streptococcus, Bacillus subtilis, Bacillus anthracoides) and gram-negative organisms (Shigella, Pseudomonas aeruginosa, Escherichia coli, Salmonella), as well as against hospital strains multiresistant to antibiotics. Miramistin provides antimicrobial and fungicidal action, enhances the functional activity of immune cells, while stimulating local (nonspecific) immunity, accelerates the wound healing process, reduces the resistance of pathogenic microorganisms to antibiotic therapy, and activates protective reactions at the application site through the activation of the absorbing and digestive functions of phagocytes.

The analgesic effect is due to the presence of lidocaine anaesthetic, which has a local anaesthetic effect, blocks potential-dependent sodium channels, thereby preventing the generation of impulses in the endings of sensory nerves and conduction of impulses along nerve fibres. The anaesthetic action of lidocaine is 2 – 6 times stronger than that of novocaine and procaine. When applied locally, it dilates blood vessels and has no irritating local action.

The study was aimed at researching the specific wound healing activity of the drug with a comprehensive therapeutic effect on a model of a planar infected wound.

Material and Methods

The drug is a gel for external use, containing per 100 g: himopsin – 0.2 g, miramistin – 0.05 g, chitosan – 2 g, lidocaine – 0.1 g, polyacrylamide – 0.1 g, hydroxypropylmethylcellulose – 2 g, glycerin – 5.0 g, and water – up to 100 g.

A group of 45 male rats of the Wistar line, weighing 200 – 240 g, (Stolbovaya branch of the FSBUN NCBMT FMBA of Russia) were used in the study.

The bacterial strains of the American Type Culture Collection (ATCC) obtained from the ACM (All-Russian Collection of Microorganisms) of Moscow were used in the experiment. The species composition was represented by the following microorganisms: *Escherichia coli* (ATCC 25922); *Staphylococcus aureus* (ATCC 6538-R).

The antibacterial effect of the studied objects was studied by the "wells" method. Bacteria were cultivated in L-broth at 37°C for 20 hours. Then, the number of cells per 1 ml of the initial suspension was determined using the Koch method. For further research, dilutions were used, providing the medium contamination on a Petri dish of 10^4 and 10^6 CFU/ml, which corresponded to the initial dissemination of the wound.

From the obtained dilutions, previously thoroughly mixed, bacteria were sown on the surface of the agar plate in a Petri dish with a sterile pipette in the amount of 0.1 ml. The volume of the applied suspension was distributed over the surface of the medium with a sterile spatula.

The septic wound was modelled for the animals under anaesthesia under sterile conditions as per the P.I. Tolstykh method (1976) [2]. For this purpose, the skin with subcutaneous tissue 25 mm in diameter was dissected into on the antiseptic-treated back section shaved from wool. A gauze plug containing 1.2 x 10^9 microbial bodies of the *S. aureus* daily culture or 2.6 x 10^9 *E. coli* was introduced into the resulting wound, and the wound was sutured. On the following day (24 hours), after modelling, the abscess with all the characteristic signs of inflammation was formed in all animals. After the stitches' removal, the upper skin flap was removed, the gauze plug was removed, and the purulence was evacuated. The area of the original wound was determined by applying a contour to a transparent film.

Treatment of the wound with the test drug or Levomekol reference product was started 24 hours after the septic wound modelling. The drugs were applied for 18 days. During the treatment, the diameter of the wound was measured every three days. Planimetric method was used to assess the course of the wound process in experimental animals.

Results

The animals were divided into groups, in accordance with the doses of the injected substance (Table 1).

Table 1: Groups of animals

No.	Infection type	Drug	Number of animals	Drug dose, g/kg
1.	*S. aureus*	Gel, 0.2 g/200 g	3♂	1
2.	*E. coli*		3♂	
3.	*S. aureus*	Gel, 0.1 g/200 g	3♂	5
4.	*E. coli*		3♂	
5.	*S. aureus*	Levomekol, 0.2 g/200 g	3♂	1
6.	*E. coli*		3♂	
7.	*S. aureus*	Levomekol, 1 g/200 g	3♂	5
8.	*E. coli*		3♂	
9.	*S. aureus*	Without drug	3♂	-
10.	*E. coli*		3♂	
11.	Without infection	Gel, 0.2 g/200 g	3♂	1
12.		Gel, 1 g/200 g	3♂	5
13.		Levomekol, 0.2 g/200 g	3♂	1
14.		Levomekol, 1 g/200 g	3♂	5 g/kg
15.		Without drug	3♂	-

After modelling a septic wound, rats in all groups of the test drug, the Levomekol reference

drug, and the wounds without drugs were sluggish in the first two days of the study. The wound at the beginning of the study is presented in Figure 1.

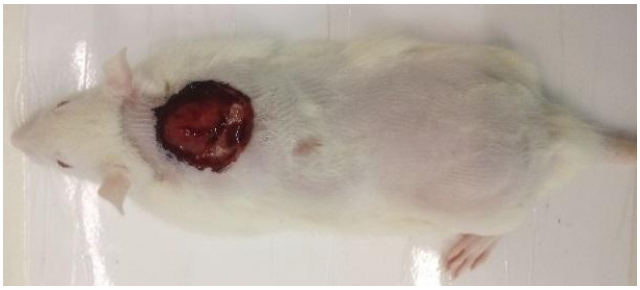

Figure 1: Wound at the beginning of the study

The wound diameter during the treatment was measured planimetrically every three days. The data are shown in Table 2.

Table 2: The result of measuring the size of the wound during the study

No.	Infection type	Drug	Wound size, cm						
			Days of infection						
			1	3	6	9	12	15	18
1.	*S. aureus*	Test drug, 0.2 g/200 g	2.7	2.5	2.1	2.0	1.2	1.0	0.5
			2.6	2.5	2.0	1.8	1.0	0.7	0.3
			2.5	2.3	2.1	1.9	1.4	1.1	0.7
		Average:	*2.6*	*2.4*	*2.06*	*1.9*	*1.2*	*0.93*	*0.5*
2.	*E. coli*	Test drug, 0.2 g/200 g	2.3	2.3	2.0	1.9	1.3	1.1	0.6
			2.5	2.5	2.3	2.2	1.2	1.0	0.5
			2.3	2.3	2.0	1.8	0.9	0.7	0.3
		Average:	*2.36*	*2.36*	*2.1*	*1.96*	*1.16*	*0.93*	*0.46*
3.	*S. aureus*	Test drug, 1 g/200 g	2.6	2.6	2.1	2	1.3	1	0.7
			2.6	2.5	2.1	1.8	1.1	1.1	0.3
			2.7	2.4	2	1.8	1.3	1	0.6
		Average:	*2.63*	*2.5*	*2.07*	*1.87*	*1.23*	*1.03*	*0.53*
4.	*E. coli*	Test drug	2.4	2.2	2	1.9	1.4	1	0.6
		1 g/200 g	2.4	2.4	2.2	2.1	1.2	1	0.6
			2.3	2.3	2.1	2	0.9	0.8	0.3
		Average:	*2.37*	*2.3*	*4.9*	*2.0*	*1.17*	*0.93*	*0.5*
5.	*S. aureus*	Levomekol, 0.2 g/200 g	2.8	2.6	2.4	2.0	1.4	1.1	0.9
			2.8	2.6	2.3	2.2	1.2	1.0	0.8
			2.3	2.2	2.1	2.0	1.3	1.1	0.7
		Average:	*2.63*	*2.5*	*2.27*	*2.07*	*1.3*	*1.07*	*0.8*
6.	*E. coli*	Levomekol, 0.2 g/200 g	2.5	2.3	2.3	2.0	1.6	1.2	1.0
			2.5	2.5	2.4	2.2	1.5	1.1	0.7
			2.6	2.5	2.4	2.2	1.4	1.1	0.6
		Average:	*2.53*	*2.43*	*5.57*	*2.13*	*1.5*	*1.13*	*0.77*
7.	*S. aureus*	Levomekol, 1 g/200 g	2.7	2.5	2.2	2.0	1.3	1.1	0.8
			2.6	2.6	2.4	2.1	1.3	1.1	0.7
			2.8	2.3	2.2	2.1	1.3	1.0	0.8
		Average:	*2.7*	*2.46*	*2.27*	*2.07*	*1.3*	*1.07*	*0.76*
8.	*E. coli*	Levomekol, 1 g/200 g	2.5	2.3	2.3	2.0	1.4	1.1	0.9
			2.6	2.4	2.4	2.1	1.5	1.1	0.8
			2.6	2.4	2.4	2.1	1.5	1.1	0.6
		Average:	*2.57*	*2.36*	*2.36*	*2.07*	*1.47*	*1.1*	*0.8*
9.	*S. aureus*	Without drugs	2.5	2.5	2.3	2.0	1.8	1.2	1.1
			2.6	2.5	2.4	2.3	2.0	1.6	1.2
			2.6	2.6	2.5	2.4	1.5	1.3	1.0
		Average:	*2.57*	*2.53*	*2.4*	*2.23*	*1.77*	*1.37*	*1.1*
10.	*E. coli*	Without drugs	2.6	2.5	2.5	2.2	1.8	1.6	1.2
			2.6	2.6	2.4	2.3	2	1.5	1
			2.7	2.8	2.5	2.3	1.7	1.5	1.1
		Average:	*2.63*	*2.63*	*2.47*	*2.27*	*1.83*	*1.53*	*1.1*
11.	Without infection	Test drug, 0.2 g/200 g	2.4	2.4	2.2	2.0	1.3	1.2	0.8
			2.7	2.6	2.2	2.0	1.0	0.9	0.6
			2.8	2.7	2.4	1.8	1.2	1.0	0.7
		Average:	*2.6*	*2.57*	*2.27*	*1.93*	*1.17*	*1.03*	*0.7*
12.		Test drug, 1 g/200 g	2.5	2.5	2.3	2.0	1.3	1.1	0.8
			2.7	2.6	2.3	2.0	1.3	1.0	0.7
			2.6	2.6	2.4	1.9	1.3	1.0	0.6
		Average:	*2.6*	*2.57*	*2.33*	*1.97*	*1.3*	*1.03*	*0.6*
13.		Levomekol, 0.2 g/200 g	2.6	2.6	2.4	1.8	1.4	1.2	1.1
			2.8	2.7	2.6	2.0	1.3	1.1	0.7
			2.7	2.7	2.2	1.4	1.2	1.0	0.6
		Average:	*2.7*	*2.67*	*2.4*	*1.73*	*1.3*	*1.1*	*0.8*
14.		Levomekol, 1 g/200 g	2.7	2.6	2.4	1.9	1.4	1.1	0.7
			2.7	2.6	2.4	1.9	1.4	1.1	0.9
			2.5	2.5	2.3	2.0	1.3	1.0	0.8
		Average:	*2.63*	*2.57*	*2.37*	*1.87*	*1.7*	*1.07*	*0.7*
15.		Without drugs	2.8	2.7	2.4	2.0	1.4	1.2	1.0
			2.7	2.7	2.6	2.3	1.8	1.5	1.2
			2.8	2.8	2.6	1.6	1.4	1.2	0.7
		Average:	*2.77*	*2.73*	*2.53*	*1.97*	*1.53*	*1.3*	*0.97*

Discussion

Levomekol was used as a reference drug. In the international practice, it has been known since the 70s as "Chloramphenicol + Methyluracil", and is a combined drug for local administration, having an anti-inflammatory effect. It is also active against gram-positive and gram-negative microbes (staphylococci, Pseudomonas bacilli and e-coli), penetrates tissues without damaging biological membranes and stimulates regeneration processes (Azuma, 2015; Dai, 2011) [3], [4]. Levomekol is widely used in surgical practice for any type of tissue damage, mainly infected ones, with mixed microflora in the first purulo-necrotic phase of the wound process (Brkich et al., 2018; Shipovskaia, Zudina, Fomina, 2015) [5], [6].

The model of septic wounds in rats as per the P.I. Tolstykh method was used during the study of the specific activity of the drug with a comprehensive therapeutic effect based on derivatives of glucosamine and acrylic polymers for the treatment of infected wounds (Tolstykh et al., 2013) [2]. The infection was performed with the *S. aureus* and *E. coli strains.* The study lasted 18 days, and during this period no full scarring occurred. The wound diameter was chosen as the effectiveness criterion.

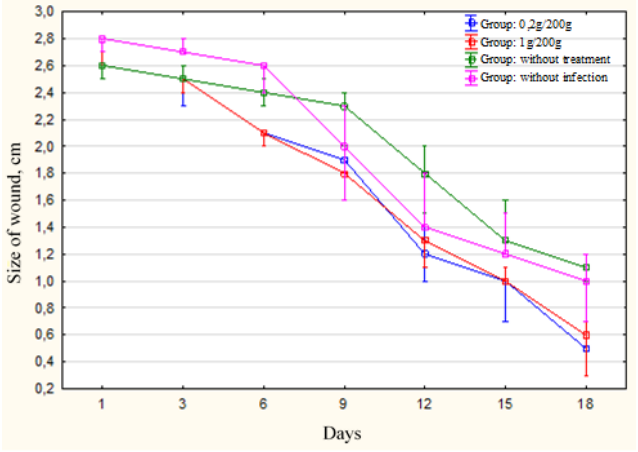

Figure 2: The wound healing dynamics in the Test Drug group in the case of S. Aureus infection

The bacterially infected wounds healed worse than the uninfected ones. On the 18th day of the study, the average diameter of the wounds infected with *S. aureus* (group 9) or *E. coli.* (group 10) was 1.1 cm, and in group 15 – 0.97 cm.

The healing rate of an uninfected and untreated wound was 0.1 cm/day (Table 2). The average diameter of the wounds without infection to which the preparations were applied on the first day of the study was 2.66 cm (groups 11 – 15). The rate of wound healing without infection after the application of the test drug was 0.105 cm/day for a dose of 0.2 g/200 g (group 11), and 0.111 for a dose of 1 g/200 g (group 12). The rate of wound healing with the

application of Levomekol was 0.105 cm/day for a dose of 0.2 g/200 g (group 13), and 0.107 cm/day for a dose of 0.2 g/200 g (group 14). The rate of healing of the uninfected wounds when applying a larger amount of the test or the reference drug was slightly higher. In general, the rate of healing of the uninfected wounds when applying the test drug was 1.11 times greater than the healing of the uninfected untreated wounds, and 1.07 times greater for the reference drug.

The size of the wounds on day 1 of the study after the infection with *S. aureus* in five groups of animals (1, 3, 5, 7, 9) averaged 2.63 cm. The dynamics of healing are presented in Figure 2.

The size of the wound on the 18th day of the study without the use of drugs was 1.1 cm. On the 18th day of the experiment, after applying the test drug to the wounds, the wound diameter decreased to 0.5 cm (dose of 0.2 g/200 g of the live weight) and 0.6 cm (dose of 1 g/200 g of the live weight). The wound healing rate was 0.117 cm/day for groups 1 and 3, which suggests that increasing the drug dose by more than 0.2 g per 200 g of the live weight lacked greater healing effect on wounds infected with *S. aureus.* For group 9 (healing with infection, without applying gels), the wound healing rate was 0.082 cm/day. The use of the studied gel increases the wound healing rate 1.43 times compared to the group of animals with the infected wounds, on which the gel was not applied.

The size of the wounds on day 1 of the study after the infection with *E. coli* in five groups of animals (2, 4, 6, 8, 10) averaged 2.63 cm. The dynamics of healing are presented in Figure 3.

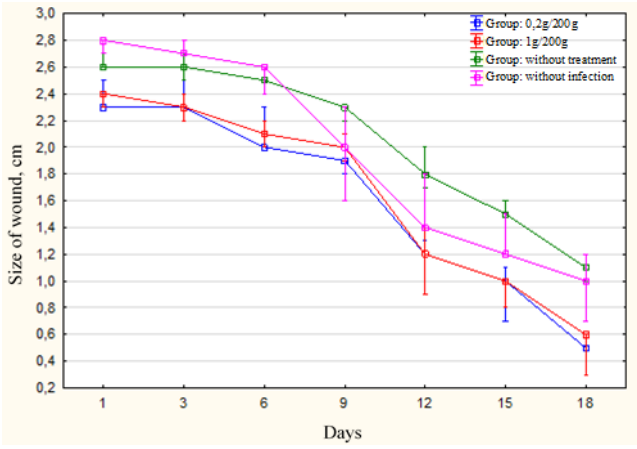

Figure 3: The wound healing dynamics in the Test Drug group in the case of E. coli infection

The size of the wound on the 18th day of the study without the use of drugs was 1.1 cm, as in case of infection with *E. coli* (healing rate – 0.077 cm/day). On the 18th day of the experiment, after applying the test drug to the wounds, the wound diameter decreased to 0.46 cm (dose of 0.2 g/200 g of the live weight) and 0.5 cm (dose of 1 g/200 g of the live weight). The wound healing rate was 0.105 cm/day for group 2 and 0.104 for group 4, which suggests that increasing the drug dose by more than 0.2 g per 200 g of the live weight lacked greater healing effect on the wounds infected with *E. coli.* The use of the studied gel increases the wound healing rate 1.36 times compared to the group of animals with the infected wounds, on which the gel was not applied.

After applying the Levomekol reference test drug to the wounds infected with *S. aureus,* on the 18th day of the experiment, the wound diameter decreased to 0.8 cm (dose of 0.2 g/200 g of the live weight) and 0.76 cm (dose of 1 g/200 g of the live weight). The data are presented in Figure 4.

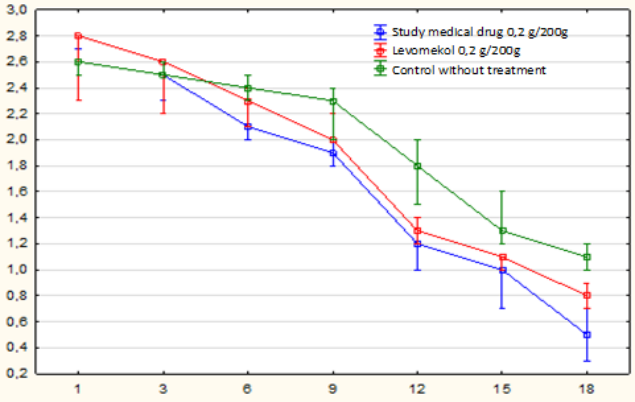

Figure 4: The wound healing rate in S. aureus infection

The wound healing rate was 0.102 cm/day for group 5 and 0.104 for group 7, which suggests that increasing the drug dose by more than 0.2 g per 200 g of the live weight lacked greater healing effect on the wounds. The use of Levomekol increases the healing rate of the wounds infected with *S. aureus* 1.27 times compared to the group of animals with the infected wounds to which the gel was not applied.

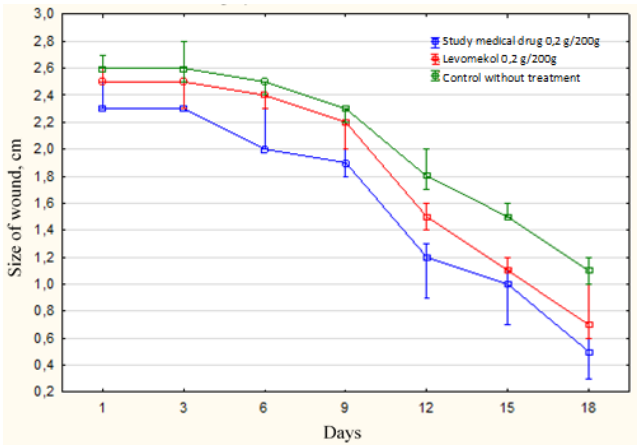

Figure 5: The wound healing rate in E. coli infection

In case of the wound infection with *S. aureus*, the healing rate in the group with the test drug was higher than in the group with the reference Levomekol drug by 1.2 mm/day in diameter. The wound healing rate in the group with the test drug was statistically significantly higher than that in the group of animals

with the infected and untreated wounds (group 9).

After applying the Levomekol reference test drug to the wounds infected with *S. aureus,* on the 18th day of the experiment, the wound diameter decreased to 0.8 cm (dose of 0.2 g/200 g of the live weight) and 0.76 cm (dose of 1 g/200 g of the live weight.). The data are presented in Figure 5.

The wound healing rate for groups 6 and 8 was 0.098 cm/day. The use of Levomekol increased the healing rate of the wounds infected with E. coli 1.27 times, as well as of the wounds infected with *S. aureus*, compared to the group of animals with the infected wounds, on which the gel was not applied, indicating the same effectiveness of Levomekol on this model of septic wounds.

In case of the wound infection with *E. coli*, the healing rate in the group with the test drug was higher than that in the group with the reference Levomekol drug by 1.2 mm/day in diameter. The wound healing rate in the group with the test drug was statistically significantly higher than that in the group of animals with the infected and untreated wounds (group 10).

Discussion

The obtained data prove the specific action of the drug with a comprehensive therapeutic effect based on derivatives of glucosamine and acrylic polymers to treat the infected wounds of various origins. The study has shown that the bacterially infected wounds healed worse than the noninfected ones. Both types of wounds — infected and uninfected ones — healed faster when applying the test drug or Levomekol ointment. When infected with *S. aureus* and *E. coli*, the studied drug showed the best result in wound healing compared with Levomekol. Increasing the dose of drugs more than 0.2 g per 200 g of the live weight lacked greater healing effect both in *S. aureus* and in *E. coli* infection.

On a model of a planar infected wound, the developed drug with comprehensive therapeutic effect has shown better wound healing effect compared with the Levomekol reference drug.

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Basic Science

Procalcitonin and Proinflammatory Cytokines in Early Diagnosis of Bacterial Infections after Bronchoscopy

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Keywords: Calcitonin; Tumor Necrosis Factor-alpha; Bronchoscopy; Cytokines; Interleukin-6; Interleukin-8; Bronchoalveolar Lavage

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Abstract

BACKGROUND: Fiberoptic bronchoscopy (FOB) guided bronchoalveolar lavage (BAL) remains as the chief diagnostic tool in respiratory disorders. 1.2-16% of patients frequently experience fever after bronchoscopy. To exclude the need for multiple antibiotic prescribing in patients with post-bronchoscopy fever, the presence of the self-limiting inflammatory responses should be excluded.

AIM: The current study was conducted to test the serum of patients undergoing bronchoscopy for some proinflammatory cytokines including Tumor Necrosis Factor-alpha (TNF-α), Interleukin-1beta (IL-1β), Interleukin-8 (IL-8) and Interleukin-6 (IL-6) and the value of Procalcitonin (PCT).

MATERIAL AND METHODS: Current case-control study was conducted at the National Research Institute of Tuberculosis and Lung Disease in Iran. Nineteen patients (48.72%) that attended with a reasonable sign for a diagnostic bronchoscopy from January 2016 to December 2017 were included in the case group. The control group consisted of 20 patients who underwent a simple bronchoscopy and without FOB-BAL. The laboratory findings for PCT concentrations and cytokine levels in the three serum samples (before FOB-BAL (t0), after 6 hr. (t1), and at 24 hr. past (t2) FOB-BAL) were compared between two groups.

RESULTS: The frequency of post-bronchoscopy fever was 5.12, and the prevalence of post-bronchoscopy infectious fever was 2.56%. PCT level was considerably higher in the patient with a confirmed bacterial infection when compared to other participants (p-value < 0. 05). Interestingly, IL-8 level in the bacterial infection proven fever patient was higher than in other patients (p < 0.001). IL-8 levels displayed a specificity of 72.7% and a sensitivity of 100%, at the threshold point of 5.820 pg/ml. PCT levels had a specificity of 84% and a sensitivity of 81%, at the threshold point of 0.5 ng/ml.

CONCLUSION: The present findings show that in patients with fever after bronchoscopy, PCT levels and IL-8 levels are valuable indicators for antibiotic therapy, proving adequate proof for bacterial infection. The current findings also illustrate that to monitor the serum levels of PCT and proinflammatory cytokines in the patients undergoing FOB-BAL, the best time is the 24-hour postoperative bronchoscopy.

Introduction

In numerous pulmonary and respiratory invasive techniques such as bronchoscopy are required to be applied. Bronchoscopy is generally a well-tolerated technique by most patients [1]. However, rare side effects such as severe arrhythmia, bleeding, pneumonia or pneumothorax are observed. Serious complications of bronchoscopic infections include spreading the infectious agents from one patient during bronchoscopy, transferring to the next procedures. Treatment for an invasive bacterial or viral infection is mandatory in such cases [2], [3].

However, one-third of patients develop fever, and sepsis-like syndrome after fiberoptic bronchoscopy (FOB) guided bronchoalveolar lavage (BAL) for yet unknown reasons and as a systemic inflammatory response [2], [3], [4]. Therefore, patients

with post-interventional fever should undergo further evaluations for observing the exact reason for the fever. The standard gold technique to detect systemic bacterial infection in patients with fever after bronchoscopy is blood cultures. However, such microbiological workup is time-consuming. In recent years, the usage of circulating proinflammatory mediators such as C reactive protein (CRP) and Procalcitonin (PCT) as alternative rapid predictive parameters have been broad [5], [6], [7].

In patients with sepsis serum levels are TNFα, IL-1β and IL-6 are increased [8]. Moreover, PCT is evaluated in response to intermediates or endotoxins released against bacterial infection (IL -6, TNF-α, IL-8, IL-1β), and is strongly associated with the amount and severity of bacterial infections.

Procalcitonin (PCT), a calcitonin hormone precursor, is produced by C cells in the thyroid gland, or by neuroendocrine cells in the lung and intestine, and its serum levels in healthy individuals are less than 0.15 ng/ml [7]. Expression of Procalcitonin is related to IFNγ, which is the released cytokine in response to viral infections is reduced. Therefore, PCT is a specific indicator of bacterial infections, and it may help to differentiate bacterial infections from viruses. PCT with a half-life of 25 to 30 hours has been described as a predictor of disease severity and antibiotic efficacy. Procalcitonin levels increase significantly in bacterial infections. In cases of viral infections or non-infectious febrile illnesses, procalcitonin levels are low or normal [9].

The production of PCT is associated with inflammation in response to inflammatory cytokines, which is characterised by a rapid increase in PCT levels. Endogenous systemic inflammatory responses stimulate alveolar macrophages to release increased cytokine concentrations. Some well-known inflammatory cytokines include Tumor Necrosis Factor-alpha (TNF-α), Interleukin-1beta (IL-1β), Interleukin-8 (IL-8) and Interleukin-6 (IL-6) [10].

In inflammatory reactions, TNF increases and reaches its maximum value in 90 minutes. While IL-6 reaches its maximum value in 180 minutes. The levels of PCT in sputum and serum elevate only after 3 to 6 hours of inflammation and reaches to its highest peaks at less than 6 to 8 hours, following a pattern similar to that of acute bacterial infection [11].

In previous studies, non-pneumonia subjects, with a rise in temperature above 1°C, were compared to subjects without increasing the temperature after BAL. The non-pneumonia group showed an elevated serum PCT and IL-6 levels that were secreted within 12 hours and was resolved after 24 hours [5].

Considering the heterogeneous and scattered reports of changes in serum proxlotonin levels and inflammatory markers after bronchoscopy and BAL, it seems that further research is required in a non-randomized clinical trial in this regard. The current study was conducted to research this field.

Hence, in the current approach, we tested the prevalence of patients with fever because of infection after FOB-BAL at the interventional pulmonology ward. Next, to clinical signs and symptoms, we assessed PCT value in the serum sample, in three intervals. In parallel, to check the self-limiting inflammatory responses and to exclude the need for antibiotic prescribing in patients with post-bronchoscopy fever, we assessed the concentrations of the proinflammatory cytokines TNF-α, IL-1β, IL-8 and IL-6 in serum.

Material and Methods

Study Design

The current case-control study is a performed approach at the interventional pulmonology ward, National Research Institute of Tuberculosis and Lung Disease (NRITLD) of Iran. The research committee and the ethics of the Shahid Beheshti University of Medical Sciences (IR.SBMU.NRITLD.REC.1396.375) confirmed stages of the study. An expert methodologist estimated the least sample size of 20 individuals in each of case and control groups by taking the statistical assumptions and formula [12].

We screened every patient that attended with a reasonable sign for a diagnostic bronchoscopy during January 2016 for inclusion and exclusion criteria. We entered patients if they were above 18 years old and with no infectious diseases or pneumonia. We excluded individuals with cardiac arrhythmia and acute from the study. We included the patients with an urgent diagnosis of bronchoscopy in the case group. Patients who did not need immediate diagnostic bronchoscopy included in the control group. Hence, none of the patients experienced bronchoscopy without a necessary reason.

After receiving signed consent letters, thirty-nine patients with respiratory disorders joined the current approach. The case group comprised 19 patients (48.72%) an immediate need for diagnostic FOB-BAL. A group of 20 selected patients with precise diagnosis and no need for diagnostic FOB-BAL (51.28%) entered the study as the control group.

We documented the demographic and laboratory information and recorded axillary body temperature before FOB-BAL (t0), after 6 hr (t1), and at 24 hr past (t2) FOB-BAL. We documented fever when the body temperature was ≥ 38°C. Data collection including age, sex, the cause of bronchoscopy, underlying disease, corticosteroid use, antibiotic use, body temperature, performed laboratory tests findings before the study and the laboratory findings at 6 and 24 hours after the intervention.

Serum culture was performed to evaluate the bacterial contamination following bronchoscopy.

In this study, we considered positive fever patients with positive serum culture and concurrent elevation of PCT serum levels, as the pneumonia cases. The laboratory findings for PCT concentrations and cytokine levels in the three serum samples (before FOB-BAL (t0), after 6 hr. (t1), and at 24 hr. past (t2) FOB-BAL) were compared between two groups.

Blood sampling and FOB-BAL

Three arterial blood samples were collected in sterile and with no additive tubes before, 12 hr and 24 hr after BAL. After centrifuging at 3.56 G for 10 min, we stored serum at -70C° for further use on processing day. We performed BAL, along with the former guideline using a flexible fiberoptic bronchoscope (Olympus; Tokyo, Japan) [13].

Laboratory Assay

Solid phase enzyme-linked immunosorbent assay (ELISA) (Biosource, Camarillo, CA) detected plasma concentrations of cytokines based on the earlier protocols [14]. We assessed serum PCT value using Electrochemiluminescence immunoassay (ECLIA) method (Boditech, South Korea) based on the company instructions. We considered elevated Serum cytokine value when it was above the upper limit value of the control group (≥ 5 pg/ml for IL-6; ≥ 20 pg/ml for TNF-a ≥ 15 pg/ml for IL-1b; ≥ 29 pg/ml for IL-8). PCT serum values above 0.5 ng/ml defined positive values and a bacterial infection in the patient.

Statistical analysis

Statistical Package for the Social Sciences (SPSS) (ver. 22.0; SPSS Inc. Chicago, IL, USA) software regulated the statistical analysis. Frequency, percentage, means (± standard deviation) or median (least- greatest) expressed the continuous variables. Also, for categorical variables, frequencies and percentages were the presented results. Spearman's rank correlation coefficient test showed the correlations between limits. Nonparametric Mann-Whitney U-test compared the comparisons between groups with continuous variables. Otherwise, the chi-square test analysed the comparisons between groups with categorical variables. Student's t-test and the Kruskal-Wallis test represented differences in the mean with a p-value of < 0.05 for a significant value.

Results

Demographic features

Table 1 represents the baseline demographic features in the study case and control groups. The mean age in the case group was 56 ± 16 (25 to 84 years) and 49 ± 15 in the control group (25-81 years).

Table 1: Baseline characteristics of the study patients

Characteristic	All patients	Case group	Control group
Number of patients, n (%)	(n = 39)	(n = 19, 48.72%)	(n = 20, 51.28%)
Age (yr), mean ± (SD) (interquartile range)		56 ± 16 (25-84)	49 ± 15 (25- 81)
Gender, n (%)	Male	12 (30.8%)	7 (17.9%)
	Female	7 (17.9%)	13 (33.3%)

The gender of the studied population was; 19 male (48.7%) and 20 female patients (51.3%). Two groups of case and control were similar in the frequency's distribution on the sex of participants (Pearson Chi-Square, p-value = 0.079). Post-bronchoscopy fever developed in two of 39 patients (5.12%) (Table2).

Table 2: The prevalence of post-bronchoscopic fever in study subjects

Body temperature (°C), mean (SD)	
Before bronchoscopy	36.8 (0.38)
After bronchoscopy, 6 h	37.4 (0.71)
After bronchoscopy, 24 h	36.9 (0.37)
Fever after bronchoscopy (Temp ≥ 38 °C), n (%)	2 (5.12)

The indications of FOB-BAL were for diagnosis of COPD in four cases, suspected of tuberculosis in one case, Lung cancer in 6 cases, Tracheostomy in one case and respiratory hypertension in seven subjects.

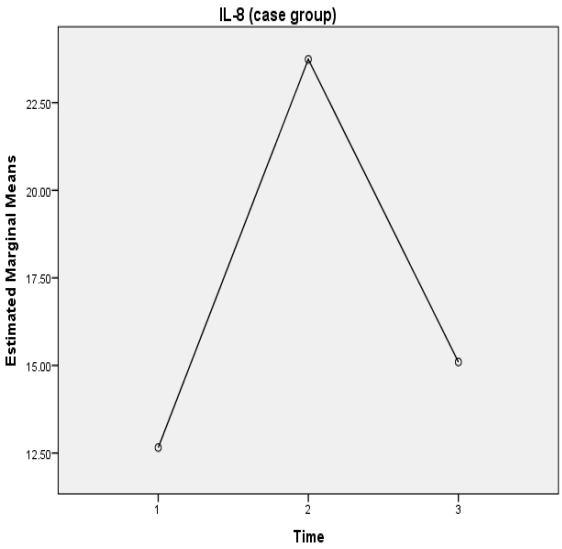

*Figure 1: Differences in IL8 concentration of case group during three intervals (P-value = 0.006); *: variance by Greenhouse-Geisser test was applied*

The concentration of IL-8, patients in the case group, presented significantly increased, when compared with the control cases (254.2 vs 1731.5

pg/ml P < 0.0001). Concentrations of IL-8 significantly increased, during the three episodes of sampling (P-value = 0.006) (Figure 1). Elevation of IL8 did not relate to gender (value of P = 0. 833) (Figure 2).

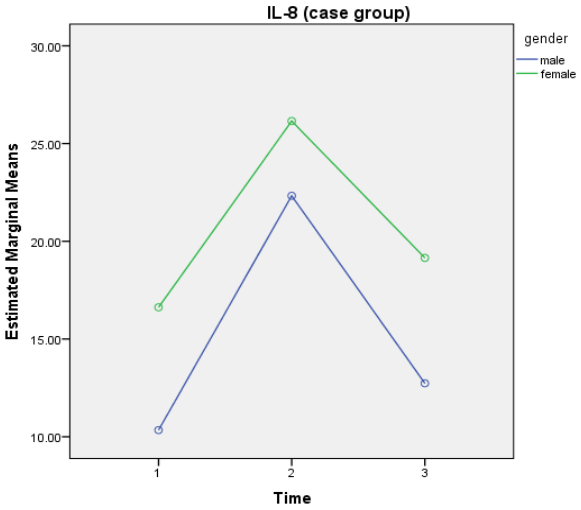

Figure 2: Correlations of IL8 concentration in three samples with sex in the case group (P-value=0. 833)

IL-6 concentrations have increased in the second specimens and reached the normal value in the third specimen. The increasing value of IL-6 in the second sample in the case group differed significantly from the mean value of IL-6 in the control group (19.38 vs 6.28 pg/ml P-value = 0.023, Mann-Whitney U test was applied) (Figure 3).

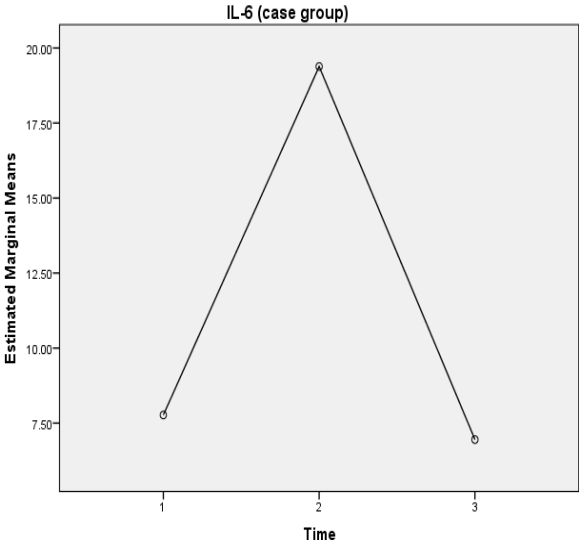

Figure 3: Differences in IL-6 level of case group in three samples (P-value = 0.023)

However, IL-6 levels did not represent significant differences in subjects with fever and without fever. Elevation of IL-6 did not relate to sex (value of P-value = 0. 593) (Figure 4). Also, in case patients with a temperature above 1°C after BAL, non-pneumonia and pneumonia subjects represented an increase in levels of interleukin 6 with no significant differences.

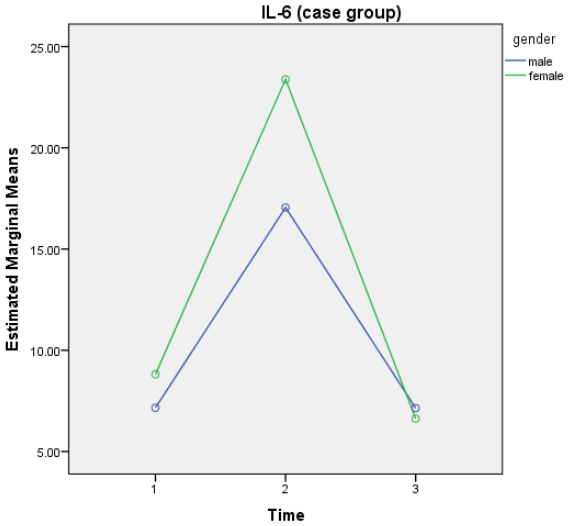

Figure 4: Correlations of IL-6 level in three samples with sex in the case group (P-value = 0. 593)

Levels of TNF-α and IL-1β decreased in the case group compared to the control group. IL-1β levels significantly decreased in the case group. However, this trend was not significant for TNF-α (P-value = 0.032 Mann-Whitney U test, P-value = 0.136, Mauchly's Test of Sphericity, respectively). Serum levels of TNFα and IL-1β were not related to the gender of the subjects (P-value = 0.833, P-value = 0.796 respectively) (Figure 5).

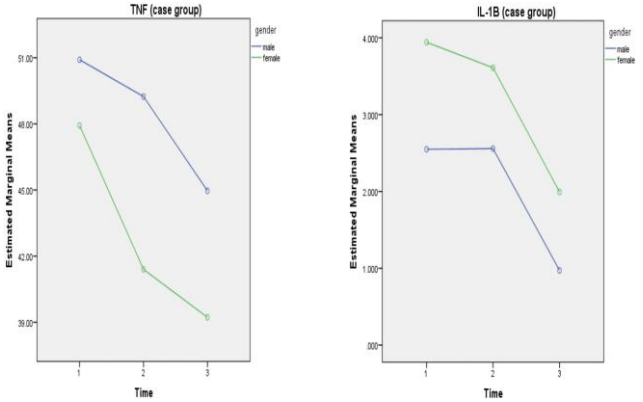

Figure 5: Correlations of TNF-α and IL-1β serum levels in three samples with the gender of the subjects (P-value = 0.833, P-value = 0.796 respectively)

Reduction of IL-1β level in the third sample was significantly different between the two groups (1.34 vs 3.40 pg/ml, P-value = 0.21). Also, TNF-α level was significantly different in all three samples of the case group compared to the control group (P-value < 0.001, Mann-Whitney U test).

Of two cases with fever after FOB-BAL, only one subject (5.3% of patients in case group) represented an elevated level of PCT in t2. PCT represented none significant differences in the case group, neither regarding the gender of the participants

or the interval of the sampling. According to the results, PCT values were normal in all 20 participants of the control group and every 3 samples of each. Laboratory findings for serum procalcitonin levels are presented in Table 3. Pearson correlation coefficient of PCT level and smoking did not represent a significant linear relationship. There was no relationship between the duration of bronchoscopy and PCT levels in any of the participants.

Table 3: Laboratory findings for serum procalcitonin levels

	Min.	Max.	Mean	Standard deviation
Procalcitonin	0.10	23.00	1.29	3.53

Current results represented that the levels of PCT in the fever positive patients with high levels of IL-6 and IL-8 are significantly higher than in patients with fever alone or in patients with isolated elevation of serum IL-6 and IL-8 protein levels (P < 0.01).

ESR levels represented an increase at t1 and reached the normal level at t2. The mean ESR level was not significantly different between the two cases and control groups at any of the three sampling times (P-value = 0.328, Mann-Whitney U test) (Figure 6).

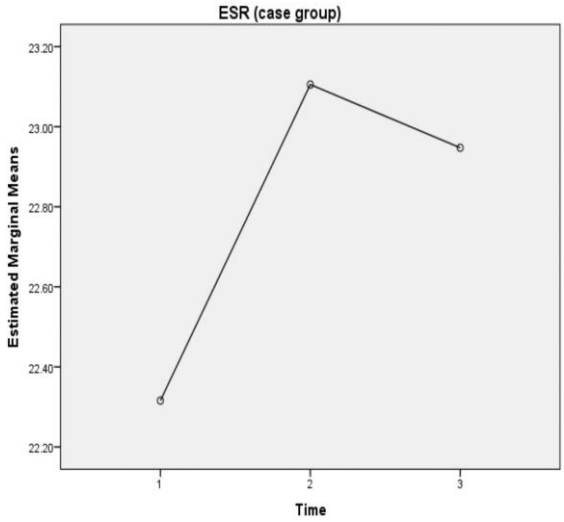

*Figure 6: ESR level in case group during three intervals (P-value > 05); *: Mann-Whitney U test*

Variations of ESR levels were similar in women and men of the case group (p-value = 0.651). As well, changes in ESR levels of three samples did not correlate with the sex of participants in the case group (p-value > 0. 05). Table 4 represents the laboratory findings of the study population.

Table 4: Laboratory findings of the study population

Group		N	Mean	Std. Deviation	p-value
ESR Time1	case	19	22.3158	10.53593	0.163*
	control	20	18.4500	6.05653	
ESR Time2	case	19	23.1053	10.51398	.072*
	control	20	18.4500	6.05653	
ESR Time3	case	19	22.9474	10.01928	.091*
	control	20	18.4500	6.05653	

*: Mann-Whitney U test.

The evaluated mean procedure time was 20.22 ± 5.62 minutes (15.00-30.00). The mean procedure time was not significantly different between the two cases and control groups and fever and unfevered cases (p-value > 0. 05).

The frequency of post-bronchoscopy fever was 5.12%. Microbiological analysis was positive for *Pseudomonas aeruginosa* in the serum culture of one subject (2.56%). Therefore, the percentage of unspecific fever after bronchoscopy was 2.56%.

PCT level was considerably higher in the patient with a confirmed bacterial infection when compared to other participants (p-value < 0.05). Interestingly, IL-8 level in the bacterial infection proven fever patient was higher than other patients (p < 0.001).

There was a moderate relationship between PCT and IL-6 concentrations in the fever cohort (Spearman rho = 0.463; p ≤ 0.001). However, there was no association between PCT and TNF-α or IL-1β, (p-value > 0. 05). We also found a strong correlation between elevated procalcitonin results with elevated IL-8 levels in the patient with positive serum culture when compared to the patients with negative culture, using exact Fisher test (P Value < 0.01).

At the threshold, the point of 3.706 pg/ml IL-6 concentrations showed a sensitivity of 100% and a specificity of 71.1%. IL-8 levels displayed a specificity of 72.7% and a sensitivity of 100%, at the threshold point of 5.820 pg/ml. Moreover, PCT levels had a specificity of 84% and a sensitivity of 81%, at the threshold point of 0.5 ng/ml. Finally, the best time to figure the diagnostic levels of procalcitonin and proinflammatory cytokines to predict a bacterial infection after bronchoscopy was 24 hours after the bronchoscopy.

Discussion

While a post-interventional fever can be due to bacterial or viral contamination, the use of antibiotics in such cases has been a matter of debate for years. Although prescribing antibiotics based on the observed fever after bronchoscopy is simple and practical, many authors believe that using these criteria results in antibiotic overuse, antibiotic-related adverse reactions and antibiotic-resistant bacteria without a thorough microbiologic study. On the other hand, serum culture and microbiologic studies are time-consuming and sometimes expensive.

PCT is a valuable alternative marker to diagnose bacterial infections because its serum values elevate as early as 3 to 4 hours after infection, much faster than other inflammatory markers such as ESR and C-reactive protein [15]. Current results show a 5.12% frequency of post-bronchoscopy fever that is in line with earlier reports, presenting a prevalence of

post-bronchoscopy fever in 1.2 – 16% of patients [2], [16].

Procalcitonin has also been a well-recognised marker for antibiotic therapy since 1993 [17], [8].

Bronchoscopy-BAL results in a vast acute phase reaction, such as peripheral neutrophilia and raised values of CRP [18]. About changes in IL-6 concentration, the findings of the current study were consistent with the results of previous studies [19], [20]. In the study by Krause et al., on 50 patients with and without BAL, IL-1β and IL-6 showed an elevation within 6 hours of operation [21].

With the concentrations of IL-8, the patients in the study group represented a significant elevation compared to the control group ($P < 0.0001$). This is in opposition to the reports by Huang et al. This contradiction is because of the differences in the population studied in two studies. Huang et al. observed over 50% elevation in neutrophils and over 7 times elevation in CRP value after bronchoscopy. However, they reported no changes in serum concentrations of IL-8 post-bronchoscopy. They performed their study on 28 healthy subjects to investigate the natural effects of Bronchoscopy with BAL [18].

In the current study, TNF-α concentrations elevated to a high peak at 24 hr. After bronchoscopy and decreases to an undetectable value by 48 hr. [18]. Here we applied the three collection steps of serum samples because of serum. TNF-α concentrations are detectable as early as 4 hr. after bronchoscopy [18]. Current results show that the prevalence of post-bronchoscopy infectious fever was 2.56%. The current findings like earlier studies emphasise on broader sterilisation to remove transmission of nosocomial infection during bronchoscopy operations [22].

In former studies, PCT was not reported as a valuable postoperative interpreter in immediate postnatal [23]. On the other hand, it was reported as a highly sensitive biomarker in the prediction of the severe community-acquired pneumonia [24].

However, the present study showed that in patients with fever after bronchoscopy, PCT levels and IL-8 levels are valuable indicators for antibiotic therapy, proving adequate proof for bacterial infection. The current findings illustrate that to check the serum levels of PCT and proinflammatory cytokines in the patients undergoing FOB-BAL, the best time is the 24-hour postoperative bronchoscopy. Some limitations of the study may be the lack of investigation of the impact of local anaesthesia. Therefore, we recommend further studies on this topic with some larger sample size and inclusion of different local anaesthesia to merge the findings of this study.

In conclusion, the present findings show that in patients with fever after bronchoscopy, PCT levels and IL-8 levels are valuable indicators for antibiotic therapy, proving adequate proof for bacterial infection. So, we recommend considering the results of procalcitonin besides the results of routine tests, in the protocol to start the antibiotic administration after bronchoscopy to reduce unnecessary antibiotic use in non-pneumonia individuals. The current findings also illustrate that to monitor the serum levels of PCT and proinflammatory cytokines in the patients undergoing FOB-BAL, the best time is the 24-hour postoperative bronchoscopy.

Acknowledgement

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Basic Science

Prevalence and Correlation between Diet and Dysmenorrhea among High School and College Students in Saint Vincent and Grenadines

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Keywords: Dysmenorrhea; Menstrual pain; Sugar; Diet; Lifestyle

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Abstract

BACKGROUND: Dysmenorrhea occurs as one of the symptoms of menstruation. While not necessarily a condition that plagues every woman, it is known to cause significant distress. Absenteeism from school and work as well as general discomfort are some of its adverse effects.

AIM: This study aims to investigate the effects of certain diets on the prevalence and severity of dysmenorrhea.

METHODS: Questionnaires was given to 478 women ranging from ages 1-55. The survey was centred around the age of menarche, presence and incidence of dysmenorrhea as well as how it is related to certain diets.

RESULTS: Majority of the participants (81.74%) belonged to the age groups of 11-15 and 16-20. 45.5% of the participants attested to dysmenorrhea at each menstrual cycle. statistical correlation between diet and dysmenorrhea was insignificant ($p > 0.05$). Consumption of caffeinated beverages correlated with dysmenorrhea ($p < 0.05$). Although not statistically significant ($p > 0.05$), the study reported dysmenorrhea in a large proportion of participants who consumed high quantities of sugars.

CONCLUSION: No relationship was established between diet and the incidence and severity of dysmenorrhea amongst the sample screened in Saint Vincent and the Grenadines. However, it appears that diet high in sugars might benefit from further research.

Introduction

Dysmenorrhea is pain induced by menstruation which occurs at the site of the lower abdomen. Its incidence is usually within the first 6-12 months after menarche [1]. It is also observed to occur within the first 2 years of menstruation. This is when a steady ovulatory cycle is usually established in females [2]. Dysmenorrhea may be comorbid with other symptoms such as; headaches, nausea, and vomiting [3]. Dysmenorrhea is an increasingly prevalent condition among premenopausal women. Its social implications include absenteeism from places of school and work [4]. There is however a rarity of physician consultations with dysmenorrhea cited as a chief complaint [2]. The combination of these factors makes this condition one of significance.

There are two types of Dysmenorrhea; Primary and Secondary. Primary Dysmenorrhea is menstrual pain that occurs in the absence of any underlying pelvic pathology. Its aetiology is the increase of myometrial production of prostaglandins and Leukotrienes. The end of ovulation triggers the synthesis and accumulation of fatty acids in the cell membrane. Progesterone levels decrease to signal the beginning of menstruation, allowing the release of these fatty acids. One of the synthesised fatty acids is arachidonic acid which is a precursor to the production of prostaglandins like E2 and F2α and

leukotrienes. Effects of the prostaglandins E2 and F2α are vasoconstriction as well as uterine contractions which induce the symptoms of dysmenorrhea [1].

Secondary Dysmenorrhea is a condition which occurs as a symptom of an existing pelvic pathology. Possible causes of this condition include but are not limited to; Endometriosis, Pelvic inflammatory diseases, adhesions, abscesses, Mullerian anomalies, and ovarian cysts [2]. A study by Harel 2008 singled Endometriosis as the more frequent cause of secondary Dysmenorrhea amongst adolescents. Endometriosis is a syndrome characterised by the presence of the uterine lining outside the uterine cavity. One of the more prevalent symptoms which enable diagnosis of endometriosis is Dysmenorrhea [2].

Possible therapeutic options for treatment of Dysmenorrhea include the use of Non-Steroidal Anti-Inflammatory Drugs (NSAIDS), analgesics, Oral Contraceptive pills as well as injectable long-acting contraceptives (Harel, 2002) [5]. Some non-pharmacological options have also been associated with success. Some of these are herbs, acupuncture and heat therapy [5].

Several factors have been linked to an increased risk of Dysmenorrhea. A couple of these factors are: earlier age of menarche increased bleeding intensity, and longer lasting flow [5]. Some have tried to find possible relationships between diet and dysmenorrhea [1], [6], [7]. Some foods are associated with reduced risk of dysmenorrhea. Foods containing Omega-3 fatty acids are a prime example of this. It is believed that Omega-3 fatty acids competitively bind to the Omega-6 sites in the cell membrane [1].

This inhibits the production of arachidonic acid and by proxy the prostaglandins and leukotriene involved in the pathogenesis of Dysmenorrhea. Conversely, foods containing Omega-6 fatty acid have been shown to have an inverse relationship with the severity of Dysmenorrhea [1]. Other foods that may affect the severity of Dysmenorrhea are dietary fibres. Dietary fibres have been observed to decrease estrogen levels. This would, in turn, decrease the chances of the occurrence of an ovulatory cycle. Thus, decreasing the risk of Dysmenorrhea [8]. Consumption of calcium-magnesium supplements has also been observed to have a positive effect on Dysmenorrhea. It was noted to cause a decrease in the severity of pain in individuals with primary dysmenorrhea [7].

This study aims to identify possible associations between dysmenorrhea in a sample population in Saint Vincent and Grenadines.

Methods

Location

Saint Vincent and the Grenadines is a southern Caribbean nation with its main island, Saint Vincent, and a chain of smaller islands. It has an estimated population of 109, 897. The island is home to many educational parastatals including some international institutions. The study was carried out on the main island with a focus in the capital, Kingstown, as well as other major locations like Arnos Vale and Belair.

Participants

The studies were conducted between January to March 2018. A total of 600 females was approached of which only 539 agreed to participate. The criteria for the study were females aged 11 to 55 years old. Participants that attested to medical conditions such as hormonal imbalance, endometriosis, pelvic inflammatory disease and sexually transmitted infection were excluded from the study. As a result, 61 individuals were left out of the study. Four hundred seventy-eight females from different institutions, ranging from high schools to college with signed consents participated in this study. All participants were presented with self-administered questionnaires.

Data collection

Published articles in reputable journals were extensively reviewed before the development of the questionnaire. Questions spanned from eating habits, gynaecological history, age, sexual history, marital status, medical history and the onset of menstruation; these were highly considered as it relates to menstrual pain.

Menstrual Pain was graded on a scale of 1 to 10 severity, 1 being the least and 10 being the worst, while the frequency of pain was graded as yes, No, and occasionally. The menstrual flow was graded as Moderate, very heavy and very light.

Diet was categorised into cereals, diaries, proteins, veggies, and carbohydrates. Use of Sugar during and before each period was considered and graded as moderate, frequent and rarely. Though few participants were indifferent about the intake of sugar and its effects on the pain, they experienced during their periods. Women who took caffeinated beverages were also identified and were graded as frequent/regular, normal/moderate, rarely/occasionally.

The statistical package for social sciences (SPSS) version 25.0 (Chicago, IL, USA) was used to analyse the data. Descriptive analysis was used to

simplify the data with the occasional use of graphs. Logistic regression analysis, chi-square and one-way ANOVA test were used for statistical analysis. A value of $P < 0.05$ was considered statistically significant.

Ethical approval

Necessary steps were carried out to acquire adequate permission for the study. The study was approved by the Research and Ethics committee of All Saints University, school of medicine.

Results

A total of 478 female respondents participated in this research. The age of participants ranged from 11-55 years, while a total of 81.74% of participants belonged to the age groups 11-15 and 16-20. The mean weight of participants were (61.8 ± 14.8) kg; other demographic data were also collected and categorised (Table 1).

Table 1: Demographic summary of participants

		Count	Percentage
Age	11-15	192	41.74%
	16-20	184	40.00%
	21-25	59	12.83%
	26-30	12	2.61%
	31-35	7	1.52%
	36-40	5	1.09%
	41-50	0	0.00%
	50-55	1	0.22%
Marital Status	Single	390	87.64%
	Married	16	3.60%
	Others	39	8.76%
Weight		61.8 ± 14.8 (95% CI 58.60-65.10)	
Height		5.0 ± 0.40 (95%CI 4.96-5.05)	

A group of 23.6% of the participants reported that they were sexually active while 72.2% reported otherwise. Age at first menstruation ranged from 8-18 years with a mean of 11.81 ± 1.41 years (Figure 1).

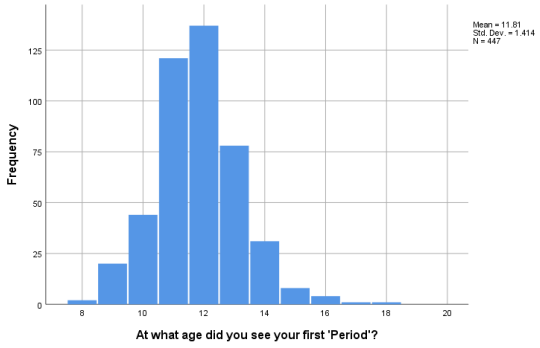

Figure 1: Histogram showing the summary of Menarche among participants

A group of 81.4% (381) participants experience menstrual flow every month since the first menstruation. Duration of period varied amongst the participants with most participants reporting the duration of 3-5 days (53.4%) and 5-7days (37.4%). Two thirds (75.3%) of the study participants reported having moderate menstrual flow while 77% of respondents reported pain during their period. More participants in the age class 16-20 and 21-25 had menstrual pain compared to others. Among the participants who reported pain during their period, most reported pain (31.6%) on the first day of their period (Table 2).

Table 2: Time of onset of menstrual pain

The onset of Menstrual Pain	Frequency	Per cent
2 days before menstruation	38	7.9
A day before menstruation	48	10.0
1st Day	151	31.6
2nd Day	39	8.2
3rd Day	13	2.7
5th Day	1	0.2
Throughout my period	123	25.7

Not all of the participants had menstrual pain during each period, 138 and 103 participants had pain occasionally and rarely with each period, respectively (Table 3). It was observed that there was a significant relationship between age and menstrual pain.

Table 3: Age crosstabulation of participants who answered the question, "Do you always have pain each time you see your period?"

			Age							Total
			11-15	16-20	21-25	26-30	31-35	36-40	50-55	
Do you always have pain each time you see your period?	Yes	Frequency	70	90	30	5	2	3	1	201
		% within Age	38.0%	50.8%	52.6%	45.5%	28.6%	60.0%	100.0%	45.5%
	Occasionally	Frequency	62	61	11	2	2	0	0	138
		% within Age	33.7%	34.5%	19.3%	18.2%	28.6%	0.0%	0.0%	31.2%
	Rarely	Frequency	52	26	16	4	3	2	0	103
		% within Age	28.3%	14.7%	28.1%	36.4%	42.9%	40.0%	0.0%	23.3%

The respondents were asked to rate the menstrual pain on a scale of 1 to 10, and no significant difference was observed across the different grouping based on period duration however increased pain was observed with increasing duration of menstruation as shown in the box plot (Figure 2).

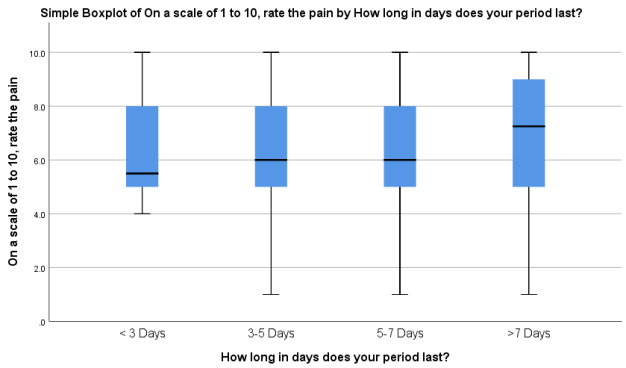

Figure 2: Correlating pain intensity and pain duration

Participants who reported consuming sugar frequently also reported more pain as compared to other groups; however, the difference was not statistically significant, p > 0.05 (Figure 3).

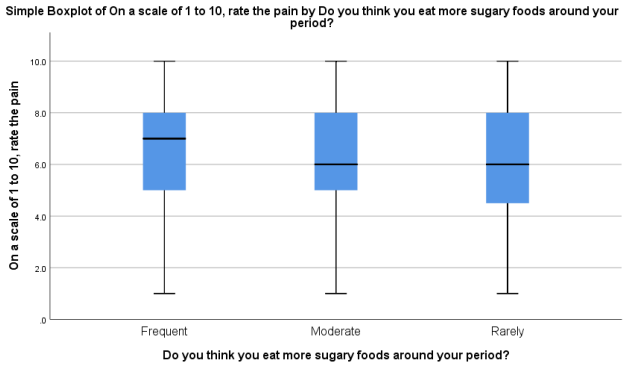

Figure 3: Correlating sugary food consumption and pain intensity

Individuals who reported consumption of caffeinated beverages also reported significantly higher pain than those who did not (p < 0.05). Participants who did not have sugar affected pain had a higher index of menstrual pain among the participants who reported having menstrual pain had their dominant food type collected and analysed (Table 4).

Table 4: Food type among participants who reported menstrual pain

		N	P Value
What Foods do you eat most-Cereals	No	256	0.116
	Yes	222	
What Foods do you eat most-Dairies	No	321	0.799
	Yes	157	
What Foods do you eat most-Proteins	No	268	0.93
	Yes	210	
What Foods do you eat most-Vegetables	No	360	0.244
	Yes	118	
	Yes	144	
What Foods do you eat most-Confections	No	322	0.862
	Yes	156	

Discussion

Primary Dysmenorrhea is common amongst young female; it is a menstrual pain without any medical conditions and impacts the quality of life of the individuals, dysmenorrhea has been linked to diet and lifestyle with an underlying physiologic mechanism of hormonal imbalance [9].

The findings from our study did not correlate diet to dysmenorrhea. Nevertheless, individuals who consumed foods high in sugar reported dysmenorrhea. A few studies have been done to assess the influence of diet on dysmenorrhea. Certain foods are said to interfere with the level of estrogen and prostaglandin in the blood. Elevated levels of Prostaglandins have been seen to be associated with dysmenorrheal [4], [8], [10]. Prostaglandins are said to cause endometrial contraction which causes menstrual cramps [10]. Individuals who consumed high vegetable diet have higher levels of Steroid-hormone binding globulins(SHBG)[4] which causes decreased estrogen and decreases stimulation of uterine endometrium and prostaglandin levels.

Though our study consisting of 478 participants was not able to show a statistical correlation between diet and dysmenorrhea, previous studies have demonstrated that after surveying 2561 females, feeding habit correlated with dysmenorrhea [11]. Another study surveying females also reported similar observations [12], [13]. The study also indicated that skipping breakfast caused dysmenorrhea when compared to participants that took breakfast irrespective of food class [12]. Some other studies show low consumption of fruit and vegetables increases dysmenorrheal, and high fibre diet with low-fat decreases dysmenorrheal [4], [14]. This study also showed that consumption of caffeinated drinks correlated significantly with dysmenorrhea (P < 0.05). Other studies have also correlated caffeine intake with dysmenorrehea [15], while it is unclear how caffeine cause dysmenorrhea, caffeine has powerful vasoconstriction effect implicated in pelvic pain, it is also associated with headaches [16].

Limitations: This study consists of across-sectional design which limits any conclusions about directionality. We also could not ascertain if people changed their diet close to their period, also if the diet affects the severity or length of pain.

We also could not assess other risk factors like smoking, family history, BMI which could be a confounding factor for primary dysmenorrhea. We could not explicitly state if non-Caribbean had their native meals or ate Caribbean food. While Caffeinated drinks were significant with dysmenorrhea, we could not ascertain the level of caffeine intake and severity of pain or specifically and if those who take caffeine also had high levels of starchy foods. Further studies will still be required.

In conclusion, while we could not identify a correlation between feeding habit and dysmenorrhea, we do know that dysmenorrhea significantly affect the quality of life of young females and also causes socio-economic stress to the nation at large, and possible ways to decrease menstrual pain via dietary interventions will positively impact not just the individual but also the nation.

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Basic Science

AM-1241 CB2 Receptor Agonist Attenuates Inflammation, Apoptosis and Stimulate Progenitor Cells in Bile Duct Ligated Rats

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Keywords: Cannabinoid receptor 2; AM1241; Hepatic progenitor cells; IL-10; p53; Bile duct ligation; Liver fibrosis; CD34

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Abstract

BACKGROUND: The cannabinoid receptor 2 (CB2) plays a pleiotropic role in the innate immunity and is considered a crucial mediator of liver disease. Cannabinoid CB2 receptor activation has been reported to attenuate liver fibrosis in CCl4 exposed mice and also plays a potential role in liver regeneration in a mouse model of I/R and protection against alcohol-induced liver injury.

AIM: In this study, we investigated the impact of CB2 receptors on the antifibrotic and regenerative process associated with cholestatic liver injury.

METHODS: Twenty-six rats had bile duct ligation co-treated with silymarin and AM1241 for 3 consecutive weeks. Serum hepatotoxicity markers were determined, and histopathological evaluation was performed.

RESULTS: Following bile duct ligation (BDL) for 3 weeks, there was increased aminotransferase levels, marked inflammatory infiltration and hepatocyte apoptosis with induced oxidative stress, as reflected by increased lipid peroxidation. Conversely, following treatment with the CB2 agonist, AM-1241, BDL rats displayed a reduction in liver injury and attenuation of fibrosis as reflected by expression of hydroxyproline and α-smooth muscle actin. AM1241 treatment also significantly attenuated lipid peroxidation end-products, p53-dependent apoptosis and also attenuated inflammatory process by stimulating IL-10 production. Moreover, AM1241 treated rats were associated with significant expression of hepatic progenitor/oval cell markers.

CONCLUSION: In conclusion, this study points out that CB2 receptors reduce liver injury and promote liver regeneration via distinct mechanisms including IL-10 dependent inhibition of inflammation, reduction of p53-reliant apoptosis and through stimulation of oval/progenitor cells. These results suggest that CB2 agonists display potent hepatoregenrative properties, in addition to their antifibrogenic effects.

Introduction

Liver fibrosis is a clinical condition, characterised by an accumulation of extracellular matrix proteins as a result of prolonged liver injury. At the same time, there is a continued stimulus for regeneration, prompting further mutilation of the hepatic architecture and vascular structures. If left untreated, fibrosis can progress into cirrhosis, hepatocellular carcinoma and liver failure. The incidence of liver fibrosis has been increased significantly in recent years [1]. Until now, patients suffering from hepatic fibrosis are treated in such a way to compensate such impaired hepatic functions. For quite a long time, liver fibrosis had been considered irreversible.

Nonetheless, there is aggregating clinical and experimental data to propose otherwise. Histological evaluation of biopsies from patients with chronic liver injury and animal models of fibrosis demonstrates that fibrosis is a dynamic, bi-directional process. Thus remodelling of fibrous scar and recovery is conceivable [2].

There is growing evidence suggesting that endocannabinoids may regulate the pathophysiology of liver diseases and plays an important role in apoptosis, tissue homeostasis, cell differentiation and proliferation. Under normal conditions, the endocannabinoid system is quite inactive and CB1, and CB2 receptors are barely expressed, partially because they are not expressed in hepatocytes. However, many studies have demonstrated the up-regulation of the expression of CB1 and CB2 receptors in hepatic myofibroblasts and vascular endothelial cells, as well as the increased concentration of endocannabinoids, especially anandamide, in the liver in the course of chronic

progressive liver diseases [3]. CB1 receptors possess a pro-fibrogenic effect in the liver and have also been implicated in the pathogenesis of alcoholic and nonalcoholic fatty liver diseases [4], [5]. On the other hand, CB2 receptors protect the liver against the development of fibrosis in CCl4 exposed mice [6], [7] and also play a potential role in liver regeneration in mouse model I/R [8] and protection against alcohol-induced liver injury.

Although, CB2 receptor agonists were reported to afford hepatic protection only in a model of I/R and CCl4 [9], [10]. In this study, we have investigated the effects of a CB2 receptor agonist (AM-1241) on a well-established rat model of cholestasis-induced fibrosis induced by three weeks' ligation of bile duct [11], [12], [13]. We have also explored the effects of AM-1241 on the production of the key immune-regulatory cytokine, IL-10 that affect hepatic inflammatory cells and also on oxidative lipid peroxidation and hepatocyte p53- dependent apoptosis. Besides, we have also explored the effects of CB2 receptors on regenerative effect through stimulation of liver progenitor (oval) cells. The latter, in turn, caused further attenuation of liver injury with replacement with healthy hepatocytes. Our findings strengthen the potential of CB2 receptors for the treatment of BDL injury and other fibrotic disorders.

Results were compared to the effects of silymarin, a drug commonly used as liver support during the treatment of cirrhosis. Silymarin has a great activity against a wide range of animal models of hepatic injury due to its antioxidant activity and radical scavenging. Silymarin was also reported to exert anti-inflammatory effects through the reduction of TNF-α [14].

The aim of the study is to evaluate the hepatoprotective effects of the Cannabinoid 2 receptor agonist, AM-1241, on hepatic fibrosis induced by common bile duct ligation of adult rats and to investigate its anti-inflammatory, antioxidant and anti-apoptotic potentials.

Material and Methods

Animals

Twenty-four; Adult male Wistar albino rats, of approximately 180-220 g body weight, were obtained from the animal house colony, National Research Centre, Giza, Egypt. All animals were housed in metal cages in a well-ventilated environment at (22 ± 3°C, 55 ± 5% humidity and 12h dark & light cycles) and were provided with a standard pellet diet (containing not less than 20% protein, 5% fiber, 3.5% fat, 6.5% ash and a vitamin mixture) and water ad libitum. All animal care and experimental procedures were approved by Ethical Committee of National research centre, Egypt (registration number: 16-036) and followed the ARRIVE (Animal Research: Reporting In-Vivo Experiments) guidelines [15].

Material

AM1241 was purchased from CaymanChem, Czech Republic. Silymarin was granted from CID, Egypt. Diethyl ether, Formaldehyde solution 34-38%, DMSO and PBS were obtained from Sigma, Egypt. Ketamine, Thylacine and Ceftriaxone have obtained from Egyptian companies; SIGMA TEC, ADWIA and EPICO respectively.

General procedures

Bile duct ligation (BDL) has been widely used for experimental induction of liver fibrosis in rats [16]. For this purpose, rats were anaesthetized with ketamine (50 mg/kg, i.p) and thylacine (5 mg/kg, i.p) [17] then the abdomen was shaved and disinfected. The common bile duct was exposed and twice ligated with 3-0 silk suture. Sham operation was performed by gently touching the bile duct without ligation. The abdomen was closed in layers. The animals were allowed to recover on a heating pad. Rats were injected with ceftriaxone (30 mg/kg, im) 30 minutes before surgery for prophylaxis against infection [18]. The experimenters were blinded to the treatments given to the animals and the biochemical and histological analyses and the data analyses.

Experimental design

The day after the operation, animals were randomly divided into four groups of six rats each and treated for three consecutive weeks as follows: The first group was sham-operated and served as the non-treated control, receiving vehicle only. The second group had a bile duct ligation then it was given the vehicle (BPS/DMSO solution in a ratio of 2:1), intraperitoneally. The third group also had bile duct ligation then was given silymarin daily in a dose of 100 mg/kg dissolved in the vehicle, intraperitoneally [19]. The fourth group as well had bile duct ligation then was given AM1241 daily in a dose of 3 mg/kg dissolved in the vehicle, intraperitoneally [20]. After three weeks (at 24 h after the last injection), blood samples were collected from the tail vein, and serum was separated by centrifugation at 3000 × g for 10 min and was used for the biochemical assessment. Rats were sacrificed by cervical dislocation, and livers were dissected, weighed, and liver to body weight ratio was calculated.

Serum biochemistry of liver transaminases, ALP and bilirubin levels

Serum concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total and direct bilirubin were determined using colourimetric kits (Biodiagnostic, Cairo, Egypt) [21], [22], [23].

ELISA for hepatic MDA, GSH, TNF-α, IL-10 and hydroxyproline content

Liver tissues were washed and homogenised in ice-cold PBS (pH = 7.4) to obtain a 20% homogenate (w·v 1), which was then centrifuged for 15 min at 3000 x g and 4°C. The supernatant obtained was used for measuring MDA (Bioassay co., China), GSH (Biotech co., China), TNF-α (Cusabio co., China), IL-10 (Cusabio co., China) and hydroxyproline (Cusabio co., China) using sandwich ELISA kits according to the manufacturer's instructions. Results were expressed for GSH, TNF and IL-10 as pg/mg protein while for MDA were expressed as nmol/mg and for hydroxyproline as ng/g [24], [25], [26], [27].

qRT-PCR analysis of α-FP and CD34 gene expression

Total RNA was isolated from rat liver samples using the RNeasy Mini Kit (Qiagen, Hilden, Germany). Reverse transcription and quantitative real-time PCR (qRT-PCR) were performed using SYBR® Premix

Ex TaqTM (TaKaRa, Biotech. Co. Ltd.). The cycling conditions were as follows: reverse transcription at 50°C for 30 min, initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s, annealing at 55°C for 30 s and extension at 72°C for 30 s. The relative mRNA level of the target genes was calculated by the comparative threshold cycle (Ct) method and was normalised on the bases of β-actin expression. The fold change in the expression of each target gene was calculated by the following formula: relative quantification = 2ΔΔCt. The following primer sequences were used: CD34 forward primer: 5′- AGC CCT ACA GGA GAA AGG CTG -3′, CD34 reverse primer: 5′- TCA CAG TTC TGT GTC AGC CAC -3′, β-actin forward primer: 5′- TTT GCA GCT CCT TCG TTG CC-3′, β-actin reverse primer: 5′- CGG TTG GCC TTA GGG TTC AGG GGG G-3′, α-FP forward primer: 5′- AGC GAG GAG AAA TGG TCC GG -3′, α-FP reverse primer: 5′- GGA CAT CTT CAC CAT GTG G -3′ (Metabion, Germany) [28], [29].

Histopathological examination

Autopsy samples were taken from the liver of rats in different groups and fixed in 10% formol saline for twenty-four hours. Washing was done in tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degrees in a hot air oven for twenty-four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns' thickness by sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin &eosin stain as well as Masson Trichrome for routine examination through the electric light microscope [30]. The severity of histopathological alternation was semi-quantitatively assessed based on liver histology evaluated by a blinded pathologist using a scoring system in which score 0 indicated no alternation; score 1, mild alternation; score 2, alternation activity; score 3, severe alternation.

Immunohistochemical analysis of α-SMA, p53, α-FP and CD34

Paraffin-embedded liver sections were deparaffinized and hydrated. Immunohistochemical analyses were performed by a standard streptavidin-biotin-peroxidase procedure. The paraffin sections were heated in a microwave oven (25 min at 720 W) for antigen retrieval and incubated with one of the following primary antibodies: rabbit polyclonal anti-rat p53, rabbit polyclonal anti-rat a-FP, rabbit monoclonal anti-rat CD34 and rabbit monoclonal anti-rat α-SMA (1:50 dilution; Abcam, Cambridge, MA, USA) and incubated overnight at 4°C. After washing with PBS, followed by incubation with the corresponding biotinylated secondary antibody (1:200 dilutions; Dako Corp.) and streptavidin/alkaline phosphatase complex (1:200 dilutions; Dako Corp.) for 30 min at room temperature, the binding sites of antibody were visualised with DAB (Sigma). After washing with PBS, the samples were counterstained with H&E for 2–3 min, and dehydrated by transferring them through increasing ethanol solutions (30%, 50%, 70%, 80%, 95%, and 100% ethanol). Following dehydration, the slices were soaked twice in xylene at room temperature for 5 min, mounted, examined, and evaluated by a high-power light microscope [31].

Quantitative morphometric analysis of Masson's trichrome, α-SMA, p53, α-FP and CD34

Quantitative morphometric analysis for Masson's trichrome, α-SMA, P53, α-FP and CD34 was performed at the Pathology Department, National Research Center by measuring the percentage of the positive stained area using the Leica Qwin 500 Image Analyzer (LEICA Imaging Systems Ltd, Cambridge, England). Morphometric measurements were performed on real-time image from the microscope that was visualised on the video monitor at high power magnification (200 ×). The marker colour to be detected was selected, then the software formed a binary image for the area of stained by the marker.

This area is determined as an area per field in micrometre square, area fraction and area percentage by using the interactive measurement software of the system. The results appeared in the form of mean, standard deviation, standard error, the minimum area and the maximum area measured. Quantitative image analysis for the selected marker was expressed as the percentage of stained area averaged across 5 different fields for each rat of at least six rats [32].

Data and statistical analysis

The data and statistical analysis in this study comply with the recommendations on the experimental analysis in pharmacology [33]. Results are expressed as mean ± SE. Multiple comparisons were performed using two-way ANOVA followed by Tukey-Kramer as a post hoc test. $P < 0.05$ was considered statistically significant. All analyses and graphs were performed using GraphPad Prism software (version 7).

Results

AM1241 treatment amended BDL-induced hepatic damage

As compared with the sham group, ALT, AST and ALP serum levels were significantly elevated in the BDL group. On the other hand, the levels of ALT, AST and ALP were significantly less elevated with AM1241 treated rats by 21%, 31% and 29% respectively, compared to that of the BDL group levels but they remained significantly higher than the sham values. In line with the cholestatic injury, the BDL group also showed a significant rise in total and direct bilirubin, compared to the control as they are not excreted through the obstructed bile duct and due to damaged hepatocytes. However, these values were significantly less elevated in AM1241 treated rats by 19%, 27% respectively than nontreated BDL group; moreover, it remained significantly higher than the silymarin group.

Table 1: Effect of AM1241 (3 mg/kg b.wt.) and silymarin (50 mg/kg b.wt.) treatment for three weeks on hepatotoxicity indices, liver and body weight ratio in bile duct ligated (BDL) rats

Groups	ALT	AST	ALP	Total Bilirubin	Direct Bilirubin	Change in body weight	Liver to body weight ratio
Sham	22.83 ± 0.61^{bc}	24.33 ± 0.92^{bc}	41.67 ± 0.71^{bc}	0.61 ± 0.06^{bc}	0.17 ± 0.014^{bc}	32.5 ± 8.04^{bc}	0.031 ± 0.001^{bc}
BDL	82 ± 2.35^{ac}	114 ± 2.89^{ac}	132.33 ± 2.47^{ac}	21.81± 0.90^{ac}	10.58 ± 0.54^{ac}	-23 ± 9.61^{a}	0.065 ± 0.002^{ac}
BDL + Silymarin	56 ± 1.34^{ab}	66.33 ± 2.33^{ab}	82.67 ± 2.23^{ab}	12.23 ± 1.6^{ab}	6.5 ± 0.44^{ab}	-17.27 ± 14.02^{a}	0.056 ± 0.002^{ab}
BDL + AM1241	64.83 ± 2.91^{abc}	78.87 ± 2.29^{abc}	94 ± 1.63^{abc}	17.63 ± 0.56^{abc}	7.7 ± 0.51^{ab}	-17.5 ± 10.01^{a}	0.051 ± 0.002^{ab}

Bile duct ligated rats significantly lost weight as compared to sham rats, moreover AM1241 and silymarin treated rats also lost weight without a difference with BDL group. Regarding liver to weight ratio, the increase observed in the BDL group, compared to the sham group, was significantly reduced by the administration of AM1241 and silymarin (Table 1).

Moreover, liver from bile duct ligated rat liver showed a nodular yellow brownish surface with oedema formation, distended bile cyst and fatty changes. However, treatment with AM1241 and silymarin produced a smooth, faint brownish surface (Figure 1).

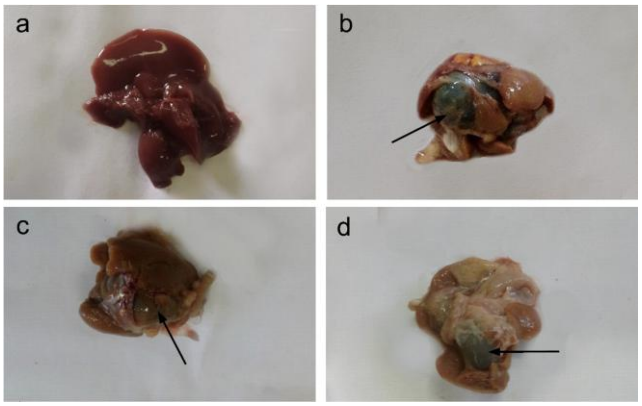

Figure 1: Effect of AM1241 (3 mg/kg) and silymarin (100 mg/kg) treatment for three weeks on gross liver morphology in bile duct ligated (BDL) rats. Liver from sham rat shows normal brownish surface (A), bile duct-ligated rat liver shows a nodular yellow brownish surface with oedema formation, distended bile cyst (arrow) and fatty changes (B), while liver treated with silymarin shows a smooth faint brownish surface with distended bile cyst (arrow) (C). Moreover, AM1241 treated rats show similar smooth, faint brownish surface (D)

AM1241 treatment inhibited histopathological deterioration induced by BDL

H&E stained liver sections obtained from sham group revealed no histopathological alteration and the normal histological structure of the central vein and the portal area with the portal vein, hepatic artery and bile ducts were recorded in (Figure 2a and 2b). However, in bile duct ligated rats, coagulative necrosis was observed in some hepatocytes which were characterised by deep eosinophilic cytoplasm and pyknotic deep blue nuclei in association with another dysplastic one with karyocytomegaly (Figure 2c, 2d and 2e). The hepatic capsule (Glisson's Capsule) showed thickening associated with a fatty change in the underlying hepatocytes (Figure 2f and 2g). Massive inflammatory cells infiltration was detected in the portal area associated with hyperplasia with the atypical epithelium of the bile ducts extended to the hepatic parenchyma (Figure 2h and 2i). There was neocholangiolar proliferation of the bile ducts characterised by hyperplasia and hypertrophy with ductular and glandular structure presented in between the damage hepatocytes as regenerative effects (Figure 2j).

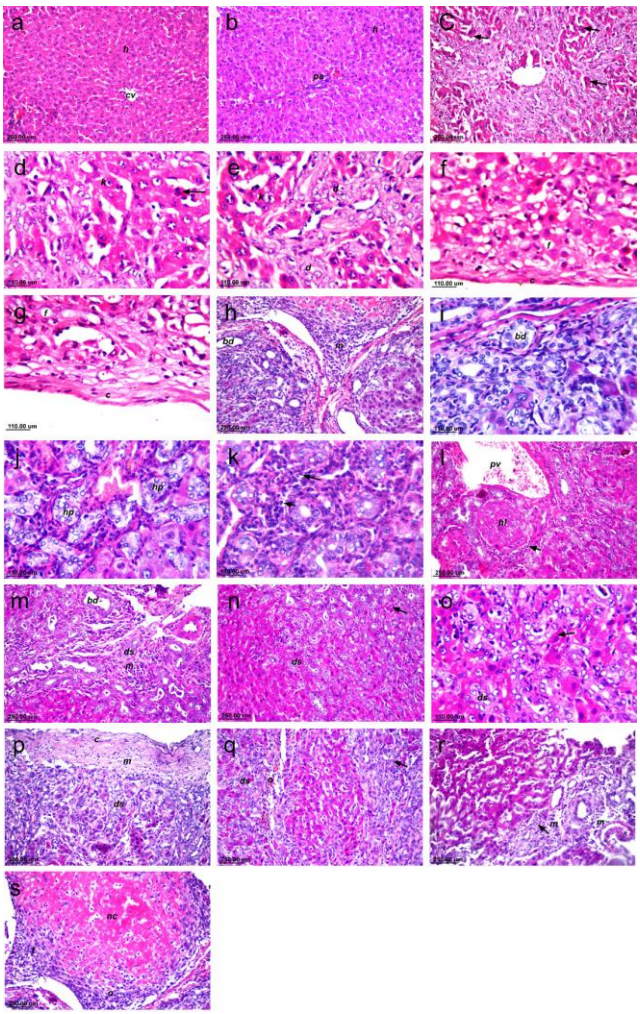

Figure 2: AM1241 treatment (3 mg/kg for 3 weeks) alleviated histopathological deterioration using H&E staining (×40 & ×80) in hepatic fibrosis associated with bile duct ligation (BDL). Liver sections of (a & b) sham rats showing the normal histological structure of the central vein (cv), surrounding hepatocytes (h) in the parenchyma, and normal portal area (pa). (c, d & e) BDL liver tissue is showing coagulative necrosis (arrow) with deeply eosinophilic cytoplasm and pyknotic nuclei hepatocytes while other dysplastic (d) with disfiguration and cytomegaly and karyomegalocytic cells (k). (f & g) BDL liver tissue is showing thick capsule (c) with fatty changes (f) in underlying hepatocytes. (h & i) BDL liver tissue is showing massive inflammatory cell infiltration (m) in the portal area with hyperplastic bile duct cells resembling hepatocytes, biliary epithelium and extending to the parenchyma (bd). (j) BDL liver tissue is showing hyperplasia and hypertrophy (hp) with ductular glandular structure presented in freshly damage liver cells as a regenerative effect (neocholangiolar proliferation). (k) BDL liver tissue is showing oval or spindle cell (arrow) basophilic extended in between the hyperplastic hypertrophic bile ducts in the hepatic parenchyma. (l) silymarin treated liver tissue showing dilated portal vein (pv) with lobulation of the hepatocytes (hl) with multiple proliferated oval or spindle cells (arrow) and dysplasia (ds) in the other with prominent nucleoli. (m) silymarin treated liver tissue showing increase proliferation of the bile ducts (bd), hypertrophic biliary epithelium, atypia and inflammatory cell infiltrate (m) in portal area. (n & o) silymarin treated liver tissue showing coagulative necrosis in some of the hepatocytes, dysplasia in other with enlarged spindle cells extended in between in the parenchyma. (p) AM1241 treated liver tissue showing thickening (c) with inflammatory cells infiltration (m) in the hepatic capsule with dysplasia (ds) in the underlying hepatocytes and atrophy in other. (q) AM1241 treated liver tissue showing oval cells proliferation (o) in between the dysplastic hepatocytes (arrow). (r) AM1241 treated liver tissue showing inflammatory cells infiltration (m) with few fibroblastic cells proliferation in the portal area (arrow). (s) AM1241 treated liver tissue showing focal loss of cell detail and architecture as necrosis (ne) surrounded by fibrosis (f) and oval cells (o)

Oval or basophilic spindle cells were extended in between the hyperplastic hypertrophied ducts in the parenchyma (Figure 2k). In silymarin treated rats, hepatic capsule showed thickening with inflammatory cells infiltration while the underlying parenchyma had dysplasia and atrophy (Fig. 2p). There was oval cells proliferation in between the dysplastic hepatocytes (Figure 2q). Inflammatory cells infiltration with few fibroblastic cells proliferation was detected in the portal area (Figure 3r). Focal necrosis with fibrosis in the surrounding was detected in the hepatic parenchyma (Figure 2s). Rat treated with AM1241 showed dilatation was observed in the portal vein associated with the proliferation of the oval or spindles cells which were separated the dysplastic with prominent nucleoli hepatocytes into lobules (Figure 2l). There was an increase in the bile ducts proliferation with hypertrophic biliary epithelium, atypia and inflammatory cells infiltration (Figure 2m). Coagulative necrosis was detected in some of the hepatocytes associated with dysplasia in others as well as an extension of spindle cells proliferation in between (Figure 2n & 2o). Table 2 summarise the histopathological alterations demonstrated in liver tissue of BDL rats.

Table 2: Effect of administration of AM1241 (3 mg/kg b.wt.) and silymarin (50 mg/kg b.wt.) for three weeks on histopathological alternations of hepatic tissue in bile duct ligated (BDL) rats

Groups	Hepatic capsule thickening	Degeneration and necrosis of hepatic parenchyma	Dysplasia of hepatocyte	Hyperplasia, hypertrophy and neocholangiolar formation with atypia, cytomegaly	Portal inflammatory reaction	Oval cell proliferation	Fibrosis
Sham	0	0	0	0	0	0	0
BDL	1	3	3	3	3	2	2
BDL + Silymarin	2	2	2	2	2	2	2
BDL + AM1241	0	2	2	2	1	3	1

AM1241 treatment mitigated BDL-induced hepatic fibrogenesis

ELISA analysis showed that that bile duct ligation caused a significant rise in hepatic hydroxyproline levels (up to 3 folds), compared to that of the control group. However, silymarin group didn't show significant difference with BDL rats, AM1241 treatment successfully reduced the elevation of hydroxyproline levels, compared to that of the BDL group (Table 3).

Table 3: AM1241 treatment (3 mg/kg for 3 weeks) alleviated hepatic fibrogenesis in bile duct ligated (BDL) rats

Groups	HP	MT %	SMA %
Sham	1.03 ± 0.06^{bc}	17.12 ± 1.88^{bc}	7.11 ± 1.29^{bc}
BDL	4.09 ± 0.09^{a}	39.76 ± 2.10^{a}	39.03 ± 2.30^{ac}
BDL + Silymarin	4.02 ± 0.20^{a}	32.61 ± 2.12^{a}	16.55 ± 1.98^{ab}
BDL + AM1241	3.29 ± 0.31^{ab}	30.04 ± 2.25^{ab}	14.30 ± 1.29^{ab}

These results matched those obtained from histomorphometric measurements of Masson Trichrome staining. It was clear that only traces of connective tissue elements were present in normal

hepatic tissues (Figure 3a), while bile duct ligation increased markedly the fibrous components associated with portal fibrosis (Figure 3b). Treatment of bile duct ligated rats with silymarin produced a less significant reduction of fibrosis than that of AM1241 group (Figure 3c and 3d). However, treatment with AM1241 significantly decreased fibrosis by 24% compared to the nontreated BDL group (Figure 3e, 3f and 3g).

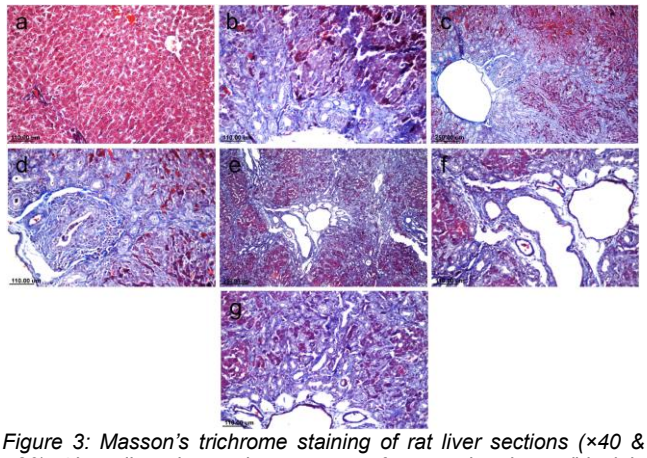

Figure 3: Masson's trichrome staining of rat liver sections (×40 & ×80). Sham liver tissue shows traces of connective tissue (blue) in the normal hepatic tissue present mainly around the main blood vessels (a). Bile duct-ligated rat liver tissue shows massive fibrosis in liver tissue with thick fibrous tissue expanded along the portal tract and extended into the periportal region. Lost normal architecture and dilated bile ducts was also observed (b). Moreover, the liver treated with silymarin shows also marked decrease in connective tissue (c & d). However, liver treated with AM1241 shows only a few blue stained collagen bundles surrounding hepatic nodules (e, f & g)

Moreover, quantitative morphometric investigation of liver sections immunostained with α-SMA, a marker of fibroblastic cells, i.e. activated hepatic stellate cells and portal myofibroblasts revealed that only traces of α-SMA expression was present in normal hepatic tissues (Figure 4a). In contrast, bile duct ligation produced the highest expression of α-SMA in hepatic tissue (Figure 4b and 4c). Treatment with silymarin produced a similar insignificant effect to that of AM1241 treated rats (Figure 4d). However, treatment with AM1241 significantly decreased α-SMA expression by 63%, compared to that of the nontreated BDL group (Figure 4e and 4f).

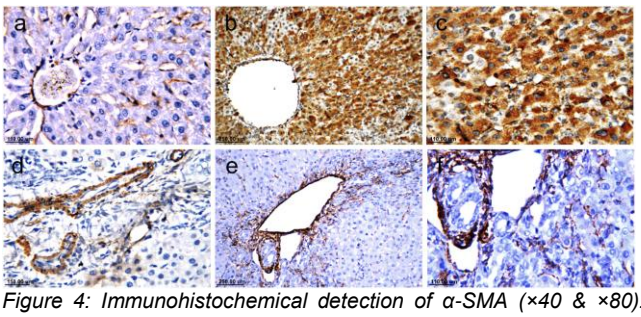

Figure 4: Immunohistochemical detection of α-SMA (×40 & ×80). Sham group shows a negligible positive reaction (a), bile duct ligated group shows highest reaction (b & c), group treated with silymarin shows decrease in reaction (d), while AM1241 treated group shows a more noticeable decrease in reaction (e & f)

AM1241 treatment attenuated BDL-induced oxidative stress

The effect of AM1241 on oxidative stress was investigated by measuring hepatic levels of the lipid peroxidation end-product (MDA; malondialdehyde) and the antioxidant molecule (GSH). GSH levels were markedly reduced while levels of lipid peroxides were significantly elevated in the BDL group, compared to that of the control group. Interestingly, Treatment with AM1241 significantly prevented the elevation of MDA by 45% and restored GSH levels up to 5 more folds than the nontreated BDL group (Table 4). Moreover, AM1241 exhibited a superior effect when compared to that of silymarin treated group.

Table 4: Effect of administration of AM1241 (3 mg/kg b. wt.) and silymarin (50 mg/kg b. wt.) for three weeks on tissue oxidative stress markers in bile duct ligated (BDL) rats

Groups	MDA (nmol/mg)	GSH (pg/mg)
Sham	31.67 ± 1.09^{bc}	122.67 ± 2.45^{bc}
BDL	182.167 ± 6.41^{ac}	14.87 ± 0.89^{ac}
BDL + Silymarin	134.67 ± 3.99^{ab}	70.57 ± 3.94^{ab}
BDL + AM1241	99 ± 3.02^{abc}	89.8 ± 2.89^{abc}

AM1241 treatment alleviated BDL-induced hepatic inflammation

The anti-inflammatory potential of AM1241 was determined by measuring levels of the pro-inflammatory cytokine (TNF-α) and the anti-inflammatory marker (IL-10). As shown in Table 3, the marked rise in serum TNF-α levels observed in the BDL group was significantly decreased (33%) by AM1241 treatment. Silymarin produced a more significant decrease in TNF-α levels, compared to that of AM1241 group.

Regarding IL-10 serum levels, bile duct ligated rats showed a significant decrease, compared to the control group, whereas treatment with AM1241 markedly restored these levels up to 5.6 more folds than the nontreated BDL group. Further, treatment with silymarin showed a significant inferior effect when compared to AM1241 group (Table 5).

Table 5: Effect of administration of AM1241 (3 mg/kg b.wt.) and silymarin (50 mg/kg b.wt.) for three weeks on tissue inflammatory response in bile duct ligated (BDL) rats

Groups	TNF-α (pg/mg)	IL-10 (pg/mg)
Sham	37.27 ± 1.28^{bc}	124.37 ± 1.78^{bc}
BDL	223.53 ± 3.75^{ac}	14.43 ± 0.85^{ac}
BDL + Silymarin	130.8 ± 6.15^{ab}	66.2 ± 3.82^{ab}
BDL + AM1241	149.2 ± 4.48^{abc}	95.83 ± 2.18^{abc}

AM1241 treatment alleviated p53-dependent hepatocyte apoptosis

Table 6 summarizes the quantitative morphometric investigation of liver sections immunostained with p53 of BDL rats. Liver sections of normal hepatic tissues showed a negligible positive

reaction of p53 expression (Figure 5a). Bile duct ligation produced a large increase in p53 immunoreactivity (Figure 5b and 5c).

Table 6: AM1241 treatment (3 mg/kg for 3 weeks) alleviated hepatic fibrogenesis in bile duct ligated (BDL) rats

Groups	P53
Sham	5.53 ± 0.53[bc]
BDL	32.77 ± 2.44[ac]
BDL + Silymarin	23.85 ± 1.84[ab]
BDL + AM1241	15.89 ± 1.89[abc]

Silymarin treatment was less effective in reducing p53 induction (Figure 5d), and treatment with AM1241 significantly decreased p53 expression significantly by 51%, compared to that of the nontreated BDL group (Figure 5e).

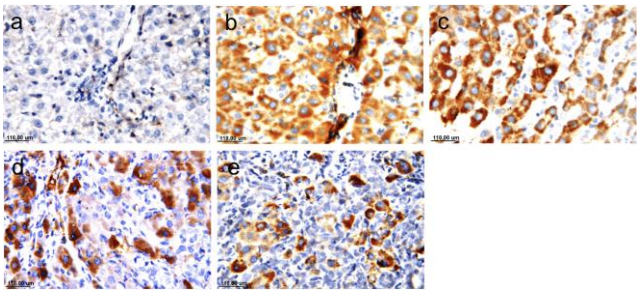

Figure 5: Immunohistochemical detection of p53 (×40 & ×80). Sham group shows a negligible positive reaction (a), bile duct ligated group shows highest reaction (b & c), group treated with silymarin shows decrease in reaction (d), while AM1241 treated group shows a more noticeable decrease in reaction (e)

AM1241 treatment augmented liver regeneration by activating hepatic progenitor cell proliferation

The effect of AM1241 on liver regeneration through activation of hepatic progenitor cells proliferation was determined by measuring levels of its surface markers, CD34 and α-FP. qRT-PCR analysis of CD34 and α-FP mRNA expression in the bile duct ligated group showed no significant changes from the control group. Silymarin treatment didn't show any significant effect over the nontreated BDL rats. Strikingly, treatment with AM1241 significantly up-regulated the genetic expression of CD34 and α-FP (252% and 89% respectively), compared to that of the nontreated BDL group (Table 7).

Table 7: AM1241 treatment (3 mg/kg for 3 weeks) augmented hepatic progenitor cell expression in bile duct ligated (BDL) rats

Groups	AFP %	AFP PCR	CD34 %	CD34 PCR
Sham	14.904 ± 3.32[bc]	0.39 ± 0.04[bc]	3.70 ± 0.81[bc]	1.14 ± 0.11
BDL	34.30 ± 3.09[a]	0.83 ± 0.03[a]	17.55 ± 2.15[a]	2.32 ± 0.43
BDL + Silymarin	41.69 ± 2.50[a]	0.755 ± 0.036[a]	23.59 ± 2.66[a]	2.20 ± 0.49
BDL + AM1241	55.03 ± 2.23[abc]	1.57 ± 0.07[abc]	38.18 ± 1.91[abc]	8.17 ± 1.021[abc]

Histomorphometric measurement for CD34 and α-FP immunostained liver sections demonstrated a very similar pattern as the qRT-PCR measurements. Only traces of CD34 and α-FP were found in normal hepatic tissues (Figure 6a, 6b and 7a). Bile duct ligated treated rats showed mild expression of CD34 (Figure 6c, 6d, 7b and 7c). Moreover, silymarin treated rats showed also a mild expression of when stained with CD34 (Figure 7d and 7e).

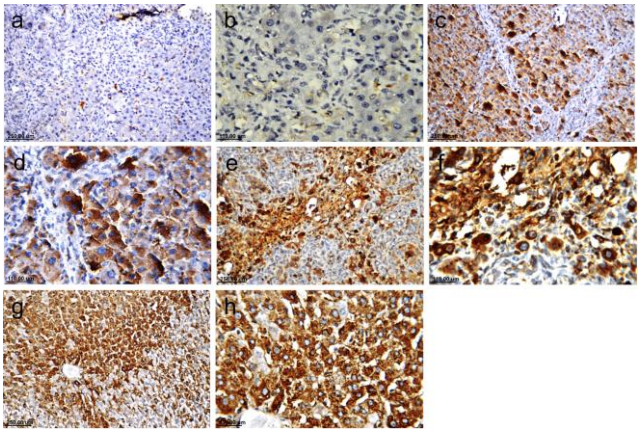

Figure 6: Immunohistochemical detection of α-FP (×40 & ×80). Sham group shows the negligible positive reaction of this stain (a & b), a noticeable increase in the reaction in BDL group (c & d) and silymarin treated group (e & f) and highest reaction in AM1241 treated group (g & h)

However, it revealed more significant expression when stained with α-FP (Figure 6e and 6f). Treatment with AM1241 showed the highest expression of CD34 and α-FP (117% and 60% respectively, compared to BDL group) with observed oval hepatic progenitor cells (Figure 6g, 6h, 7f, 7g).

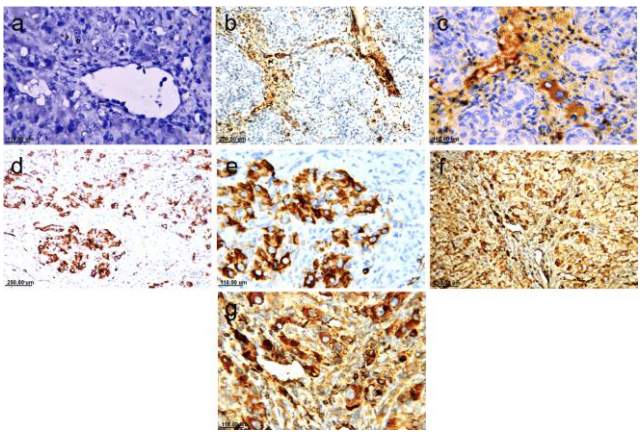

Figure 7: Immunohistochemical detection of CD34 (×40 & ×80). Sham group shows no positive reaction (a), mild increase in the reaction in both BDL (b & c) and silymarin groups treated groups (d & e) and highest reaction in the group treated with AM1241 (f & g). Notice the hepatic progenitor cells is well expressed in AM1241 treated group

Discussion

The current study reports the potential antifibrotic mechanisms of CB2 receptors activation through studying the key events involved in hepatic

fibrosis such as oxidative stress, inflammation and apoptosis. This study is the first to investigate the potential effect of CB2 receptor on liver regeneration associated with hepatic progenitor cells stimulation. These effects largely depend on its direct impact on CB2-expressing hepatic immune cells and hepatic myofibroblasts and its indirect effect on hepatocytes, which do not express CB2 [8], [34].

BDL is the classic experimental model for induction of cholestatic liver fibrosis in rodents. In our study following 3 weeks of bile duct ligation, there was a marked decrease in body weight associated with an increase in liver weight. Reduced body weight could be attributed to reduced intestinal bile secretion and food absorption while increased liver weight is referred to as hepatic congestion and swelling [35]. AM1241 treatment significantly attenuated body weight loss and liver weight increase.

Biliary obstruction causes an irregular flux of bilirubin and bile acids in the liver. Toxic hydrophobic bile salts subsequently accumulate within hepatocytes causing progressive hepatocellular necrosis and apoptosis via free radical generation. Early studies using rodent hepatocytes assumed that cholestatic liver injury is mainly caused by direct toxicity of hydrophobic bile acids, which induce mitochondrial oxidative stress and apoptosis [36]. However, the predominant bile acids in rodents are not cytotoxic; they promote inflammatory gene expression which is responsible for hepatic neutrophil recruitment and an inflammatory injury. Neutrophil cytotoxicity involves extensive neutrophil-derived oxidative stress and hepatocyte necrosis [37]. This was reflected in the present study by the elevation in serum transaminases, ALP and bilirubin in the BDL group.

Moreover, Histopathological examination revealed coagulative necrosis in association with many dysplastic cells and hepatocytes showing fatty changes. Massive inflammatory cells infiltration was detected in the portal area associated with the neo-cholangiolar proliferation of the bile ducts as regenerative effects. Oval basophilic cells were extended in between the hyperplastic ducts. Daily administration of AM1241 produced significant decrease in serum transaminases, ALP and bilirubin levels with no significant difference from silymarin group. However, histopathological examination still reveals areas of coagulative necrosis in some of the hepatocytes associated with few dysplastic cells as well as proliferation of oval cells in between. Moderate Inflammatory cells infiltration with few fibroblastic cells proliferation was detected in the portal area**.** These results confirm the previous findings by Batkai, Osei-Hyiaman [9] who reported that administration of CB2 agonist, JWH-133 protected against hepatic I/R (ischemia reperfusion) injury whereas, CB2 deficient mice displayed enhanced liver injury and inflammation following I/R. The reported mechanism involved decreased inflammatory cell infiltration, reduced lipid peroxidation and expression of pro-inflammatory cytokines. Moreover, ***Batkai, Osei-Hyiaman [9]*** experiments in cultured sinusoidal endothelial cells indicated that CB2 activation reduced tumor necrosis factor alpha-induced adhesion molecules with decreased adhesion of neutrophils to endothelial cells.

Liver fibrosis of any aetiology is characterized by progressive accumulation of collagenous fibrous tissue in the liver parenchyma with the proliferation of collagenous secreting activated hepatic stellate cells (HSCs) and portal myofibroblasts [38]. In this context, BDL in our study produced marked collagenous filaments deposition around portal tract as indicated by Masson's trichrome stained fibrous bands, increased liver hydroxyproline content (a marker of collagen deposition) and also by increased α-SMA expression (a marker of HSCs). The present study demonstrates that CB2 activation successfully decreased BDL-induced fibrogenesis. This was concluded by the significant reduction in liver hydroxyproline levels, measured with ELISA and the decrease of collagen deposition, measured with morphometric analysis of Masson's stain. This also was coincided with a significant reduction in α-SMA expression. These results confirm the previous findings by Julien, Grenard [6] who reported that CB2 knockout mice showed a diminished cirrhosis when exposed to CCl4, as assessed by morphometric analysis of Sirius red-stained slides, and by measurement of collagenous protein spectrophotometrically, whereas Munoz-Luque, Ros [39] reported that administration of the CB2 agonist JWH-133 to CCl4 rats improved liver fibrosis, decreases the inflammatory infiltrate and reduces the density of hepatic myofibroblasts.

Moreover, Julien et al**.,** 2005 also reported that activation of CB2 receptors in cultured activated hepatic stellate cells reduced cell accumulation and triggered its apoptosis by stimulating both COX-2 and oxidative stress. They reported that THC increased COX-2 protein expression and ROS activity that induced HSCs apoptosis. However, the apoptosis was diminished by selective COX-2 inhibitor and two potent antioxidants, respectively.

CB2 receptors expressed on hepatic inflammatory cells play a major role in amelioration of hepatic injury and fibrogenesis. Previous literature showed that CB2 agonists prevented the switch of Kupffer cells to a pro-inflammatory M1 phenotype, and enhances transition towards the anti-inflammatory M2 phenotype, via a mechanism involving activation of heme oxygenase-1 [40]. While others reported that CB2 receptors activation downregulated the production of the profibrogenic cytokine IL17 by Th17 lymphocytes [41], [42]. Moreover, our finding showed that CB2 receptors produced a marked elevation of immunoregulatory IL-10 as compared to both BDL and silymarin groups. IL-10 produced by M2b macrophages and Th2 lymphocytes is a potent stimulator of HO-1 with subsequent enhancing polarisation of macrophages toward

immunoregulatory M2c phenotype [43], [44], [45]. Also, IL-10 produces apoptosis of pathogenic Th17 and decreases the release of inflammatory IL-17 [46], [47], [48]. This was evident in our experiment by a significant decrease of TNF-a, a marker of pro-inflammatory M1-macrophages and by a significant increase of IL-10, a marker of anti-inflammatory M2-macrophages.

Neutrophil accumulation in the liver is a common feature of the cholestatic liver disease and is mediated by several adhesion molecules and inflammatory cytokines released from kupffer cells. These inflammatory cells attach to hepatocytes and release ROS and other oxidants. These changes, in turn, trigger intracellular oxidative stress and lipid peroxidation inside the hepatocytes [49], [50]. This was evident in the present study by the remarkable increase in lipid peroxidation expressed as MDA content and the depletion of GSH levels. Oxidative stress is found to play a major role in the pathogenesis of cholestasis as it induces hepatocyte death (both apoptotic and necrotic), amplifies the inflammatory response. Treatment of BDL rats with CB2 agonist in the present study effectively reduced the lipid peroxidation and restored the hepatic antioxidant defence system as shown by the marked improvement of MDA and GSH activity which was coincided with a decreased number of inflammatory cells as shown by H&E stain, compared to both BDL and silymarin groups. This goes in line with previous studies which reported that activation of CB2 receptors by JWH133 and cannabidiol decreased hepatic ROS and lipid peroxidation end-products by attenuating neutrophil infiltration and expression of neutrophil adhesion molecules in an animal model of I/R and alcohol-induced injury, respectively [9], [51].

Moreover, CB2 receptors were reported to play a role in reducing oxidative stress markers in hydrogen peroxide stimulated cultured RAW 264.7.7 macrophages [52].

p53 is an oncoprotein, potentially stimulate cell growth arrest and apoptosis and is upregulated in many liver diseases, ranging from fatty liver disease to HCC [53]. p53-dependent mitochondrial stress is found to induce apoptosis associated with increased Bax and Bcl2 in bile acid-induced liver toxicity in both in-vivo and in-vitro [54], [55]. It is also associated with increased lipid peroxidation and mitochondrial stress in CCL4 rats [56], [57], [58]. Likely, BDL in the present study showed a marked increase in the apoptotic process with strong cytoplasmic immunoreactions for p53. However, daily treatment with AM1241 produced a significant reduction in expression of p53 as compared to both BDL and silymarin groups. Previous studies also reported that 2-AG-mediated CB2 signalling by MAGL inhibition protects against hepatocyte apoptosis elicited by acute liver injury [59]. However, CB2 receptors are not expressed inside the hepatocytes, their impact on modulation of the proinflammatory cytokines and oxidative stress may explain an indirect and paracrine effect on hepatocyte apoptosis.

A heated debate raged for years as regard to the role of oval/progenitor cells in liver regeneration which is capable of generating both hepatocytes and biliary cells following BDL [60], [61], [62]. Our results showed that sham rats showed negative expression of CD34 and α-FP by immunostaining and gene expression with no detection of oval cells by H&E. However, BDL and silymarin groups showed moderate expression of these oval cell markers and its gene expression with observed oval cells by H&E. Interestingly, treatment with AM1241 produced a significant expression of α-FP and CD34 immunoreaction and enhanced its gene expression with more detected oval cells by H&E as compared to both BDL and silymarin group. Moreover, certain bile acids and growth factors were found to be a potent stimulator of progenitor profile of mesenchymal cells [63] where others reported that kupffer cells modulation initiate oval cells stimulation [64]. Whether CB2-dependent modulation of kupffer cells activity and growth factors plays a role in progenitor cells stimulation would be further investigated. Moreover, CB2 receptors were previously reported to promote neural and cardiac progenitor cells regeneration. Palazuelos, Aguado [65] reported that stimulation of CB2 receptors enhanced the proliferation of neural progenitor cells, both in vitro and in vivo while Wang, Ma [66] reported that AM1241 promoted proliferation of ki-67 immunostained cardiac progenitor cells (CPCs) and encouraged cardiomyocyte regeneration in post-myocardial infarction.

In conclusion, our data demonstrate that CB2 receptors reduce liver injury and fibrogenesis following bile duct ligation with explaining new distinct mechanisms originating from hepatic myofibroblasts and immune cells. These include IL-10 dependent inhibition of inflammatory mediators, reduction of lipid peroxidation inside hepatic cells and inhibition of hepatocyte p53-dependent apoptosis. Besides, CB2 receptors are shown to possess a regenerative effect through stimulation of HPCs stimulation. These results suggest that CB2 agonists display potent hepatoregenrative properties, in addition to their antifibrogenic effects.

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Comorbidities as Risk Factors for Acute and Recurrent Erysipelas

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Abstract

BACKGROUND: Erysipelas is a common infectious skin disease. A typical feature of erysipelas, especially on the lower limbs, is the tendency to reoccur and the study aimed to define the comorbidities associated with it.

AIM: We aimed to investigate systemic and local comorbidities in patients diagnosed with erysipelas on the lower limbs.

MATERIAL AND METHODS: We conducted a retrospectively-prospective, population-based cohort study which included all patients diagnosed with erysipelas on the lower limbs, during two years. Patients were divided into two groups: patients with first episode and patients with recurrent erysipelas. These two groups were compared, with particular emphasis on systemic and local comorbidities.

RESULTS: The study included 313 patients, of which 187 with the first episode of erysipelas and 126 with a recurrent. Regarding the analyzed systemic risk factors, the recurrent erysipelas was significantly associated with obesity ($p < 0.0001$), insulin dependent diabetes mellitus ($p = 0.0015$), history of malignant disease ($p = 0.02$) and tonsillectomy ($p = 0.000001$). For a p-value < 0.0001, significantly more frequent finding of peripheral arterial occlusive disease, chronic oedema/lymphoedema, fungal infections of the affected leg and chronic ulcer was confirmed in recurrent erysipelas. Neuropathy had 23% of the recurrent cases and 8.6% in patients without recurrence, and the difference was found to be significant for $p = 0.0003$. The only dissection of the lymph nodes was found more frequently in recurrent erysipelas ($p = 0.017$), but no associations with other analysed local surgery on the affected leg. Patients with recurrent erysipelas had ipsilateral coexisting dermatitis $p = 0.00003$ significantly more frequent. Minor trauma often preceded the first episode of erysipelas $p = 0.005$.

CONCLUSION: Identification and treatment of modifiable risk factors are expected to reduce the risk of a subsequent episode of erysipelas on the lower limbs.

Introduction

Erysipelas is an acute bacterial nonpurulent infection of the superficial layer of the skin, with significant inflammation of the lymphatic vessels (lymphangitis), whose main clinical feature is demarcated elevated, warm erythema with pronounced systemic symptoms [1], [2]. Cellulitis is a soft tissue infection that affects the deep dermis and subcutaneous tissue [2], [3], [4]. The historical distinction between cellulitis and erysipelas based on a different bacterial aetiology, and therefore therapeutic modalities, is outdated with the growth of evidence suggesting overlapping of these two entities [5], [6]. Epidemiological studies show an increase in the incidence of erysipelas [7], [8], [9]. It is thought to be 200 per 100,000 people per year, and there is no gender difference. The highest incidence was observed in the oldest age groups [9]. The most common anatomical localisation of erysipelas are the lower limbs [9]. Women are at greater risk for erysipelas on the trunk, and men are erysipelas on the lower limbs [9]. These infections are caused by

streptococci, most commonly in Group A, but also from the groups B, C, F, or G. The diagnosis is primarily clinical and is based empirically on the cutaneous manifestations, and systemic signs of infection [10]. Typically for erysipelas, especially the lower limbs, is the tendency to recur. The incidence of recurrent erysipelas varies from study to study. Namely, 10-30% of patients who have had erysipelas, will have relapses at different time intervals, several weeks to years [11], [12]. The relapse rate is 8% to 20% per year [13]. In studies with a longer follow-up period, the rate of relapse is significantly higher, so in a retrospective three-year study it is more than 45% [14]. The recurrent erysipelas of the lower limbs is thought to be a result of the repetitive bacterial invasion of the skin through injuries to its protective barrier [14]. Accordingly, the potential points of entry of the infection were analyzed as risk factors for relapse in several clinical studies-disruption of the cutaneous barrier (ulcer, trauma), coexisting dermatoses of the lower limbs, lymphoedema, surgical interventions of the lymphatic/venous system, peripheral arterial occlusive disease, chronic venous insufficiency [8], [12], [15]. General risk factors include obesity, history of malignant disease and diabetes mellitus and smoking [12], [13], [14], [15], [16], [17], [18]. Treatment for an initial and recurrent episode of erysipelas do not differ and are described in several existing protocols [19], [20], [21], [22]. These protocols reinforce the significance of long-term antibiotic prophylaxis as a method for the reduction of recurrent erysipelas [23], [24], [25], but proclaim as well rigorous control of predisposing risk factors [13], [14], [16], [17].

We aimed to investigate systemic and local comorbidities in patients diagnosed with erysipelas on the lower limbs.

Methods

We conducted a retrospectively-prospective, population-based cohort study, conducted in a dermatology department in two years. All patients aged ≥ 18 years were recruited, with a diagnosis of acute erysipelas on the lower limbs. All types of necrotising skin and soft tissue infections (SSTI) have been excluded, skin infections in severely immunocompromised patients, and infectious complications of severe injuries to soft tissues. Patients with a first episode of erysipelas on the lower extremities were followed for at least one year from the initial episode, for the development of a recurrent one.

Upon completion of the study and the follow-up period, patients were divided into two groups, cohorts. First group (no recurrence group – NE)-patients with the first episode of erysipelas defined only on clinical findings which included the area of erythema, swelling, warmth and pain, fever was not needed to meet the definition and did not experience a recurrent episode during the follow-up period. Second group-patients with recurrent erysipelas (RE), defined as a second/multiple episodes of erysipelas that meets the criteria of the first episode, at the same anatomical localisation, at least 1 month to one year from the initial diagnosis. This group will also include all patients with at least a second episode that has occurred during the study and out of the study (through anamnestic data or medical documentation for it). These two groups will be compared with particular emphasis on general and local risk factors/comorbidities. The required data for all recruited patients was obtained through clinical examination and patient interview, as well as medical records. The analysed variables were classified in local and general risk factor/comorbidities. Obesity is defined if BMI ≥ 30 [26]. Alcohol abuse is considered if the consumption of 14 units of alcohol per week is exceeded-in men and 7 units weekly for women [27].

Point of entry was detected by clinical examination (wound, chronic ulcer, coexisting chronic pruritic dermatoses and fungal infections of the ipsilateral extremity). Chronic oedema/lymphoedema defined as chronic progressive swelling of the affected lower limb longer than 3 months [28] present on clinical examination or in the medical records. The following variables will be considered present if pointed in an interview or medical records-history of regional surgery; neurological diseases, history of phlebitis, diabetes mellitus, chronic renal failure, hepatic cirrhosis, cardiovascular diseases, history of malignancy, rheumatic and autoimmune diseases, peripheral arterial occlusive disease (PAOD), chronic venous insufficiency (CVI). Regarding statistics, the Kolmogorov-Smirnov test was used to test the distribution of data. The categorical variables are represented by distribution on frequencies. Quantitative variables with symmetric distribution are shown with mean values, and the media was used to display quantitative data with asymmetric distribution. Pearson Chi-square test, Yates Pearson Chi-square test (Student t-test for independent samples and Mann-Whitney test) were used to compare groups with first and recurrent erysipelas. The statistical significance was defined on the level of $p < 0.05$.

Results

Comparison of general and local risk factors between

The study included 313 subjects, of which 187 were with a first episode of erysipelas (NE), and 126 with recurrent erysipelas (RE).

Table 1: Comparison of comorbidities – general risk factors

Variable	Ne group	Re group	P-level
Bmi			
Mean ± SD	30.01 ± 16.5	31.49 ± 6.5	P = 0.34
Min - max	20.8 – 247	22.7 – 53.8	
Obesity	65 (34.76)	73 (57.94)	P < 0.0001
Diabetes mellitus			
Insulin dependant	39 (20.86)	48 (38.10)	P = 0.0015
On oral hypoglycemic agents	38 (20.32)	27 (21.43)	
Chronic kidney disease	9 (4.81)	8 (6.35)	P = 0.56
Cirrhosis hepatis	4 (2.14)	5 (3.97)	P = 0.34
History of malignancy	10 (5.35)	16 (12.7)	P = 0.02
Autoimmune disease	4 (2.14)	5 (3.97)	P = 0.34
Tonsilectomy	4.8 (9)	23.8 (30)	P = 0.000001
Alcohol excess	28 (14.97)	16 (12.7)	P = 0.57
I.v. drug use	0	3 (2.38)	P = 0.13
Actueal smoking	42 (22.46)	35 (27.78)	P = 0.28
Cerebro vascular disease	4 (2.14)	5 (3.97)	P = 0.34
Copd	10 (5.35)	11 (8.73)	P = 0.24
Congesticve heart failure	42 (22.46)	33 (26.19)	P = 0.45
Ischaemic heart disease	21 (11.23)	23 (18.25)	P = 0.08

All the analyzed systemic risk factors (Table 1), with the exception of alcoholism, were more commonly reported in RE patients, but a significant difference between the two groups was confirmed regarding this risk factors-obesity (p < 0.0001), diabetes mellitus (p = 0.0015), history of malignant disease (p = 0.02) and tonsillectomy (p = 0.000001) (Figure 1).

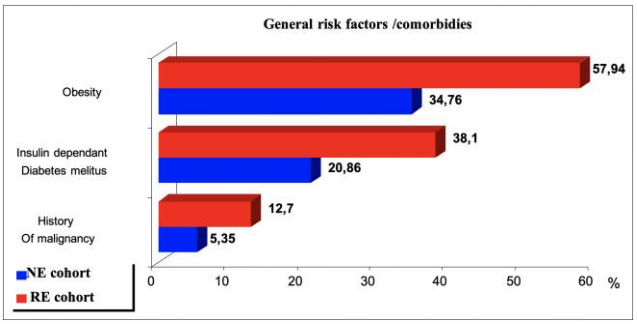

Figure 1: Graphic representation of general risk factors in the two cohorts

About 34.8% of patients with NE were obese, and 57.9% were patients with RE. Insulin-dependent DM had 20.9% of patients in the NE group and 38.1% with RE. 5.35% of the NE patients had a history of the malignant disease and 12.7% in the RE group. Tonsillectomy was performed in 4.8% of patients without and 23.8% of patients with RE.

Table 2: Comparison of comorbidities – local risk factors

Variable	NE group	RE group	p-level
CVI	92 (49.2)	65 (51.59)	P = 0.68
PAOD	41 (21.93)	52 (41.27)	P < 0.0001
Chronic oedema/lymphoedema	64 (34.22)	109 (86.51)	P < 0.0001
History of an ulcer	24 (12.83)	64 (50.79)	P < 0.0001
History of flebitis	44 (23.53)	35 (27.78)	P = 0.39
Neuropathy	16 (8.56)	29 (23.02)	P = 0.0003
The surgical intervention of the blood and lymph. Vessels			
Saphenectomy	6 (3.21)	11 (8.73)	P = 0.08
Endovascular and other surgical intervention of the blood and lymph.Vessels	26 (13.9)	20 (15.87)	
Local orthopaedic surgery			
Endoprotesis of knee and hip joint	8 (4.28)	7 (5.56)	P = 0.051
Fractures or other orthopaedic interventions	14 (7.49)	20 (15.87)	
Dissection of regional lymph nodes	1 (0.53)	7 (5.56)	P = 0.017
Surgery of the skin and soft tissue			
Skin grafting	1 (0.53)	5 (3.97)	
incision, drainage and other procedures	1 (0.53)	4 (3.17)	
Fungal infection			
Onichomycosis	7 (3.74)	25 (19.84)	P < 0.0001
Tinea pedis	21 (11.23)	41 (32.54)	
Preceding trauma	54 (28.88)	19 (15.08)	P = 0.005
Chronic ulcer	29 (15.51)	48 (38.1)	P < 0.0001
Ipsilateral coexisting dermatitis	49 (26.2)	62 (49.21)	P = 0.00003

Peripheral arterial occlusive disease (PAOD), chronic oedema/lymphoedema, history of an ulcer, neuropathy, lymph node dissection, preceding trauma, chronic ulcer fungal infections and coexisting dermatitis on the ipsilateral are local risk factors with significantly different representation in both cohorts (Table 2). For p < 0.0001 values, significantly more frequent finding of PAOD was confirmed in RE (51.6% vs 49.2%) in NE patients, chronic edema/ lymphoedema (86.5% vs 34.2%), fungal infections of the affected limb t (52.4% vs 15%), and chronic ulcer (38.1% vs 15.5%). Consecutive. With p < 0.0001 significance, patients with recurrent erysipelas had a significantly more frequent history of an ulcer, compared with patients with NE (50.8% vs 12.8%). Neuropathy had 23% of patients with RE, and 8.6% in a patient with no recurrence and the difference was confirmed as significant for p = 0.0003. Dissection of lymph nodes had 8 patients, one without relapse and 7 with recurrent erysipelas (p = 0.017). Patients with RE had significantly more frequent ipsilateral coexisting dermatitis (49.2% vs 26.2%, p = 0.00003). Minor trauma significantly preceded NE, (28.9% vs 15.1%, p = 0.005).

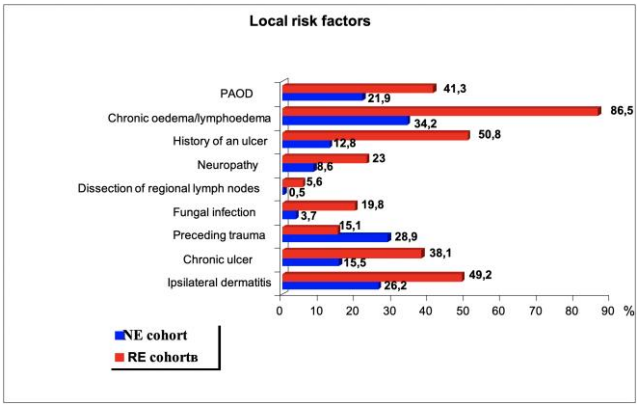

Figure 2: Graphic representation of local risk factors in the two cohorts

Discussion

The recurrence rate of erysipelas is almost 30% in a 2-4-year period [29]. There are only a few studies that analyze the risk factors in recurrent erysipelas, some of the results of our study are consistent with them [12], [13], [14], [15], [16], [17], [18], [30].In this study, we included 313 patients throughout two years, of which 187 were the first episode of erysipelas, and 126 with recurrent erysipelas. The study was a retrospective-prospective population-based cohort, and the collected data are with particular emphasis on systemic and local risk factors and with the primary goal to compare patients with first (NE) and recurrent erysipelas (RE). The

study included all patients with erysipelas on the lower extremities, hospitalised and outpatient, avoiding bias in patient selection; however, hospitalised patients are more likely to be older and have more comorbidities. In our cohort of RE, significantly more frequent systemic risk factors were-obesity, history of malignant disease, which correlates to other studies [8], [13], [14], [15]. Diabetes in the RE is present with 38.1% vs 20.9% in NE group ($p < 0.001$) and was strongly associated with recurrent erysipelas. In other studies [14], [17] this association has not been established. However, Harris et al. suggested an association with glucose intolerance [31]. Diabetes affects the healing process [32]. Hyperglycemia reduces the function of neutrophils and monocytes through impairment in the immune system cascade, primarily chemotaxis, adherence, and phagocytosis [33], [34]. People with diabetes are generally at greater risk of infection with certain microorganisms, in particular, group A and B streptococci and Staphylococcus aureus [35]. This study confirms tonsillectomy ($p = 0.000001$) as a risk factor for relapse, previously suggested by a study by Karppelin et al., from 2013 [18]. The ability of the streptococcus to survive intracellularly is suggested as a mechanism in recurrent tonsilitis and is likely the reason for the recurrent nature of erysipelas [36], [37], [38]. The most significant risk factors are local-Chronic oedema/lymphedema, history of ulcer, coexisting ipsilateral dermatitis, PAOD, chronic ulcer, fungal infections of the ipsilateral limb-all significant in other studies [12], [13], [14], [15], [16], [17]. In most of the patients who were involved in our study, the point of entry could be identified. The disruption of the cutaneous barrier is repeatedly referred to as a risk factor, namely in relation to fungal infections it is considered that they do not cause erysipelas, but in many cases of erysipelas of the lower limbs, the responsible streptococci are residents in the interdigital spaces, when they are macerated, presented with regards and fissures [39], [40], [41]. Recurrent episodes [40] have been discontinued the treatment of tinea pedis as a point of entry. Sometimes, the streptococcal reservoir is the anal canal or vagina, especially in patients with previous gynaecological carcinoma treated with surgical and radiotherapy [42]. However, only the disruption of the skin barrier usually does not lead to the onset of infection. It is considered that there must precede damage to the subcutaneous tissue and lymphatics. The authors agree that damage to the lymphatic system plays a key role in the development of acute cellulite on the lower limbs. In particular, damage to the venous and lymphatic system predisposes to the creation of an environment suitable for bacterial colonization for infections caused by β-haemolytic streptococci [12], [13], [43]. The lymphatic system plays a central role in the host's defence against skin infections and soft tissue. The damaged lymphatic clearance for microbial antigens and inflammatory mediators is proposed as a mechanism leading to a self-sustaining vicious circle of inflammation [44], [45].

In our study, the significance of CVI was not confirmed as a predictive risk factor, which is the case of multiple studies [12], [14], [30]. We've concluded that the reason is that the institution in which this study was conducted is specialised in the treatment and care of chronic wounds. All patients with first episode of erysipelas and CVI, were educated about the benefits and use of the appropriate compressive stockings/bandage (for each patient with CVI a compression bandage was applied during hospitalization) as well as skin care especially for lipodermatosclerosis and stasis dermatitis that is common in these patients and acts as point of entry.

Surgical interventions of blood and lymphatic vessels, as well as orthopaedic surgery, have not been proven as significant risk factors, unlike in other studies [15], [41] except for local lymph node dissection. This is consistent with the risk factor history of malignant disease. Malignity can be complicated by venous and lymphatic compromise, directly due to tumour effects or indirectly due to radiotherapy, and it predisposes to streptococcal infection [43]. These results indicate that erysipelas should be considered as a recurrent, potentially chronic disease [4]. In all patients with acute erysipelas the lower extremities preventive measures are required to reduce the high incidence of recurrent disease. The extent of the required prophylaxis is unknown. However, prolonged antibiotic prophylaxis in patients at high risk has a role in preventing the recurrence [24]. The crucial element in prevention of recurrent infection is elimination of risk factors such as avoiding mechanical trauma, treatment of point of entry (chronic ulcers, tinea pedis, onychomycosis, pruritic dermatitis, and CVI), use of compressive stockings/bandages, lowering Body Mass Index (BMI) and rigorous glycemic control in diabetic patients.

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Clinical Science

Assessment of Tp-Te Interval and Tp-Te/Qt Ratio in Patients with Aortic Aneurysm

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Keywords: Tp-Te interval; Transmural dispersion; Tp-Te/QT; Aortic Aneurysm

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Abstract

BACKGROUND: Arrhythmic disorders in the aortic aneurysm (AA) have been rarely reported.

AIM: The study aimed to assess the repolarisation indices of ventricular arrhythmia (VA) (mainly Tp-Te interval and Tp-Te/QT ratio) in patients with AA.

METHODS: A group of 98 patients with AA and 75 patients as control were recruited. Many of indices of ventricular arrhythmia were assessed.

RESULTS: Many of indices like QT, QTc, QTpc, Tp-Te/QT, Tp-Te/QTc, Tp-Tec/QTc, S-Tp, S-Tpc, S-Te, S-Tec and fQRS were found to be significantly different in AA group (for all $P < 0.05$). However, QTp, mean Tp-Te and Tp-Tec were not found different (for all $P < 0.05$). Aortic diameter (Ao-D) was found to have a positive correlation with QTc, QTpc, S-Tp, S-Tpc, S-Te, S-Tec, fQRS (for all $P < 0,05$) and negative correlation withTp-Te/QT ($P = 0.047$). The best cut-off level for prediction of Tp-Te ≥100 ms was found the Ao-D > 43.5 mm in ROC analysis (AUC: 0.69; $P = 0.151$) with sensitivity 60% and specificity 79.6%.

CONCLUSIONS: Although our study did not find any differences for mean Tp-Te interval between groups, many of other indexes of TDR were found to be significantly different. Ao-D was found to have significant correlations with many indices.

Introduction

Thoracic aortic diseases include degenerative, structural, acquired, genetic-based, and traumatic diseases of the aorta and aortic aneurysm (AA) is the main part of this conundrum whose diagnosis is made easily with transthoracic echocardiographic (TTE) study [1], [2]. AA had a complicated pathogenetic process with the degenerative formation and diminished significant aortic distensibility also with substantially increased of aortic wall stress and stiffness which has been demonstrated as a predictor risk factor of increased cardiovascular disease and arrhythmic events [3], [4], [5]. Although there are several case reports about disorders of atrioventricular conductivity in AA with dissection complications, however there is not enough knowledge about arrhythmic disorders in patients with AA without rupture or dissection in the literature [6], [7]. On surface Electrocardiography (ECG) image T wave is inscribed by a sum of opposite voltage gradients in three different cell layers (Epicardial, M and endocardial cells) in the ventricular wall. Tpeak-Tend (Tp-Te) interval has been considering a measure of transmural dispersion of repolarization (TDR) and prolongation of Tp-Te (≥ 100 milliseconds [ms]) as well as QTc, QT and Tp-Te/QT ratio have been found of risk factors to develop cardiac arrhythmia especially ventricular arrhythmia (VA) and sudden cardiac death (SCD) in various cardiac disease also with normal healthy individuals [8], [9],

[10], [11], [12], [13], [14]. Fragmented QRS (fQRS) is another important novel ECG risk predictor for electro-mechanical dyssynchrony, VA, SCD and poor prognosis in patients with HF and hypertrophic cardiomyopathy [15], [16]. So this study aimed to determine if mainly Tp-Te interval and other indices of TDR like QT, QTp, Tp-Te/QT and fQRS are significantly different in patients with AA compared to the healthy control group.

The study was completed between March 2017 and January 2018 with totally 173 patients. Ninety-eight patients with AA and 75 normal healthy persons were included. Baseline characteristics and history of diseases including of diabetes mellitus (DM), hypertension (HT), coronary artery disease (CAD) and as well as being on any treatment or diet were assessed at baseline. AA was evaluated according to previous guidelines with the upper limit of normal ascending aorta diameter was accepted 39 millimetres (mm) [1], [2]. Patients with prior pacemaker implantation, cancer, other major illnesses, abnormal thyroid function test, abnormal electrolyte values and on antiarrhythmic drug treatment due to may affect ECG images so make changes on T wave measurements were excluded.

Approval of the Ethics Committee

The study protocol was approved by the Ethics committee at AfyonKocatepe University, and informed consent was obtained from each patient.

ECG

All ECGs were recorded using a General Electric MAC 5000 (GE Healthcare, Milwaukee, WI, USA). All 12-lead ECGs were recorded at 25 mm/s with standard lead positions. After magnification by 200%, all indices were measured. To eliminate both interobserver variability and bias, all measurements were measured by a single observer who was blinded to all clinical findings. QT intervals were taken to be from the onset of the QRS complex to the end of the T wave. The Tp-Te interval was defined as the interval from the peak of the T wave to the end of T wave [17]. Q-Tpeak (QTp) was measured from the onset of QRS to the peak of the T wave (Figure 1). The Tp-Te value reported was the average value of obtained in all precordial leads. The Tp-Te/QT ratio was calculated as the ratio of Tp-Te in that lead to the corresponding QT interval. Other novel indices were described as S-Tend (S-Te) interval and S-Tpeak interval (S-Tp). S-Te and S-Tp were measured from nadir of S wave to peak of T wave and end of T wave in precordial limbs. Bazett's formula (n/RR) was applied to all the indices to find heart rate corrected form (c: heart rate corrected) [18].

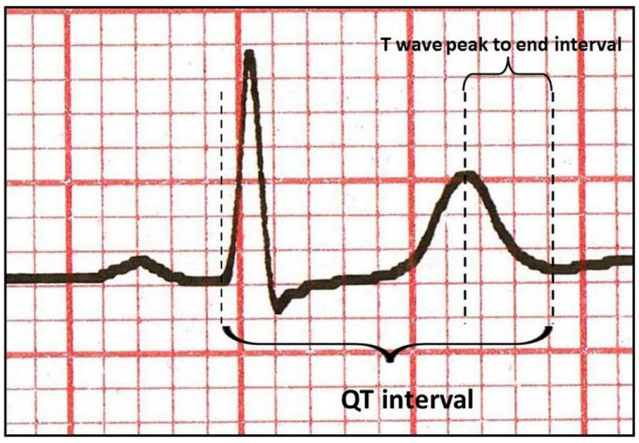

Figure 1: Demonstration of the T wave peak to end and QT intervals [17]

fQRS included various RSR patterns and was defined by the presence of an additional R wave (R prime), a notch in the nadir of the S wave, notch of the R wave, or the presence of more than one R prime (fragmentation) in two contiguous leads corresponding to a major myocardial segment [15].

Echocardiography

A Vivid 5 pro echocardiographic unit (GE, USA) with 3.5 MHz probe was used. The echocardiographic study was performed in standard position and standard measurements (M-mode, two-dimensional and Doppler echocardiography), were performed and/or reviewed by experienced staff cardiologists, compliant with the recommendation of the American Society of Echocardiography. Mitral inflow was determined by continuous and pulse wave Doppler echocardiography at the tips of the mitral leaflets. Early diastolic mitral peak flow velocity (E), late diastolic mitral peak flow velocity (A), E/A ratio were measured. Left ventricular diastolic dysfunction (LVD-Dys) was defined as a mitral continuous-wave (CW) Doppler E < A as stated in previous guidelines [19], [20].

Statistical analysis

Continuous variables were expressed as mean ± SD (Standard deviation), and categorical variables were presented as frequencies (%, per cent). Continuous and categorical measures were compared with *t*-tests or 2 statistics, as appropriated. For correlations, appropriate calculations were done. *A p* value < 0.05 was accepted as a statistically significant. All analyses were performed using SPSS Version 16.0 (SPSS Inc. Chicago, IL, USA).

Results

A group of 173 patients were included in our study (98 patients with AA and 75 patients in the control group). Some of the baseline features were displayed in Table 1 and 2.

Table 1: Baseline characteristic features and echocardiographic measures of groups

Baseline Features	Counts	Patients (n = 98. 100%)	Control (n = 75. 100%)	Total (n = 173. 100%)	$p^{¥}$
Women	Count & percent in total	42 (24.2%)	24 (13.8%)		0.145 *
Male	Count & percent in total	56 (32.2%)	51 (29.4%)		
Age	Mean ± SD	70 ± 13.9 (min = 31. max = 96)	43 ± 10.5 (min= 28. max= 81)		< 0.0001 #
S- BP (mmHg)	Mean ± SD	138 ± 20.9	118 ± 10.7		< 0.0001 #
	Median (25%-75%)	140 (120-160)	120(110-130)		
D-BP (mmHg)	Mean ± SD	73 ± 10.7	68 ± 7.4		0.005 #
	Median (25%-75%)	80 (70-80)	70 (60-70)		
Pulse rate (per minute)	Mean ± SD	84 ± 25.2	79 ± 13.0		0.610 #
	Median (25%-75%)	80 (68-100)	78 (71-90)		
LDL-chol	Mean ± SD	129.2 ± 42.3	139.1 ± 41.3		0.178 #
	Median (25%-75%)	127 (98-158)	131.5 (110.5-171.2)		
Glucose	Mean ± SD	116.7 ± 32.4	97.5 ± 9.1		< 0.0001 #
	Median (25%-75%)	104.5 (95-124.5)	98 (91-104)		
BUN	Mean ± SD	27.2 ± 18.9	13.2 ± 3.1		< 0.0001 #
	Median (25%-75%)	21 (15-32.4)	13 (11-15)		
DM	Count and % within total population	28 (16.1%)	0 (0%)		< 0.0001 *
Hypertension	Count and % within total population	73 (42.1%)	0 (0%)		< 0.0001 *
CAD	Count and % within total population	33 (19.0%)	0(0%)		< 0.0001 *
LVEF %	Mean ± SD Min: 18 Max: 58	53 ± 9.3	60 ± 0		< 0.0001 #
	Median (25%-75%)	60 (47-60)	60 (60-60)		
LVDD (mm)	Mean ± SD	50 ± 7.3	46 ± 4.7		< 0.0001 #
	Median (25%-75%)	51 (46-54)	46 (44-50)		
LVSD (mm)	Mean ± SD	32 ± 8.1	28 ± 4.7		0.01 #
	Median (25%-75%)	32 (27-39)	28 (25-32)		
IVS (mm)	Mean ± SD	10 ± 1.8	9 ± 1.1		< 0.0001 #
	Median (25%-75%)	11 (9.5-12)	10 (9-10)		
Ao-D (mm)	Mean ± SD	41.8 ± 3.0 (min = 39. max = 54)	27.8 ± 3.2 (min = 20. max = 35)		< 0.0001 #
	Median (25%-75%)	41 (39-43)	27 (26-31)		

*: Chi-Square test. #: Independent samples non-parametric Mann-Whitney U test. ¥:*P* < 0.05 is accepted statistically significant. SD: Standard deviation, mm: millimeter, S-BP: Systolic blood pressure, D-BP: Diastolic Blood pressure, LDL-chol: LDL cholesterol, BUN: Blood Urea Nitrogen, DM: Diabetes Mellitus, CAD: Coronary Artery Disease, LVEF: Left ventricular ejection fraction, LVDD: Left ventricular end-diastolic diameter, LVSD: Left ventricular end-systolic diameter, IVS: interventricular septum, Ao-D: Aortic diameter.

Many baseline parameters were found to be significantly different in AA group comparing to control group except LDL cholesterol (LDL-chol) and pulse rate (for LDL-chol *P* = 0.178; for pulse rate *P* = 0.610 and all others *P* < 0.05).

Table 2: Comparing to some of ECG features and mitral E/A ratio between groups

Features	Counts	Patients (n = 98)	Control (n = 75)	Total (n = 173)	$p^{¥}$
PW (mm)	Mean ± SD	15.8 ± 5.4	9.2 ± 1.1		< 0.0001 #
	Median (25%-75%)	11 (9-12)	10 (8-10)		
LA (mm)	Mean ± SD	41.7 ± 7.2	34.3 ± 4.6		< 0.0001 #
	Median (25%-75%)	41 (36.2-46)	35 (32-37.7)		
RV (mm)	Mean ± SD	27.7 ± 5.6	28.4 ± 4.4		0.382 #
	Median (25%-75%)	27 (24-31)	28 (25-32)		
RA (mm)	Mean ± SD	30.3 ± 7.3	29.9 ± 4.8		0.792 #
	Median (25%-75%)	30 (23.2-35)	29 (27-33.2)		
P time (ms)	Mean ± SD	77.4 ± 42.1	86.8 ± 17.0		0.656 #
	Median (25%-75%)	80 (67.5-110.5)	80 (80-100)		
QRS time (ms)	Mean ± SD	88.0 ± 12.8	85.4 ± 10.7		0.111 #
	Median (25%-75%)	86 (80-100)	80 (80-94)		
T time (ms)	Mean ± SD	132.9 ± 28.1	140.6 ± 27.2		0.061 #
	Median (25%-75%)	120 (120-160)	140 (120-160)		
QT time (ms)	Mean ± SD	367.5 ± 49.3	354.5 ± 30.4		0.016 #
	Median (25%-75%)	363 (343-392.5)	360 (320-364)		
E/A (missing in patients N = 44, in control n = 31)	E < A count & per cent in total	41 (23.6%)	11 (6.3%)		< 0.0001 *
	E > A count & percent in total	13 (7.5%)	33 (19.0%)		

*: Chi-Square test. #: Independent samples non-parametric Mann-Whitney U test. ¥:*P* < 0.05 is accepted statistically significant. SD: Standard deviation, mm: millimeter, ms: millisecond, PW: Left ventricular posterior wall, LA: Left atrium four-chamber diameter, RV: Right ventricular four-chamber diameter, RA: Right atrium four-chamber diameter, E/A: Early diastolic mitral peak flow velocity (E), late diastolic mitral peak flow velocity (A).

Mean ascending Ao-D was found 41.8 ± 3.0 mm in the AA group and 27.8 ± 3.2 mm in the control group (*P* < 0.0001).

Table 3: Comparing of TDR between groups with independent samples non-parametric Mann-Whitney U test

Ecg Features	Counts	Patients (n = 98)	Control (n = 75)	Total (n = 173)	$p^{¥}$
QTc (ms)	Mean ± SD	423.5 ± 55.8	403.8 ± 35.1		< 0.0001 #
	Median (25%-75%)	422 (401.7-446.2)	404 (381-424)		
QTp (ms)	Mean ± SD	294.5 ± 49.6	279.6 ± 28.1		0.08 #
	Median (25%-75%)	296 (268-320)	280 (262-300)		
Tp-Te (ms)	Mean ± SD	71.7 ± 15.1	74.8 ± 14.4		0.111 #
	Median (25%-75%)	80 (60-80)	80 (70-80)		
Tp-Te ≥ 100 ms					0.382 #
QTpc (ms)	Mean ± SD	342.7 ± 38.3	318.8 ± 35.7		< 0.0001#
	Median (25%-75%)	339 (317-360)	318 (295-341)		
Tp-Tec (ms)	Mean ± SD	84.0 ± 20.7	84.8 ± 16.3		0.497 #
	Median (25%-75%)	83 (71.7-97)	87 (74-94)		
Tp-Te/QT	Mean ± SD	0.196 ± 0.04	0.211 ± 0.038		0.011 #
	Median (25%-75%)	0.200 (0.166-0.222)	0.222 (0.187-0.232)		
Tp-Te/QTc	Mean ± SD	0.186 ± 0.11	0.186 ± 0.037		0.003 #
	Median (25%-75%)	0.177 (0.142-0.196)	0.194 (0.155-0.210)		
Tp-Tec/ QTc	Mean ± SD	0.196 ± 0.04	0.208 ± 0.04		0.035 #
	Median (25%-75%)	0.202 (0.165-0.222)	0.220 (0.187-0.232)		
PR (ms)	Mean ± SD	123.6 ± 66.6	142.5 ± 71.7		0.288 #
	Median (25%-75%)	144 (118-160)	120 (120-160)		
RR (ms)	Mean ± SD	758.7 ± 211.6	142.5 ± 71.7		0.308 #
	Median (25%-75%)	736 (599.7-876.2)	760 (666-840)		

#: Independent samples non-parametric Mann-Whitney U test. ¥:*P* < 0.05 is accepted statistically significant. SD: Standard deviation, ms: millisecond, RR: Measurement between two consequent R wave interval. PR: Measurement between the beginning of P wave to beginning to Q wave. c: Heart rate-corrected form with Bazett's formula (n/RR), min: minimum, max: maximum.

Significantly differences were found to be between groups for posterior wall (PW) and left atrium diameter (LA), QT time and mitral E < A or E > A (for all *P* < 0.05), but not for right atrium (RA), right ventricular (RV) dimensions, P- time, QRS- time and T time (all *P* > 0.05). For TDR, significant differences were found to be between groups for QTc, QTpc, Tp-Te/QT, Tp-Te/QTc, Tp-Tec/QTc (for all *P* < 0.05) except QTp, Tp-Tec and Tp-Te (for all *P* > 0.05 in Table 3).

Table 4: Comparing of TDR between groups with independent samples non-parametric Mann-Whitney U and Pearson Chi-Square tests

Ecg Features	Counts	Patients (n = 98)	Control (n = 75)	Total (n = 173)	$p^{¥}$
S-Tp (ms)	Mean ± SD	284.3 ± 34.1	231.6 ± 29.5		0.02 #
	Median (25%-75%)	254 (220-278.5)	234 (219-248)		
S-Tpc (ms)	Mean ± SD	291.2 ± 38.5	260.4 ± 30.6		< 0.0001#
	Median (25%-75%)	281 (266.7-309)	265 (241-282)		
S-Te (ms)	Mean ± SD	348.6 ± 25.7	306.9 ± 30.6		0.03 #
	Median (25%-75%)	321 (294-350)	310 (280-320)		
S-Tec (ms)	Mean ± SD	375.1 ± 38.8	347.0 ± 34.1		< 0.0001 #
	Median (25%-75%)	370 (356.7-392)	346 (329-367)		
fQRS	Present count & percent in total	50 (28.9%)	25 (14.4%)		0.020*
	None count & percent in total	48 (27.7%)	50 (28.9%)		

¥: *P* < 0.05 is accepted as statistically significant. #: Independent samples non-parametric Mann-Whitney U test. *: Chi-Square test. S-Tp: Measurement from nadir S wave to T peak. S-Te: Measurement from nadir S wave to T end. fQRS: Fragmented QRS. SD: Standard deviation, ms: millisecond, c: Heart rate-corrected form with Bazett's formula (n/RR), min: minimum, max: maximum.

When considering all patients with Tp-Te interval ≥ 100 ms, there wasn't any difference between groups (*P* = 0.382). Significant differences were also found to be between groups for S-Tp, S-Tpc, S-Teand S-Tec and fQRS (for all *P* < 0.05 in Table 4, and Figure 2).

In correlation analysis, Ao-D was found to have a positive correlation with QTc, QTpc, S-Tp, S-Tpc, S-Te, S-Tec and fQRS (for all *P* < 0.05 in Table 5). However negative correlation was found with Tp-Te/QT (r = - 0.158; *P* = 0.047).

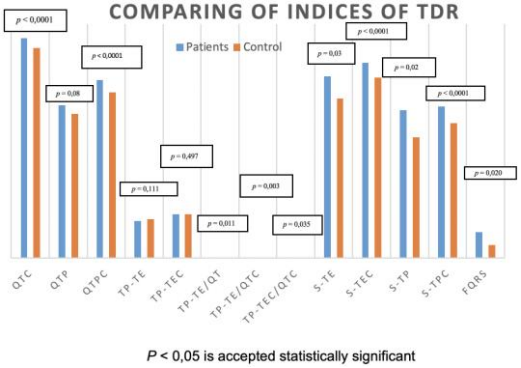

Figure 2: Comparing of indices of TDR

To determine the best cut-off, point of Ao-D for prediction of Tp-Te ≥ 100 ms, analysis of ROC (Receiver Operating Characteristics) curves demonstrated cut-off level of Ao-Dwas to be determined > 43.5 mm with the area under the curve (AUC) was 0,69 (*P* = 0.151) and sensitivity 60%, specificity 79.6%.

Discussion

A histopathological feature of AA is based on degeneration of medial muscular (consisting of main proteins of collagen and elastin) layer of vessel wall [21]. Pathogenesis of AA includes aortic wall degeneration which has passive lumen dilation and active dynamic remodelling and stiffness of aorta also plays a major role as a contributor risk factor in this pathogenetic process as well as being a result of the progress of AA [22]. Aortic stiffness with other risk factors of AA has been accepted as a risk factor for increased major cardiovascular events and some arrhythmia [4], [5]. Some reports have been published about arrhythmic consequences of aortic disease especially acute aortic dissection [6], [7]. However, there is limited information about arrhythmic events and disorders in patients with AA without dissection. TDR within the ventricular myocardium has been suggested due to three electrophysiologically different cells, endocardial, epicardial and M cells [23]. The peak of the T-wave was shown to coincide with epicardial repolarisation and the end of the T-wave with repolarisation of the M cells so that Tp-e provides a measure of TDR [24]. Prolongation of indices of TDR like QTc, QTp, Tp-Te, Tp-Tec interval and Tp-Te/QT ratio has been suggested to provide of indexes of TDR and supposed to be risk factor of VA in various clinical scenarios like patients with Brugada syndrome (BS), hypertrophic cardiomyopathy, myocardial infarction with ST-Segment elevation and HF with low ejection fraction [12], [13], [14], [25], [26], [27], [28]. In these studies, various cut-off levels for Tp-Te values ≥ 100 ms have been proposed to predict the adverse outcome [27]. In our study mean ascending Ao-D was found higher in AA group (*P* < 0.0001). As the main part of our study, we found the significant differences between groups for indices of TDR like QTc, QTpc, Tp-Te/QT ratio, Tp-Te/QTc ratio and Tp-Tec/QTc ratio except for QTp, Tp-Tec. Interestingly, the mean Tp-Te interval was not found to be different between groups (*P* = 0.111). When considering to all patients with Tp-Te ≥ 100 ms, there wasn't any difference between groups (*P* > 0.05). Newer indices S-Tp, S-Tpc, S-Te, S-Tec and fQRS were found to be significantly different (for all *P* < 0.05).

Table 5: Correlation analysis of indices of TDR with Ao-D

		Age	*LVEF*	*LVEDD*	*LVESD*	*Ao-D*	*BUN*	*Glucose*	*LDL-chol*	*DM*	*HT*	*CAD*
QTc	r	0.291	-0.391	0.183	0.297	0.295	0.265	0.006	-0.146	0.169	0.220	0.186
	p	<0.0001	<0.0001	0.016	<0.0001	<0.0001	0.003	0.949	0.158	0.026	0.040	0.014
QTpc	r	0.298	-0.270	0.122	0.200	0.303	0.140	0.007	-0.180	0.100	0.223	0.166
	p	<0.0001	<0.0001	0.109	0.008	<0 .0001	0.116	0.937	0.081	0.189	0.003	0.029
Tp-Te/QT	r	-0.160	-0.050	0.055	0.056	-0.158	0.169	-0.043	-0.046	-0.029	0.159	-0.085
	p	0.036	0.515	0.474	0.464	0.047	0.057	0.646	0.661	0.705	0.037	0.267
Tp-Te/QTc	r	0.027	0.031	0.109	0.141	0.026	0.00	-0.128	0.011	-0.140	-0.137	-0.067
	p	0.722	0.688	0.152	0.064	0.741	0.999	0.164	0.918	0.065	0.072	0.380
Tp-Tec/QTc	r	-0.135	-0.062	0.072	0.071	-0.117	0.189	-0.024	-0.018	-0.009	-0.133	-0.078
	p	0.078	0.419	0.345	0.353	0.143	0.033	0.799	0.861	0.908	0.080	0.309
S-Tp	r	0.281	0.005	0.186	0.041	0.266	-0.118	-0.127	-0.050	-0.091	0.215	0.067
	p	<0.0001	0.946	0.014	0.597	0.001	0.186	0.168	0.630	0.234	0.005	0.382
S-Tpc	r	0.376	-0.231	0.134	0.198	0.370	0.115	0.008	-0.081	0.100	0.291	0.176
	p	<0.0001	0.002	0.079	0.009	<0.0001	0.199	0.934	0.437	0.191	<0.0001	0.020
S-Te	r	0.245	-0.009	0.253	0.062	0.228	-0.085	-0.187	-0.071	-0.109	0.199	0.079
	p	0.001	0.906	0.001	0.420	0.04	0.267	0.042	0.497	0.155	0.009	0.279
S-Tec	r	0.367	-0.324	0.203	0.258	0.354	0.028	-0.005	-0.170	0.171	0.309	0.204
	p	<0.0001	<0.0001	0.070	0.001	<0.0001	0.716	0.956	0.100	0.024	<0.0001	0.007
fQRS	r	0.115	-0.284	0.034	0.149	0.203	0.076	0.132	-0.118	0.154	0.197	0.110
	p	0.132	<0.0001	0.635	0.051	0.010	0.393	0.153	0.256	0.043	0.009	0.151

LDL-chol: LDL cholesterol, BUN: Blood Urea Nitrogen, DM: Diabetes Mellitus, CAD: Coronary Artery Disease, LVEF: Left ventricular ejection fraction, LVDD: Left ventricular end-diastolic diameter, LVSD: Left ventricular end-systolic diameter, Ao-D: Aortic diameter. HT: Hypertension.

In correlation analysis, Ao-D was found to have a positive important correlation with QTc, QTpc, S-Tp, S-Tpc, S-Te, S-Tec and fQRS (all for $P < 0,05$) and negative correlation with Tp-Te/QT ratio ($P = 0.047$). To determine the best cut-off level of Ao-D for Tp-Te ≥ 100 ms interval, ROC (Receiver Operating Characteristics) curves demonstrated cut-off level > 43.5 mm with the area under the curve (AUC) was 0.69 ($P = 0.151$) and sensitivity 60%, specificity 79.6%.

Limitations: There are some important limitations to this study. This study was a cross-sectional study, and these findings need to further evaluate in a cohort study to find the importance of these indices for prediction of cardiovascular outcomes.

In conclusion, although our study did not find any differences for mean Tp-Te interval between groups many of other incidents of TDR were found to be significantly different. Ao-D was found to have significant correlations with many indices. Their clinical usages for prediction of adverse outcomes are needed to be assessed in the future.

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Effects of High - Intensity Laser in Treatment of Patients with Chronic Low Back Pain

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Abstract

BACKGROUND: Chronic low back pain lasts longer than 12 weeks and is characterised by pain, muscle weakness, reduced functional ability and psychosocial burden.

AIM: To compare the effects of two physical modalities, high-intensity laser against ultrasound therapy in the treatment of patients with chronic low back pain.

MATERIAL AND METHODS: This was a prospective, monocentric, controlled clinical study comprising a group of 54 patients at the age between 25 and 65 years. Patients were divided into two groups: an examined group of 27 patients (high-intensity laser and exercises) and a control group of 27 patients (ultrasound therapy and exercises). The results were evaluated by the Numeric Pain Rating Scale, Oswestry Disability Index and Schober's test. Clinical findings were evaluated at the same time points for all patients, before treatment, at two weeks and three months following treatment. Statistical analyses were made to compare the differences between the results obtained on admission and the two consecutive control check-ups. Statistical significance was defined as a P value < 0.05.

RESULTS: The examined group showed statistically significantly better results than the control group after completion of the treatment (at two weeks) and at follow up after three months.

CONCLUSION: This study has shown that patient with chronic low back pain treated with a high-intensity laser has significantly reduced low back pain, reduced disability and improved range of motion. Its positive effect maintained for three months. It seems to be an effective, safe and useful physical modality in the treatment of a patient with chronic low back pain.

Introduction

Pain is an unpleasant sensory and emotional experience associated with actual or potential injury of some part of the body [1]. Pain intensity is not always associated with the degree of tissue damage since pain is not only a physical but also a mental process with a wide range of repercussions and consequences.

Low back pain is a sum of symptoms from different aetiology that are manifested with a pain in the lumbar or lumbosacral spine, with or without pain radiation in the legs [2]. Low back pain is a common musculoskeletal disorder with high prevalence in the general population [3].

Chronic low back pain lasts longer than 12 weeks, and it can persist even after discontinuation of the action of nociceptive stimuli [4]. Characteristics of chronic low back pain include long-term pain, muscle weakness, reduced functional ability and psychosocial burden, which make the treatment of chronic low back pain a complex process. Therapeutic procedures for treatment of chronic low back pain comprise multidisciplinary approach, education of patients, therapeutic exercises, application of physical agents and psychological counselling [5].

Over the last years, aggressive conservative treatment has been recommended by increasing the number of therapeutic programs that include a multidisciplinary team and numerous treatment modalities [6]. Therapeutic program is individually designed for each patient depending on the local and general clinical findings in the patient.

Exercise therapy has become a standard procedure for the management of spinal pain. Combined exercises for gluteal muscles strengthening and exercises for lumbar segmental stabilisation have shown improvement in balance, larger muscle endurance and a decrease in disability pain index in patients [7].

Ultrasound therapy as a physical modality is very often practised in the treatment of chronic low back pain. Its mechanical action is considered to be predominant, and it consists of alternating ultrasound pressure that is manifested as molecular vibration in the tissue. Analgesic effect of ultrasound therapy has also been confirmed, resulting in improvement of functional disability in patients with chronic low back pain [8].

Laser therapy is a painless and non-invasive treatment that can be used in the treatment of different clinical conditions. It has been confirmed that laser therapy significantly reduces acute and chronic pain as well as rheumatoid arthritis, chronic osteoarthritis, carpal tunnel syndrome, fibromyalgia, knee injuries, pain in the shoulders and postoperative pains [9], [10]. A reduction of pain after laser treatment is a result of its anti-inflammatory effects, increase in microcirculation, and stimulation of immunological processes, nerve regeneration and increased secretion of β-endorphins [11]. Recently a high-intensity laser therapy (HILT) has been introduced in the field of physical medicine. HILT is considered to be a non-invasive and painless modality because of its high intensity and specific wavelength. By application of this type of laser larger regions and deeper tissue structures can be more effectively treated than with the other types of lasers [12]. Clinical studies have documented the anti-inflammatory, anti-edematous and analgesic effect of the high-intensity laser, thus justifying its use in patients with pain problems [12].

The aim was to compare the effects of both physical modalities, therapy with high-intensity laser versus ultrasound therapy, and to point out the differences in the analgesic effect, reducing disability and the range of motion in the lumbar spine.

Material and Methods

This prospective, monocentric, controlled clinical investigation was conducted in the Institute of physical medicine and rehabilitation. The diagnoses of the patients were established by medical history, physical examination, and x-ray findings of the lumbosacral spine. In total, 54 male and female patients between the ages of 24 and 65 were enrolled in the study. The patients were divided into two groups: Group 1 included 27 patients treated with high-intensity laser and exercises, and Group 2 (control group) included 27 patients treated with ultrasound therapy (US) and exercises. Each patient received 10 sessions in total, continuously each day in two weeks, with breaks during weekends. The research was approved by the Ethics committee of the Medical Faculty in Skopje (03-6283/1).

Patients who were not working on occupations requiring intensive effort and who had sufficient mental capacity to understand and answer the questions asked in the assessment scales were included in the study. Inclusion criteria for the study were patients with chronic low back pain that persisted for more than three months and pathological findings on lumbar X-rays. Patients agreed not to take any medication (anti-inflammatories, analgesics, or muscle relaxants) throughout the study or receive any treatment for back pain. Patients were excluded if they had a positive neurological examination (presence of positive motor or sensory abnormalities indicating spinal root compression), lumbar spine surgery, congenital malformation, trauma, metabolic disorders or cancer, inflammation, infection or known photosensitivity or other illnesses unrelated to back pain which precluded involvement for practical reasons.

Patients who met the inclusion criteria participated in the study. Taking part in the study was voluntarily, and all participants were informed in details about the purpose of this study. All subjects read and signed consent forms, by the ethical standards of the Declaration of Helsinki.

Application of the high-intensity laser therapy (HILT) was performed with the apparatus PRESTIGE LINE VIKARE 4WHL1361 (Medical Italia), the power of 4W, the intensity of 1.50 J/cm², scanning regime. Scanning was performed longitudinally in the lower-back area of L1-L5 and S1, on dry skin previously cleaned with alcohol. Laser probe was in contact with the skin. The procedure lasted for 15 minutes and the total dose of absorbed energy in the tissue was 2400 J. HILT was calibrated for constant output throughout the experiment. All protection measures for applying laser therapy were respected. The therapy is painless and with no risk for patient's health.

Control group of patients received ultrasound therapy (continuous waveform) with an intensity of 0.5 W/cm² due to the chronicity of the condition and deep position of lower back musculature. The therapy was applied for 5 minutes to the lumbar paravertebral area. The treating physical therapist, with the technique of using slow circular movements, applied the transducer head over the lumbar and dorsal muscles.

All patients in both groups performed isometric and static exercises for strengthening back, abdominal, lumbar and gluteal muscles under the supervision of a physiotherapist for 15 min once a day. The standardised program included posterior

pelvic tilts, quadriceps exercises, and posterior hip and knee muscles stretching. Core stability training for the lumbar area was applied in supine and prone positions. Participants were taught by a physiotherapist to perform the exercises correctly, and all treatment groups were given instructions to perform the exercises at home. The exercise program was designed to be easily carried out at home. Patients were asked to maintain the daily home exercises for three further months. There was no need for special equipment or access to a gym or fitness facility. The patients were informed that the key to prevent recurrences and provide functional recovery was making the exercises part of their lives.

The patients were assessed for pain, lumbar range of motion and disability. Evaluation of the measured outcomes was performed at the beginning of the study, and evaluation was repeated after 2 weeks of treatment and again after 3 months of further follow-up.

Numeric Rating Pain Scale (0-no pain and 10-worst possible pain) have been validated in the assessment of pain and were used to quantify subjective assessments [13]. In the three time points of examination, the doctor filled in the Numeric Rating Pain Scale. Schober's test for assessment of the range of motion, i.e. lumbar spine flexion was made by the researcher [14]. Schober's test was measured before, after the two-week treatment and after 3 months follow-up. Oswestry Disability Index (ODI) was used to evaluate the function of a patient with chronic back pain [15]. Subjects were evaluated before the first treatment, at the end of treatment (after 2 weeks) and 3 months follow-up after the treatment.

Statistical analysis was made with the statistical package Statistic for Windows 7.0 and SPSS 17.0. Numerical (quantitative) series were analysed by using the measures of central tendency (mean and median) and measures of dispersion (standard deviation). Chi-square test for two parameters was used for comparison of certain features between the two groups of participants as well as for determination of the association between certain features in the group of participants. Non-parametric Mann Whitney U test was used for testing the significance in the difference between the mean values in both independent groups. Statistical significance was defined as a P value < 0.05.

Results

A total of 54 patients with chronic low back pain participated, and both groups completed the study. No side effects were observed during HILT, US therapy and exercise therapy throughout the study. No subject report taking the analgesic/anti-inflammatory drug during the period of their participation in the study. There was no statistically significant difference in terms of gender distribution between the two groups (Table 1). The mean age was 55.4 ± 6.7 years in HILT group and 55.3 ± 7.2 years in US therapy group. There was no significant difference between the groups in terms of age (Mann-Whitney U Test Z = -0.5103; p = 0.6087).

Table 1. Patients demographic data

Category		HILT No (%)	Ultrasound No (%)
Gender	Men	15 (55.6%)	14 (51.8%)
	Women	12 (44.4%)	13 (52%)

Pearson Chi-square test: 0.0745; df = 1; p = 0.7849; *significant for p < 0.05.

An analysis was made of the average score on standardised Numeric Rating Pain Scale applied in both groups of patients at three-time points, on admission, at two weeks and after three months. On admission, there was no significant difference (p > 0.05) between the two groups. The analysis of pain score after two weeks and after three months showed a significant difference (p < 0.05) between the two groups. The HILT group shows greater improvement in pain score (Table 2).

Table 2. Changes in Numeric Rating Pain Scale among treatment groups

Pain Scale	Mean	Number	Standard Deviation	Median (IQR)	p
On admission					
HILT	7.22	27	0.85	7 (7-8)	Mann-Whitney U Test: Z = 0.874, p = 0.382
Ultrasound therapy	6.96	27	0.94	7 (6-8)	
2 weeks					
HILT	2.11	27	0.80	2 (2-3)	Mann-Whitney U Test: Z = -5.519, p = 0.0001*
Ultrasound therapy	4.26	27	1.06	4 (3-5)	
3 months					
HILT	1.89	27	0.64	2 (1-2)	Mann-Whitney U Test: Z = -6.271, p = 0.0001*
Ultrasound therapy	4.89	27	0.85	5 (4-5)	

* significant for p < 0.05.

The analysis by using the Oswestry Disability Index (ODI) in both groups of patients showed a significant change (p < 0.05) between the groups after two weeks and after three months (Table 3). There was a statistically significant improvement in the ODI in the group treated with HILT.

Table 3. ODI changes among treatment groups

ODI	Means	Number	Standard Deviation	Median (IQR)	P
On admission					
HILT	44.33	27	3.92	44 (41 – 46)	Mann-Whitney U Test: Z=-1.021, p=0.307
Ultrasound	45.22	27	3.91	44 (43 – 47)	
2 weeks					
HILT	16.29	27	4.83	14 (12 – 20)	Mann-Whitney U Test: Z=-5.588, p=0.0001*
Ultrasound	26.74	27	4.51	26 (22 – 30)	
3 months					
HILT	15.89	27	4.58	14 (12 – 19)	Mann-Whitney U Test: Z=-5.891, p=0.0001*
Ultrasound	26.63	27	3.73	28 (25 – 29)	

* significant for p < 0.05.

In both groups, the analysis showed no significant difference (p > 0.05) in the Schober's test on admission and after two weeks. There was a significant difference (p < 0.05) in the range of motion by Schober's test after three months, that means better lumbar flexion in the group treated with HILT

(Table 4).

Table 4. Changes in Schober's test among treatment groups

Schober's test	Means	Number	Standard Deviation	Mediana (IQR)	P
On admission					
HILT	4.67	27	0.71	4.3 (4.1-5.3)	Mann-Whitney U Test:
Ultrasound Therapy	4.58	27	0.76	4.3 (4-5.4)	Z = 0.562, p = 0.574
2 weeks					
HILT	6.42	27	0.82	6 (5.8-7.2)	Mann-Whitney U Test:
Ultrasound therapy	6.13	27	0.69	6 (5.7-6.5)	Z = 1.375, p = 0.169
3 months					
HILT	6.48	27	0.88	6 (5.8-7.2)	Mann-Whitney U Test:
Ultrasound Therapy	6.08	27	0.74	5.9 (5.7-5.4)	Z = 1.859, p = 0.044*

* significant for $p < 0.05$.

Discussion

This study was conducted to compare the efficacy of two different physical modalities and to determine which of them gives better results in achieving the analgesic effect, disability of patients and flexibility of the lumbar spine. The study included 54 patients with chronic low back pain treated with high-intensity laser, ultrasound therapy and exercise therapy. The comparison of the parameters in the examined group (patients treated with high-intensity laser and exercises) at the beginning of therapy, at two weeks and three months after completion of therapy revealed significant changes in the results obtained by the Numeric Rating Pain Scale, Schober's test and Oswestry Disability Index. The group of patients who were treated with high-intensity laser and exercises showed statistically significantly better results in all three parameters when compared to the control group of patients treated with ultrasound therapy and exercises. High-intensity laser in combination with exercise therapy proved to be effective in patients with chronic low back pain. Its analgesic effects after ten days of application were maintained in the next three months. It resulted in a better functional performance in patients and improved flexibility in the lumbar spine.

Low-intensity laser therapy is still being used in the treatment of chronic low back pain and with its analgesic effects contributes to the better functional ability of patients and better range of lumbar spine motion [16]. Since recently, the new type of lasers, the high-intensity ones, has been introduced in the physical rehabilitation medicine as a non-invasive and safe physical modality. The use of high-intensity laser in physical medicine is a relatively new technology, which is continuously developing. By its high-intensity power and specific wavelength, it enables treatment of different clinical conditions. It is used in the treatment of shoulder pain [12], degenerative knee disease [17], and chronic pain in the ankle [18]. Clinical studies have confirmed the use of high-intensity laser in the treatment of acute and chronic pain associated with chronic arthritis, tendinitis, fibromyalgia as well as knee injuries [9], [10]. The analgesic effect is due to inhibition of painful sensation at different levels. Histamine and bradykinin release from inflammatory tissue [19] is reduced, and the pain threshold is increased. Also, laser light reduces the secretion of substance P from peripheral nociceptors, thus reducing the pain relay and preventing the development of hyperalgesia [20]. The laser analgesic effect is due to increased secretion of endogenous opioids such as β-endorphins, by which the pain is centrally inhibited [21]. Absorbed laser light in the tissue increases the mitochondrial oxidative process; hence the production of ATP, RNA and DNA is increased resulting in a photobiological effect [22].

This study aimed to determine the impact of the high-intensity laser on pain and functionality of patients with chronic low back pain. Alayat et al., 2014, reported that a combined treatment of exercises and high-intensity laser gave a better range of lumbar spine motion, reduced pain and better functionality in patients than that with a high-intensity laser, but without exercises and placebo laser with exercises. They found out that four weeks of treatment with laser and exercises resulted in statistically significant improvement on the VAS scale. Functional ability of these patients, which was measured by the Oswestry Disability Index, also showed a statistically significant difference compared to the other two groups of patients [23].

Angelova A and Ilieva EM, 2016, in their pilot, a randomised clinical study from 2016 investigated the analgesic effect of high-intensity laser in patients with osteoarthritis. Pain intensity was compared by VAS scale and dolorimeter, which showed a significant pain decrease in patients after seven days [24]. Efficacy of high-intensity laser in patients with knee osteoarthritis was proved by Gppl-Joo Kim et al., 2016 [25]. They showed that therapy with high-intensity laser was more effective in these patients than conventional physical therapy. Kheshie et al. demonstrated significantly better results of high-intensity laser than of low-intensity one in the treatment of chronic pain associated with knee osteoarthritis [26].

Laser therapy has rarely been presented in the management of patients who have fibromyalgia in spite of the benefits described in two controlled studies conducted by Gür et al., [27], [10]. These researchers have demonstrated that low-intensity laser alone and in combination with amitriptyline was safe and effective in the treatment of fibromyalgia if administered every day in 2 weeks.

A large number of studies have examined the treatment options of osteoporosis, such as exercise therapy, vibration therapy, pulsed electromagnetic field therapy and low-level laser therapy [28]. Laser beams can stimulate the proliferation by increasing the synthesis of DNA and RNA of osteoblasts in vitro

[29]. High-intensity laser combined with exercises is more effective in decreasing pain and in improving quality of life in male patients with osteopenia or osteoporosis [30].

Fiore et al., 2011, in their randomised trial have also proved the analgesic effect of high-intensity laser in patients with low back pain. Patients in their study received 15 treatment sessions of the high-intensity laser during three consecutive weeks against a control group of participants who received ultrasound therapy. The results obtained in both groups of patients were analysed by the VAS scale and Oswestry Disability Index immediately after the completed therapy and showed a significantly greater pain decrease in patients treated with a high-intensity laser [31].

It is assumed that exercises for the strengthening of spinal, abdominal and gluteal muscles, which are applied in patients with chronic low back pain, should be combined with laser therapy for achieving better results [16]. Several researchers have shown no advantages of using laser therapy alone or conducting exercises alone in the treatment of chronic pain, but these studies have analysed only the short-term effects of laser therapy [16].

It should be emphasised that ultrasound therapy as a physical agent is important in the treatment of musculoskeletal disorders [32]. Until nowadays ultrasound therapy has proved to be a good choice of physical agent in the treatment of chronic pain. The application of ultrasound energy in the treatment of low back pain was presented in the study of Safoora Ebadi et al. The results they obtained in their randomised controlled study showed the analgesic effect of ultrasound therapy, which resulted in improvement in functional disability in patients with chronic low back pain [33]. Durmus et al., 2010, also evaluated three groups of patients with low back pain that were given ultrasound therapy and exercised therapy, electric stimulation and exercise therapy and only exercise therapy. They observed that exercises combined with ultrasound therapy reduced pain when compared to patients treated with the other two physical modalities [34].

The present study indicates that exercise therapy is clinically able to decrease pain, increase ROM, and improve function. It is providing to be economical, practical, and safe to emphasise the importance of an exercise program in rehabilitation aimed at functional recovery. The combined significance, improving chronic low back pain and having this positive effect last for a period of up to 3 months.

Moreover, HILT can be useful to reduce pain and disability related, but this is important to add rehabilitation programs with the exercise of leg and spine and stretching to reduce the frequency of low back pain.

The study has several limitations. The main limitation of our study is the low number of patients included in the study. Second, we could not perfectly control the daily routine of the subjects. All patients were instructed to perform exercises at home, and report of exercise compliance was obtained from family members. Even though neither the family members nor the participants themselves reported any deficiency in the exercise prescription at home, we considered this to be a limiting factor in the present study. Also, the limitation of the study is that the occupation of patients and the body mass index have not been considered. Further studies with a larger number of patients, more demographic features and controls are required to evaluate the long-term effects of the therapies. We believe that many new studies on the effects of HILT will be required to complement the limitations of this study.

In conclusion, this study has shown that patients with chronic low back pain treated with a high-intensity laser have significantly reduced low back pain, reduced disability and improved range of motion. Its positive effect maintained for three months. It seems to be an effective, safe and useful physical modality in the treatment of a patient with chronic low back pain. Exercise therapy should never be ignored to treat and prevent lumbar back pain.

HILT is an adjuvant physical therapy modality that may provide better outcomes for a patient with chronic back pain when used in combination with exercise.

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Comparison of Effectiveness and Patient Satisfaction of Vaginal Versus Oral Misoprostol in Treatment of Missed Miscarriage

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Abstract

BACKGROUND: In recent years' medical management with misoprostol is an effective alternative to surgical evacuation. But there is a dearth of evidence to reveal the effectiveness of the different routes of misoprostol and satisfaction rate among the patients treated with these routes.

AIM: This study was conducted to compare the effectiveness and patient's satisfaction rate of vaginal versus oral misoprostol.

METHODS: It was a prospective non-interventional study. One hundred women of having missed abortion confirmed by ultrasonography examination were enrolled in the trial. Fifty-eight subjects were administered 200 mcg of oral and 42 subjects received 200 mcg of vaginal misoprostol every four hours up to four doses. If complete expulsion did not occur 12 hours after the last dose, the surgical evacuation was done.

RESULTS: There was no significant statistical difference between the effectiveness of treatment with vaginal (78.57%) and oral misoprostol (79.31%) (p = 0.928). The difference between Patients' satisfaction at the time of discharge for the vaginal group (64.29%) and oral group (65.52%) was not statistically significant (P = 0.991). There was an increase in patients' satisfaction for both groups at the follow-up session, but still, the difference was not significant (P = 0.897).

CONCLUSION: This study confirms that there is no statistical difference between the effectiveness and patient satisfaction of oral and vaginal misoprostol in the treatment of missed abortion.

Introduction

In the past, treatment for miscarriage before 14 weeks consisted of aggressive surgery [1]. But surgery is associated with many complications, such as postoperative infection [2]. However, in recent years, medical management has been introduced which is effective, safe, and acceptable [3]. Till now, so many different regimens have been tried and used, more or less successfully and it is one of the most confusing aspects of medical treatment of abortion. More than one regimen may be effective at a particular stage of pregnancy [4]. Misoprostol is an effective agent commonly used in the treatment of miscarriages especially missed miscarriage, but optimal dose and route of administration of misoprostol have not been determined by randomised trials [5]. Even the World Health Organization has not recommended a standard regimen for administration of misoprostol in the treatment of missed miscarriage [6].

A study was performed to find out the effect of misoprostol route on its pharmacokinetic profile [7]. There was the best absorption of misoprostol following vaginal administration. Small drug doses administered vaginally were capable of inducing contraction of uterus slowly and maintaining it for induction of labour. Due to the higher peak in oral administration, the side effects were more compared to that of the vaginal route [7]. Although the studies performed there is no agreement among experts in the superiority of the effectiveness of oral misoprostol over vaginal misoprostol and vice versa. Some

studies revealed that vaginal misoprostol is more effective than oral one in the expulsion of uterine content [8]. However, some other studies showed that there is not any significant difference between oral misoprostol and vaginal form of that [9]. Another important issue that should be considered in the treatment of missed abortion is the acceptability and satisfaction of the patient with the administered treatment. Because patients of having missed abortion experience grief, anxiety and depression, the method of treatment may affect their emotional state [10]. Some trials have concluded that women have higher satisfaction with oral misoprostol [11] while some other findings were against the superiority of oral misoprostol over the vaginal form of that considering the patient's satisfaction [9].

Since there is no fixed standard regimen for the treatment of missed abortion and because the way of treatment can affect the patients emotionally, further studies can be helpful. So in this study, we investigated the effectiveness of 800 mcg oral misoprostol versus vaginal misoprostol in a tertiary care hospital in India. Also, patients' satisfaction treated with vaginal versus oral misoprostol was compared at the time of discharge and in a follow-up session.

Material and Methods

Study Design

The study was a non-interventional prospective trial conducted in Sapthagiri hospital, Bangalore. Women who were eligible for the study were thoroughly counselled, and informed consent was taken orally.

Inclusion and exclusion criteria

Patients were recruited for the study based on the following inclusion criteria:

1. Females of age group 18 to 45 years, 2. Women with a gestational age of < 13 weeks of gestation from LMP, 3. Diagnosis of missed abortion by USG, 4. Mild vaginal bleeding or spotting or no bleeding and spotting at all, 5. Close cervix on pelvic examination, 6. Haemoglobin ≥ 9 gm/dl, 7. No history of asthma, liver disease on known allergy to misoprostol

Woman with any degree of cervical dilation, excessive uterine bleeding, twin gestation sac, molar pregnancy, BP ≥ 160/ 90 mmHg, signs and symptoms of infection, long-term corticosteroid therapy, and patients with high risk of uterine rupture and women who refused compliance with follow up schedule are excluded from the trial.

During a one-year study from September 2016 to September 2017, we enrolled 100 women, each with a documented missed abortion < 13 weeks of gestation through ultrasound examination. The subjects were administered mifepristone on day one followed by oral or vaginal misoprostol on day 3. Out of 100, 42 patients received 200 mcg of vaginal misoprostol, and 58 subjects received 200 mcg of oral misoprostol every four hours up to four doses. The patients were examined for complete expulsion of uterine content. If complete expulsion did not occur 12 hours after the last dose, the surgical evacuation was done.

Study Procedure

All patients were monitored for vaginal bleeding and expulsion of uterine content. In case of any expulsion, the POCs were examined by the gynaecologists. Also, a bimanual pelvic examination was performed to determine any retained gestational material. If complete abortion occurred before the completion of all doses, the next doses were not given.

Clinical outcomes had been considered before the initiation of the trial as:

- The effectiveness of trail had been defined as the expulsion of uterine content completely without the need for surgery.

- Failure was defined as the need for surgery for completing the course of treatment.

Clinical outcomes were recorded 12 hours after the last dose of misoprostol. The surgical evacuation was done in case of severe pain, infection, heavy vaginal bleeding or failure of complete expulsion of POCs after administration of the last dose of misoprostol.

Subjects were observed for 12 hours after complete abortion and then discharged. All women were then asked to return to hospital 14 days after discharge for examination with USG to make sure that there was no retention of any conception product in the uterine, also for assessing their satisfaction. The subjects were asked to fill a multiple- choice questionnaire by themselves at two-time points: one at the time of discharge from hospital and one at follow-up session 14 days after their discharge from the hospital.

Statistical analysis

The data were recorded in mean ± SD. Statistical significance was determined by Chi-square test for complete evacuation and patient satisfaction. $P < 0.05$ was considered statistically significant. The SPSS 16.0 statistical package was used for analysing the data.

Results

As mentioned in Table 1, baseline characteristics of both groups in terms of age, parity and period of gestation were comparable.

Table 1: Characteristics of the patients

Characteristics	Vaginal group (n = 42)	Oral group (n = 58)
Age (years)		
18-27	14 (33.33)	20 (34.48)
28-37	22 (52.38)	30 (51.72)
38-45	6 (14.28)	8 (13.79)
Mean ± SD	32.41 ± 3.52	34.61 ± 3.14
Parity		
1	8 (19.04)	11 (18.96)
2	10 (23.80)	14 (24.13)
3	20 (47.61)	25 (43.10)
4	4 (9.52)	8 (13.79)
mean± SD	2.47 ± 0.90	2.51 ± 0.95
Gestation duration (weeks)		
5-6	1 (2.38)	0 (0)
7-8	9 (21.43)	23 (39.66)
9-10	22 (52.38)	17 (29.31)
11-12	10 (23.81)	18 (31.03)
Mean ± SD	9.35 ± 1.34	9.34 ± 1.52

Values are given a number or number (percentage) unless otherwise indicated.

There was no significant difference between oral and vaginal route (Table 2) in success of treatment ($\chi 2 = 0.008$; P = 0.928; df = 1).

Table 2: Effectiveness of vaginal and oral misoprostol in the treatment of missed abortion

	Success	Failure	$\chi 2$ (p-value)
Vaginal	33 (78.57)	9 (79.31)	0.008
Oral	46 (21.43)	12 (20.69)	(P= 0.928)

Values are given a number or number (percentage) unless otherwise indicated.

There was no significant difference of patient's satisfaction between oral and vaginal misoprostol (Table 3) at time of discharge ($\chi 2 = 0.0162$; P = 0.991; df = 2).

Table 3: Patients satisfaction at the time of discharge

	Satisfied	Unsatisfied	Don't know (P value)	$\chi 2$
Vaginal	27 (64.29)	6 (14.29)	9 (21.43)	0.016
Oral	38 (65.52)	8 (13.79)	12 (20.69)	(0.991)

Values are given a number or number (percentage) unless otherwise indicated.

There was no significant difference of patient's satisfaction between oral and vaginal misoprostol (Table 4) at follow-up session ($\chi 2 = 4.822$; P = 0.897; df = 2).

Table 4: Patient satisfaction at a follow-up session

	Satisfied	Unsatisfied	Don't know $\chi 2$ (P value)
Vaginal	31 (73.81)	2 (4.76)	9 (21.43)
Oral	49 (84.48)	5 (8.62)	4.82 (0.897) 9 (6.90)

Values are given a number or number (percentage) unless otherwise indicated.

The complete abortion rate in the vaginal group was 78.57%, while it was 79.31% in the oral group. The abortion rate was higher in the oral group. However, the difference was not statistically significant (P = 0.928)

The questionnaires filled by the patients at two times of discharge showed 64.29% patients' satisfaction for vaginal treatment and 65.52% for oral treatment. But the difference was not significant statistically (P = 0.991). Patients' satisfaction for both groups increased at follow-up session and at this time point the result of patients' satisfaction of oral treatment (84.48%) was higher than that of the vaginal group (73.81%), but the difference was not statistically significant (P = 0.897).

Discussion

Misoprostol is an effective agent commonly used in the treatment of miscarriages but in spite of the studies that have been conducted the optimal dose and route of administration of misoprostol have not been determined yet [5]. Studies showed that the degree of absorption of misoprostol and its effect on uterine contractility after vaginal misoprostol was more long-lasting and more continuously increasing uterine contractility comparing to the time when oral misoprostol was administered After vaginal administration [3]. The difference between AUC values of orally and vaginally administered misoprostol is likely due to pre-systemic gastrointestinal or hepatic metabolism of oral misoprostol that will not happen in vaginal route [12]. So higher efficacy of vaginal misoprostol is expected due to the greater bioavailability of this route.

However, in spite of the logical explanation that vaginal misoprostol may be more effective than oral misoprostol due to their pharmacokinetic differences, in our study, there was no statistically significant difference in terms of response to treatment between oral misoprostol and vaginal misoprostol (P = 0.928). Although there are some studies that their results are contrary to our findings [1], [8], [13], [14], [15], [16], [17], there are some studies that support our findings [9], [18].

It was stated that age of gestation could influence the effectiveness of oral and vaginal misoprostol [27], but in our study, the subjects of two groups were very similar in the gestation duration (mean ± SD = 9.35 ± 1.34 for the vaginal group and mean ± SD = 9.34 ± 1.52 for the oral group).

Patient satisfaction is an important factor to be considered in the treatment of missed abortion because except the physical pain that these patients have, they are emotionally involved because of the loss of their child. They also suffer grief and depression [9]. Our study does not show a significant difference between patients' satisfaction of vaginal group and the oral group at the time of discharge (p = 0.991). Patients' satisfaction result at the second time point has increased for both groups (73.8% for the

vaginal group and 84.48% for the oral group). But still, the difference between the satisfaction of subjects of both groups is not statistically significant (P = 0.897).

From the results that we have obtained, we can infer that the emotional status of the patients in both groups may affect their response while completing the questionnaire at the time of discharge. It can be claimed so because the patient's satisfaction in both groups has increased after 14 days which is a good time for coming out of their grief. It has been stated that due to less privacy of vaginal misoprostol, patient show less satisfaction towards this route of administration [14] but our study is not in line with this finding because both groups of vaginal and oral misoprostol showed similar satisfaction towards their treatment. This similarity in the satisfaction of the subjects can be justified by considering the effectiveness of the treatment by these routes. As the difference in the effectiveness of both vaginal and oral misoprostol was not statistically significant, we can conclude that the success of the treatment is one important factor affecting patient satisfaction.

Therefore, it can be concluded that there is no difference between the vaginal and oral route of administration of misoprostol in the success of the treatment and patient satisfaction when used in the treatment of missed abortion.

The small sample size was one of the limitations of this study. Also, this study was not a controlled one. Further double-blind controlled studies with larger sample size are needed to elucidate the optimal route of misoprostol and patient satisfaction.

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The Association between the Level of Antithrombin III and Mortality in Children with Sepsis

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Abstract

BACKGROUND: Sepsis is a significant cause of morbidity and mortality in children. The diagnosis of sepsis remains continuing to develop which determines treatment and prognostic. Antithrombin III is one of the coagulation markers to evaluate the prognosis of sepsis.

AIM: To determine the association between the level of antithrombin III and mortality in children with sepsis in the Pediatric Intensive Care Unit, Haji Adam Malik General Hospital, Medan.

METHODS: A cross-sectional study was conducted in Haji Adam Malik General Hospital, Medan from April until June 2015. There were 41 children with sepsis. Sepsis was diagnosed from clinical and laboratory findings. Complete blood count, antithrombin III level, C-reactive protein and procalcitonin were an indicator of unproven sepsis that performed in the laboratory. Meanwhile blood culture was performed in the microbiology laboratory. The association between the level of antithrombin III and mortality was analysed by using chi-square test.

RESULTS: Of the 41 participants, the low antithrombin III level was 13 of 41 children (31.7%) meanwhile the normal antithrombin III level was 28 of 41 children (68.3%). There was 8 of 13 (42.1%) and 11 of 28 (57.9%) children in death cases of low and normal antithrombin III level, respectively. Samples with low antithrombin III level had 2.473 higher risk mortality than normal antithrombin III level (P = 0.184; 95% CI 0.641 to 9.5421; PR = 2.473).

CONCLUSION: There was no statistically significant association between levels of antithrombin III and mortality in children with sepsis.

Introduction

Sepsis is one of the causes of mortality amongst children worldwide. It is defined as systemic inflammatory response syndrome (SIRS) which caused by infection either proven by clinical examination, blood culture or septic markers [1], [2], [3]. The incidence of sepsis was 0.56 cases per 1000 children per year, the highest amongst infants (5.16 per 1000) and decreased with ages (0.20 per 1000 among 10-14-year-olds) [4]. The development of diagnostic of sepsis is the main key to treatment and prognostic determination [5], [6].

Antithrombin III is an inhibitor of thrombin-mediated vascular injury in the microcirculation during severe sepsis. This endogenous anticoagulant is rapidly depleted in the early phases of sepsis as a result of decreased synthesis, increased destruction, and enhanced clearance by thrombin-antithrombin complex formation [7]. Examination of antithrombin III (ATIII) will be able to be an objective and reliable examination in detecting sepsis circumstances, in determining the severity and differentiate various possible causes of sepsis and can be used to measure the response to treatment [5], [6].

A study in Turkey, 2007, showed that the value of initial antithrombin III (ATIII) level could be used as a prognostic factor in neonatal sepsis. From this study was reported that the initial antithrombin III (ATIII) level decreased in patients with sepsis and could predict mortality [7].

However, the antithrombin III level, as a prognostic factor, in children has never been reported; this is the background of this study to determine the association between antithrombin III (ATIII) level and mortality in children with sepsis.

Methods

Study Design

A cross-sectional study was conducted to determine the association between the level of antithrombin III and mortality in children with sepsis Pediatric Intensive Care Unit, Haji Adam Malik General Hospital, Medan, North Sumatera, Indonesia from April until June 2015. Sepsis was diagnosed with by clinical examination, blood culture or septic markers. The inclusion criteria are children with sepsis from 6 months to 18 years old.

The exclusion criteria are patients with blood disorders because the level of antithrombin III was decreased in blood disorders. Samples were obtained by consecutive sampling. Data were analysed by statistical software, and the result was presented in tables. This study was approved by the Health Research Ethical Committee, Medical School, University of Sumatera Utara.

Sample Recruitment

All subject who fulfilled the inclusion criteria were enrolled in this study. Data patients about sex, mean age, body weight, stature, nutrition status, data laboratory (Routine blood, antithrombin III, C-reactive protein (CRP), procalcitonin and blood culture) were collected from medical records.

On the first day of treatment, all subjects were suspected as sepsis based on clinical findings; samples had laboratory tests such as routine blood, antithrombin III, C-reactive protein (CRP), procalcitonin and blood culture. Antithrombin III (ATIII) level is part of the coagulation examination. Blood was taken at least 1.8 mL from the cubital vein. The blood inserted into a citrate tube and transported to the laboratory according to the Standard

Operating Procedure. Examination of ATIII level is carried out using Teco Coatron AT III. The 75-150% value of antithrombin III (ATIII) is a normal category, and the < 75% value of antithrombin III (ATIII) is a low category [8]. Samples were followed-up until death or discharged from the paediatric intensive care unit.

Statistical Analysis

The association between the level of antithrombin III and mortality in children with sepsis was analysed using chi-square with a 95% confidence interval and P-value < 0.05 was considered significant.

Results

Of 41 patients were enrolled in this study. Baseline characteristics samples were described in Table 1. shows the baseline characteristics of patients, including sex, mean age, body weight, stature, nutrition status, data laboratory (Routine blood, antithrombin III, C-reactive protein (CRP), procalcitonin and blood culture). There was no association between sex, mean age, nutrition status, CRP, blood culture, mean leukocyte, mean platelets, mean procalcitonin to mortality in this study. There was an association (p = 0.024) between mean haemoglobin in the death group (10.26 g/dl) and life group (11.54 g/dl).

Table 1: Baseline characteristics samples

Characteristics	Mortality	Death Life	p
Sex (n%)			
Boys	12 (52.2)	11(47.6)	0.397[a]
Girls	7 (38.9)	11(61.6)	
Mean Age (years)	7.95 (5.49)	8.71 (5.38)	0.656[b]
Nutritional Status (n%)			
Malnutrition	4 (66.7)	2 (33.3)	0.390[d]
Normal	15 (42.9)	20 (57.1)	
CRP (n%)			
Positive	18 (48)	19 (51.4)	0.610[d]
Negative	1 (25)	3 (75)	
Blood Culture (n%)			
Positive	1 (20)	4 (80)	0.350[d]
Negative	18 (50)	18(50)	
Mean Haemoglobin(g/dl)	10.26 (2.17)	11.54 (1.26)	0.024[b]
Mean leukocyte (mm^3)	15.165 (9567)	14.927 (5270)	0.979[c]
Mean Platelets (mm^3)	276.173 (188.333)	222.256 (129.778)	0.488[c]
Mean Procalcitonin	9.09 (13.44)	15.56 (41.86)	0.875[c]

[s] Chi-Square; [b] T independent; [c] Mann Whitney; [d] Fisher's Exact.

Table 2: Association between the level of antithrombin III and mortality

Antithrombin III	Mortality		P	PR	95% CI
	Yes	No			
Low	8 (42.1)	5 (22.7)	0.184	2.473	0.641-9.542
Normal	11 (57.9)	17 (77.3)			

Chi-square test was used to determine the association between the value of antithrombin III (ATIII) and mortality in children with sepsis (Table 2). Antithrombin III was classified as normal if the level of antithrombin III was 75% until 150% and low if the level of antithrombin III was < 75% [8]. According to the test's result, there was no statistically significant association between levels of antithrombin III and mortality in this study (p = 0.184).

Discussion

Sepsis is a group of signs of systemic inflammation due to infection based on clinical

examination or proven by blood culture and septic markers [1], [2], [3]. Sepsis is one of the common reason for mortality in infants and children Who reported that 70 per cent of 8 million mortality of children under five years old in developing countries was caused by infectious diseases in which ended up to sepsis [4]. In 2007, Indonesia Basic Health Research reported that sepsis was the most common reason for infant mortality (20.5%) [9].

The sepsis diagnostic approach is currently to determine early signs of sepsis, evaluate treatment and make prognosis [5], [10].

Antithrombin III is a useful prognostic marker in sepsis, due to its potential inhibitor of thrombin which occurs when there is a vascular injury in microcirculation during the process of infection. Many previous studies have reported that initial AT levels of patients with sepsis who developed septic shock and DIC are an indicator of prognosis and cases with very low AT levels have a higher mortality rate [7]. A study in Europe, 2006, showed that low activity of antithrombin III was correlated with severity and survival rate in patients with sepsis [11].

A study in Korea, 2014, showed that antithrombin and protein C were strong prognostic markers patients with DIC, sepsis or severe sepsis [12]. The same conclusion also found in a study in Thailand, 2006, where the reduced level of protein C, protein S and antithrombin might predict the survival rate of gram-negative sepsis due to Burkholderia pseudomallei [13].

This study shows that there is no statistically significant association between levels of antithrombin III and mortality in this study (p = 0.184). Low level of antithrombin III in the subjects who died was 8 subject (42.1%) and with normal levels were found in 11 subject (57.9%). Although not statistically significant, there showed that subjects with a low level of antithrombin III in patients with sepsis had 2.473 higher risk for caused mortality compared with the normal level of antithrombin III (P = 0.184; 95% CI 0.641 to 9.5421; PR = 2.473).

The limitation of this study did not compare to other haematology markers examinations, so it could not evaluated that specificity and sensitivity. This study was needed more fourth researches to determine the level of antithrombin III as a prognostic marker in children sepsis.

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Clinical Science

Investigation of Frequency of the Lethal Triad and Its 24 Hours Prognostic Value among Patients with Multiple Traumas

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Abstract

BACKGROUND: Death in multiple trauma (MT) patients is one of the serious concerns of the medical service provider. Any prediction of the likelihood of death on the assessment of the patient's condition is performed using different variables, one of the tools in the triage of patients to determine their condition.

AIM: We aimed to investigate the frequency and the predictive value of death in 24 hours triad of death in patients qualified with multiple traumas admitted to Imam Khomeini hospital.

METHODS: This was a prospective cross-sectional study to determine the prevalence and predictive value of 24-hour triad of death among patients with MT referred to an emergency department. Three factors including acidosis, hypothermia and coagulopathy and predictive value of 24-hour death were evaluated. Arterial blood gas, oral temperature and blood samples for coagulation factors were analysed. Data were analysed using SPSS version 19. Multivariate analysis (logistic regression) was used to determine the predictive value of the triad of death.

RESULTS: A group of 199 MT patients referring to Imam Khomeini hospital during the first 6 months of 2015 were evaluated for the first 24 hours of admission. Logistic regression analysis showed that using the following formula based on the triad of death can predict death in 96% of cases can be based on the triad of a death foretold death upon admission to the emergency room. It should be noted that this prediction tool as 173 people left alive after 24 hours as live predicts (100% correct).

CONCLUSION: The triad of death is one of the tools in the triage of patients to determine their condition and care plan to be used, provided valuable information to predict the prognosis of patients with a medical team.

Introduction

Death in multiple trauma patients referring to hospitals is one of the serious concerns of the medical service providers. For this reason, any prediction of the likelihood of death on an assessment of the patient's conditions performed using different variables, one of the tools in the triage of patients to determine their condition and care planning is used and as well as valuable information to predict the prognosis of the treatment team [1].

Trauma deaths divide into three categories: immediately at the scene, within the first 24 hours during initial resuscitation, and in the next 3 to 4 weeks as a result of multiple organs failure. Failure to resuscitate adequately in the emergency department can lead to acidosis, hypothermia, and coagulopathy, which can result in multiple organs failure and cause death in these patients [2], [3].

Major trauma patients may develop a tendency to bleeding diathesis, which results in defective clotting and platelet function. If patients require > 10 units of packed red blood cells (PRBC),

patients should receive PRBC in a 1:1 ratio with fresh frozen plasma (FFP). Both acidosis and hypothermia contribute to the coagulopathy and should be corrected as soon as possible [4], [5].

The fundamental problem in patients, who need damage control procedures, is the shock due to trauma. This is due to hypovolemia caused by bleeding and tissue damage caused by a large amount of energy transmitted through the affected organism. The shock caused by trauma leading to hypoperfusion and inflammatory cascade is activated. Metabolic acidosis and hypothermia result in worse induced coagulopathy or loss of coagulation factors. Although the triad of hypothermia, acidosis and coagulopathy may be fatal in certain circumstances, one or more of this death may play a protective role.

A lot of press (publications) has supported the notion that significant hypothermia may protect the organism from severe hypoperfusion. Oxygen dissociation curve is shifted to the right with acidosis. This allows better oxygen removal in the tissue [6]. The clotting cascade is comprised of serine proteases whose activity is pH dependent. It has been widely established that acidosis is a common consequence of acute injury (locally and systemically).

It is also known that severe acidosis impairs the efficiency of the clotting cascade. Thromboelastography (TEG) is a sensitive means of assessing the interaction of all parts of the clotting cascade as they work in concert. In a large trial of general surgery patients undergoing large volume blood loss surgery (500 cc), the presence of hyperchloremic acidosis correlated with the development of coagulopathy. Exvivo data evaluating the impact of high chloride solutions on the TEG profile also indicates clotting dysfunction when the serine proteases are in a Cl2 rich and academic environment [7].

Coagulability is destroyed with hypothermia. So despite normal PT and PTT, clinical coagulability may exist. Often coagulability relief when the patient is again rewarmed, although some cases of DIC rarely reported. Multi factors play a role in impairing the coagulability; consisting of blood concentration, vessel constriction and releasing of tissue thromboplastin from cold ischemic tissue. Deposited fibrinogen due to hypothermia may increase the risk of cardiac and cerebral thrombosis. Hypothermia induces bone marrow suppression and splenohepatic sequestration. This decreases platelet and leukocyte. Leucopenia and thrombocytopenia relieve when the patient is again rewarmed [8].

We aimed to investigate the frequency and the predictive value of death in 24 hours triad of death in patients qualified with multiple traumas admitted to Imam Khomeini hospital.

Methods

Study design

The present cross-sectional and prospective was carried out on the triad of death to determine the prevalence and predictive value for 24 hours in multiple trauma patients admitted to ED of Imam Khomeini Hospital, Sari, Mazandaran, Iran. The duration of the study was in the first 6 months of 2015. Protocol of this study was approved by the ethics committee of Mazandaran University of Medical Sciences after evaluation in the research council of emergency medicine specialists group. Registration thesis number is 870 at Mazandaran University of Medical Science. To maintain the confidentiality of patients' medical profile data and to adhere to ethical practice, the researchers keenly adhered to the principles introduced in the declaration of Helsinki during the study period. Information regarding the study method was given to the participants, and written consent was obtained from them before being included in the study.

Participants

The subjects in the present study consisted of patients with Multiple Trauma were enrolled due to the inclusion and exclusion criteria, who had referred to the emergency department (ED). The patients' GCSs was recorded on admission time and the mean systolic blood pressure of patients at presentation measured. In this study, the median heart rate of patients on admission accounted for the average number of respiratory rate, the median oxygen saturation (O_2sat). Among the vital signs upon arrival, only the respiratory rate had a significant correlation with the mortality rate.

Data collection

A senior emergency medicine resident was responsible for gathering data of the patients by completing a pre-designed checklist including baseline characteristics (Age, sex**,** GCS**,** systolic blood pressure, Heart Rate, Number of respiratory rates, O_2sat).

Considering that approximately 400 multiple trauma patients in Imam Khomeini hospital in Sari employ and with the prevalence of 25 to 35 per cent of the triad of death, And by taking a 30% prevalence rate for the calculation of sample size and accuracy of 4.5%, The number of samples required to determine the prevalence of the triad of death will be about 200 people. The three factors triad of the death of the patients was being evaluated at the beginning and six hours later. The first factor, acidosis, in the PH range of less than 7.36 and less than 7.15 were being assessed. The second factor is coagulopathy for

which PT; PTT & PLT were be used. The third factor is hypothermia. Follow-up of these patients will be done by the investigator (Emergency Medicine Resident), and a questionnaire was be completed by him.

Statistical Analysis

The sample size was determined based on presenting with multiple trauma patients. After data collection, the data were statistically analysed using SPSS 21 statistical software. The data describe the mean and standard deviation for quantitative variables and the number and percentage for qualitative variables were being used. To determine the predictive value of the triad of death, multivariate analysis (logistic regression) was to being used. The significance level was 0.05.

Results

In this study, 199 multiple trauma patients referring to Imam Khomeini hospital emergency department during the first 6 months of 1394 were evaluated for the first 24 hours of admission. Patients included 155 males (77.9%) and 44 women (22.1 per cent).

The average age for men was 35.5 years (SD Rules 15 years), with a minimum of 18 and a maximum of 60 years. The average female patient age was 35.6 years (SD Rules 13.8 years), with a minimum of 13 and a maximum of 60 years. Among male patients, 21 patients (13.5%) and among female patients, 5 patients (11.4%) died (Table 1).

Table 1: Gender and Death 24h Cross tabulation

			Death 24h		Total
			Alive	Death	
Gender	Female	Count	39	5	44
		% within Gender	88.6%	11.4%	100.0%
	Male	Count	134	21	155
		% within Gender	86.5%	13.5%	100.0%
Total		Count	173	26	199
		% within Gender	86.9%	13.1%	100.0%
		% within Death24h	100.0%	100.0%	100.0%

Using this formula, 19 cases predicted as mortality (73.1%) and 7 cases predicted as alive (26.9%). It should be noted that this prediction tool as 173 people left alive after 24 hours as live predicts (100% correct) (Table 2)**.**

Table 2: Death 24h Statistics

Death 24h		Age	SBP	GCS	PT	PTT	PLT	pH	T	WBC	Hb
Alive	N Valid	173	173	173	173	173	173	173	173	173	173
	Mean	35.02	117.77	14.32	12.14	30.06	238438	7.35	36.676	11097	12.72
	Median	30.00	120.00	15.00	12.00	30.00	242000	7.34	37.000	10500	12.90
	Std. Deviation	13.640	18.051	2.259	.419	2.666	75339	.048	.4940	4143	1.764
Death	N Valid	26	26	26	26	26	26	26	26	26	26
	Mean	39.46	79.81	5.23	26.93	75.77	109307	7.19	35.081	20215	8.53
	Median	37.50	67.50	5.00	27.00	64.00	90000	7.18	35.000	18350	8.50
	Std. Deviation	16.090	27.477	2.612	10.168	29.144	46063	.103	.1167	7359	.925
	P-VALUE	0.198	< 0.001	< 0.001	*< 0.00 1*	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

SBP = Standard Blood Presure; GCS = Glasgow Coma Scale; PT = Prothrombin Time; PTT = Partial Thromboplastin Time; PLT = Pelatelet; T = Temperature; WBC = White Blood Cell; Hb = Hemoglobin.

In this study, 26 patients (13.1%) died; of which all (100%) hypothermic 173 people were survived out of which 2 cases (1.2%) were hypothermic. The Difference was statistically significant (p-value <0.0001) (Table 3).

Table 3: Hypothermia Statistics

Hypo-thermia		Age	SBP	GCS	PT	PTT	PLT	pH	T	WBC	Hb
No Hypo-thermia	Valid	171	171	171	171	171	171	171	171	171	171
	Missing	0	0	0	0	0	0	0	0	0	0
	Mean	35.09	117.83	14.43	12.13	30.04	238952	7.35	36.693	11112	12.76
	Median	30.00	120.00	15.00	12.00	30.00	242000	7.34	37.000	10500	12.90
	Std. Deviation	13.694	17.901	2.020	.396	2.665	749025	0.048	0.4696	4156	1.744
Hypo-thermia	Valid	28	28	28	28	28	28	28	28	28	28
	Missing	0	0	0	0	0	0	0	0	0	0
	Mean	38.75	82.14	5.21	25.93	72.64	115392	7.20	35.089	19475	8.63
	Median	36.00	72.50	5.00	24.00	64.00	90000	7.19	35.000	15500	8.50
	Std. Deviation	15.773	28.785	2.573	10.448	30.307	56157	0.108	0.1286	7617	0.990
	p-value	0.278	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

SBP = Standard Blood Presure; GCS = Glasgow Coma Scale; PT = Prothrombin Time; PTT = Partial Thromboplastin Time; PLT = Pelatelet; T = Temperature; WBC = White Blood Cell; Hb = Hemoglobin.

In this study, 26 patients (13.1%) died. Among them, 25 patients (96.2%) had PH <7.36.173 patients (86.9%) survived, of which 114 (65.9%) had PH <7.36, respectively**.** The difference was statistically significant (p-value = 0.002) (Table 4).

Table 4: Acidosis and Death 24h Cross tabulation

			Death 24h		
			Alive	Death	Total
Acidosis	>7.36	Count	59	1	60
		% within Acidosis	98.3%	1.7%	100.0%
		% within Death24h	34.1%	3.8%	30.2%
	<7.36	Count	114	25	139
		% within Acidosis	82.0%	18.0%	100.0%
		% within Death24h	65.9%	96.2%	69.8%
Total		Count	173	26	199
		% within Acidosis	86.9%	13.1%	100.0%
		% within Death24h	100.0%	100.0%	100.0%

Using this formula, 19 cases predicted as mortality (73.1%) and 7 cases predicted as alive (26.9%). It should be noted that this prediction tool as 173 people left alive after 24 hours as live predicts (100% correct).

Discussion

In this study, the frequency and the predictive value of death in 24 hours triad of death were investigated in patients qualified with multiple trauma admitted to Imam Khomeini hospital. This centre has so far studied to determine the frequency of the triad of death which has not been done in patients with multiple trauma. Due to this fact that in sari's Imam Khomeini hospital, there is no registry trauma unit and to provide more accurate services to multiple trauma patient, it is helpful to determine the severity of injury, in this study, we investigated patients with Multiple trauma for the presence of three factors of acidosis, hypothermia and coagulopathy and aimed to find the relationship between these factors and fatality rate among Patients with Multiple Trauma [9], [10]. Trauma deaths divide to three categories: immediately at the scene, within the first 24 hours during initial

resuscitation, and in the next 3 to 4 weeks as a result of multiple organs failure [11], [12]. Failure to resuscitate adequately in the emergency department can lead to acidosis, hypothermia, and coagulopathy, which can result in multiple organs failure and cause death in these patients [6], [13]. Most hospitals obtain a standard panel of labs in every trauma patient, although in many cases, these have little impact on the initial management [14], [15]. Critical labs in patients with major trauma include a baseline hematocrit, platelet count, blood clot for typing, pregnancy test, and coagulation panel. Laboratory evaluation of the trauma patient can provide an objective measure of the adequacy of resuscitation. It also provides much-needed information for proper transfusion products and the onset of coagulopathy.

The fundamental problem in patients, who need damage control procedures, is the shock due to trauma. This is due to hypovolemia as a result of bleeding and tissue damage caused by a large amount of energy transmitted through the affected organism. The shock caused by trauma leading to hypoperfusion and inflammatory cascade is activated. Metabolic acidosis and hypothermia result in worse induced coagulopathy or loss of coagulation factors. Although the triad of hypothermia, acidosis and coagulopathy may be fatal in certain circumstances, one or more of these deaths may play a protective role 15. We have in contrast to previous studies, patients were 13-60 years.73.1% of the victims had platelet disorders, 100 per cent had abnormal PTT, and 92.3% had abnormal PT. This is in line with the results of previous studies 16 and unfortunately of 199 patients with multiple trauma triad of death, 26 people died within the first 24 hours from the moment of admission, also the primary systolic blood pressure mean was significantly low in victims [17], [18]. Similar results are found in study investigation the significant relation between anaemia and mortality and all of the patients with multiple traumas were anaemic because this study determined that Trauma is a serious global health problem, accounting for approximately one in 10 deaths worldwide. Uncontrollable bleeding accounts for 39% of trauma-related deaths and is the leading cause of potentially preventable death in patients with major trauma. While bleeding from vascular injury can usually be repaired surgically, coagulopathy-related bleeding is often more difficult to manage and may also mask the site of vascular injury. The causes of coagulopathy in patients with severe trauma are multifactorial, including consumption and dilution of platelets and coagulation factors, as well as dysfunction of platelets and the coagulation system. The interplay between hypothermia, acidosis and progressive coagulopathy, referred to as the 'lethal triad', often results in exsanguinations [19].

Similar results are found in study investigation the low primary systolic blood pressure, low GCS score and coagulopathy were associated with increased mortality [20], [21], [22].

We have in contrast to previous studies the mortality rate was 100% in patients with impaired PTT and patients who were impaired in PT and patients with platelet disorders were 77.4% and 79.2%, respectively and there was no significant correlation between age and mortality. The mean GCS for women was 13.98 (SD = 2.88). The mean GCS in men was 12.9 (SD = 4.04). The difference was statistically significant (p-value = 0.048) [23], [24], [25].

Finally, this research showed us the necessity of having a trauma registry in Sari's Imam Khomeini hospital which can be a great help in evaluating of patients with multiple trauma who have the factors with high predictive value for the 24-hour death. Identification of abnormal levels of these factors in the time and effort to correct it within 24 hours of acceptance had a major role in preventing the premature death of the patients. Some limitations was in this study.

In conclusion, this research could be the basis for further studies to examine the effects of these factors on the rate of mortality in patients with Multiple Trauma.

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Author' s Contribution

All authors passed four criteria for authorship contribution based on recommendations of the International Committee of Medical Journal Editors.

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Family Physicians' Awareness of Autism Spectrum Disorder: Results from a Survey Study

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Abstract

AIM: Autism spectrum disorder (ASD) is a common neurodevelopmental disorder in children. Family physicians with the first medical contact of children are among the most frequent physicians with ASD. We aimed to investigate family physicians' awareness of ASD.

METHODS: This study was carried out family physicians in between September 25-October 15, 2018. The questionnaire form on autism awareness prepared by the researcher was delivered to family physicians electronically and in printed form, and it was filled out by volunteers.

RESULTS: Forty-eight family physicians with an average professional experience of 16.9 ± 8.8 years participated in the study. A group of 66.7% of the participants had not previously received education on ASD, and 70.8% of them did not refer any child to child psychiatry with suspected ASD in the last 6 months. The participants stated that the most common clinical features in children with ASD were the inability to make eye contact (72.9%) and repetitive movements (47.9%), and 56.3% of them stated one or more features that are not observed in ASD. The compliance of the participants' answers about the clinical features observed in children with ASD with the DSM-5 criteria was determined to be 54.6 ± 18.4%. Significantly higher compliance rates were observed in the participants with education on autism and those working as a physician below 15 years.

CONCLUSION: In our study, family physicians' awareness of ASD was not found to be adequate. Education programs on autism awareness should be applied to family physicians who are probably the most frequently encountered physicians by children with ASD.

Introduction

Autism spectrum disorder (ASD) is a common neurodevelopmental disorder characterised by a permanent disruption in mutual communication and social interaction, repetitive behaviours, the field of interest or activities [1]. ASD has been thought to be developed by parental disinterest in the first years of its definition, and it has been understood to be a neurobiological disorder in the following years [2]. Also, recent studies have found many genetic disorders about ASD, and it has been found that ASD has a genetic basis [3], [4].

ASD appears to be one of the fastest increasing psychiatric disorder in children. In the current reports, its incidence has raised to 1 in 50 to 68 children [5], [6]. Among the reasons for this increase, changes in the diagnostic criteria have played an important role, but education and awareness works conducted in society have also contributed to this increase.

Current evidence indicates that interventions to increase the functionality of children with ASD are more effective in young ages and long term prognosis is better [7]. Therefore, training and awareness of healthcare professionals about ASD are crucial. In this study, we aimed to investigate autism awareness of family physicians that are probably first and most commonly contacted persons by autistic children.

Material and Methods

Study Population

The study was conducted with family physicians working in Edirne province of Turkey between September 25, 2018, and October 15, 2018. After receiving the necessary permissions from Edirne local health authority, a questionnaire form designed for the study was sent to family physicians working in Edirne province as electronic and printed documents. A total of 48 family physicians who accepted to participate between these dates were included in this study. Our study is a cross-sectional survey study, study protocol complies with the Declaration of Helsinki, and the study was approved by the local ethics committee.

Questionnaire Form

A 6-items questionnaire form for autism awareness was prepared by the researcher. This form included open-ended questions including “Specify 5 clinical features regarding autism”, “At which age are autistic patients are most commonly brought to a physician?” and “What is the most common cause of presentation to a physician in autistic patients?”. In addition to these questions, the questionnaire form also included the questions of “How many children you have referred to Child and Adolescent Psychiatry specialist within the last 6 months?”, “Have you ever received training about autism? If yes, do you think it was sufficient?” and “How many years do you work as a physician?”. To provide confidentiality of data and reliability of the study, age, gender and identity information was not involved in the questionnaire. Percentages of the five clinical features of autism stated by the responders that were involved in the ASD criteria in the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) textbook were calculated [1].

Statistical Analysis

Statistical analysis of this study was conducted using SPSS 16.0 (SPSS Inc., Chicago, IL, USA) software. Distribution of the variables was analysed with the Shapiro test. In the evaluation of the data, descriptive statistics were expressed as mean ± standard deviation, while categorical variables were given as a percentage (%). Comparison of continuous quantitative data between the two groups was made with a t-test. Correlation between the categorical variables was tested with Chi-square analysis. $P < 0.05$ values were considered statistically significant.

Results

A total of 48 family physicians with a mean professional experience of 16.9 ± 8.8 years. Of all participants, 66.7% were not trained for autism previously, 27.1% were trained, but they did not find the training sufficient, while 6.3% were trained and found the training sufficient (Figure 1). 70.8% of the participant physicians did not refer to any children suspected for autism to the child and adolescent psychiatrist, while 29.2% stated that they had referred 1 to 3 children for further investigations.

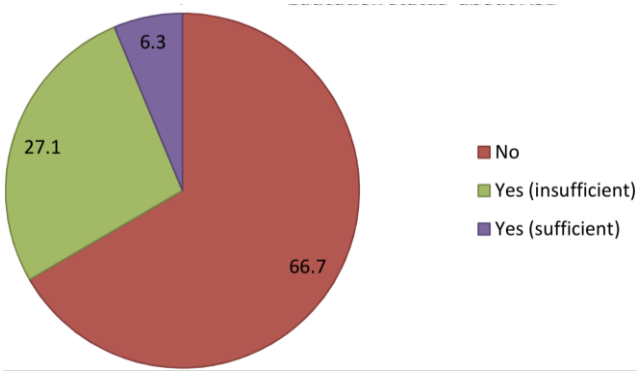

Figure 1: Participants’ status of previous training about ASD

Of the participants, 48% stated the first age when families brought autistic children as 2-3 years, and a substantial part of of the participants (37%) stated this period as 4-5 years of age (Figure 2).

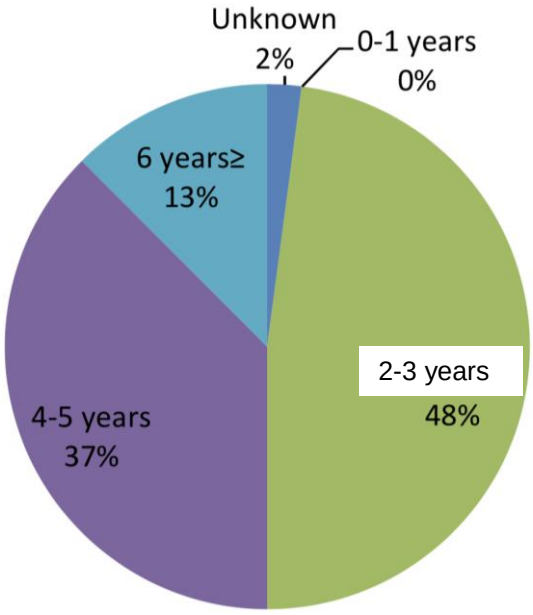

Figure 2: Distribution of age of the first presentation stated by physicians in ASD patients

The compliance of the answers given by the participants regarding five clinical features of autism with ASD diagnostic criteria of DSM-5 was found as 54.6 ± 18.4% (median: 60%). The most commonly

stated autistic features among the five clinical features of autism by the participants were the inability to make eye contact (72.9%), repetitive movements (47.9%), delayed speech (42.9 to 47.9%), and not responding to being called (41.7%) (Table 1).

Table 1: Distribution of the clinical features stated by physicians related to autism

Clinical Feature	n	%
Inability eye contact	35	72.9
Repetitive movement	23	47.9
Delayed speech development	23	47.9
Not responding to being called	20	41.7
Restricted peer relationship	11	22.9
Speech disorder	10	20.8
Inability to communicate	10	20.8
Indifference to the environment	8	16.7
Interest in rotating objects	7	14.6
Not playing with toys	5	10.4
Withdrawn	5	10.4
Obsession	3	6.3
Preferring to have a familiar routine	2	4.2
Avoids physical contact	1	2.1
Likes parts of the object	1	2.1
Unrest in the noise	1	2.1
Typical facial expression	1	2.1

Participants indicated multiple clinical features.

The most commonly given answers to the question about the most common causes of presentation in autistic children were delayed speech by 33.3%, inability to communicate by 18.8%, and inability to make eye contact by 14.6% (Figure 3).

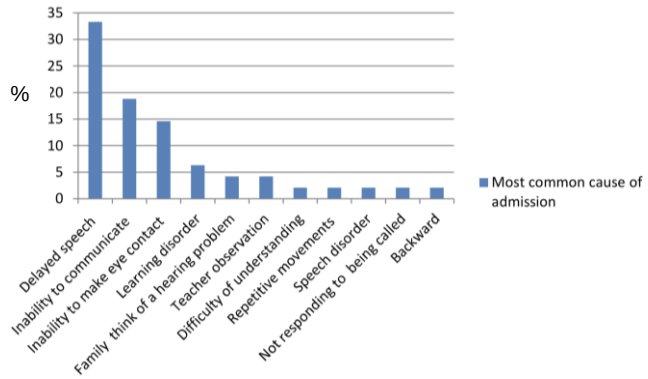

Figure 3: Distribution of the clinical findings stated by physicians as the most common cause of presentation in ASD patients

Of the participants, 56.3% (n = 27) stated one or more features that are not seen in autism, when they answer the question about five clinical features seen in autistic children. The most common answers that were not specific to autism included attention deficit disorder with hyperactivity by 34.6%, learning disability by 23.1%, singing by 11.5%, and irritability by 11.5%.

Compliance of the answers regarding clinical features of autism to DSM-5 criteria was compared between the physicians who were trained (n = 16) and the physicians who were not trained (n = 32), higher rate of conformity was observed in the trained physicians (68.7 ± 19.3 vs 56.2 ± 20, p = 0.04).

According to the duration of the work of the participants, physicians with a professional experience ≤ 15 years, which the number of participants was equal (n = 25), and those with a professional experience > 15 years were compared. Conformity of the answers regarding clinical features of autism to DSM-5 criteria was significantly higher in the physicians with a professional experience ≤ 15 years (68.7 ± 18.3 vs 52.2 ± 19.7, p = 0.006).

Compliance of the answers regarding clinical features of autism to DSM-5 criteria was compared between the physicians who have referred patients to child and adolescent psychiatrist within the last 6 months, and those have not referred, higher rate of compliance was found in the physicians who have referred patients (70 ± 23.2 vs 56.5 ± 18.1, p = 0.035).

Trained and untrained physicians about autism were compared, the trained physicians were found to refer more patients within the last 6 months with the presumed diagnosis of ASD (p = 0.004). No significant difference was found between the trained and untrained participants in terms of stating the age of presentation for autism as 2-3 years. Trained and untrained physicians were compared in terms of clinical features related to autism; trained physicians more commonly stated only limited peer relationship among typical findings of ASD, while no significant difference was found in other clinical features (Table 2).

Table 2: Comparison of some clinical variables stated by trained and untrained physicians about autism

Variables		Education (+) n (%)	Education (-) n (%)	p
Age of Autism diagnosis	2-3	7 (43.7)	16 (50)	0.68
	Other	9 (56.3)	16 (50)	
Patient referral within 6 months	Yes	9 (56.3)	5 (15.6)	
	No	7 (43.7)	27 (84.4)	0.004
Specified clinical features				
Avoiding eye contact	Yes	13 (81.3))	22 (68.8)	0.35
	No	3 (18.8)	10 (31.3)	
Repetitive movements	Yes	8 (50)	15 (46.9)	0.83
	No	8 (50)	17 (53.1)	
Delayed speech development	Yes	6 (37.5)	17 (53.1)	0.83
	No	10 (62.5)	15 (46.9)	
Not responding to being called	Yes	8 (50)	12 (37.5)	0.4
	No	8 (50)	20 (62.5)	
Restricted peer relationship	Yes	7 (43.8)	4 (12.5)	0.01
	No	9 (56.3)	28 (87.5)	
Inappropriate clinical feature	Yes	9 (56.3)	18 (56.3)	1
	No	7 (43.8)	14 (43.8)	

Discussion

Autism Spectrum Disorder is a complex, lifelong and heterogeneous neurodevelopmental disorder characterised by stereotyped and repetitive behaviors and disrupted social and communication skills [5], [6]. Whereas ASD has been etiologically linked to parent disinterest in the 1960s, today it is accepted as a neurologic disorder [2]. Many studies have been conducted in recent years about the genetics of ASD, and it has been demonstrated that

ASD has a significant genetic basis [3], [4]. In addition, there are evidence that age of parents, antenatal, prenatal and environmental factors make a contribution to the development of ASD [8], [9], [10], [11], [12].

Family physicians are the physicians found around places of family residences or the places where they can be easily reached, and to be first visited and who deliver protective healthcare services and first line diagnosis and treatment services [13]. Therefore, family physicians are the first healthcare professionals to be referred by children with ASD due to a symptom related to autism or due to any reason other than autism, especially protective healthcare services. Studies have found that first line physicians and pediatrists play a key role in the diagnosis of ASD [14], [15]. Therefore, autism awareness of family physicians is crucial for early diagnosis in children with ASD.

It was remarkable that in our study, two third of family physicians with a mean professional experience of 16.9 ± 8.8 years were not trained about ASD. Given that the incidence of ASD has been found as 1% in the current studies, it was remarkable that 70.8% of the family physicians have not referred patients suspected for ASD to the child and adolescent psychiatrists within the last 6 months [16].

An autism spectrum disorder is diagnosed with clinical findings at the presentation of the person and the history received from families. There is no pathognomic sign or laboratory test. Therefore, to establish the diagnosis, physicians must first know clinic symptoms well, assess clinical features of the child having autism, and listen to the family carefully [17].

In a study from India, two main features stated by pediatrists for the diagnosis of ASD were reported an an inability to make eye contact and social communication difficulties [18]. In our study also the most common feature stated by the participants was the inability to make eye contact. However, the feature stated by the participants as the most common cause of admission children with ASD to a physician was delayed speech. This result reflects that the reasons for admission children with ASD to a physician and the reasons for providing a physician to consider ASD in a child presented are different. Studies have found that the duration between onset of first symptom and referral of children with ASD to a physician by their families is longer [19]. Among the reasons for this, the most important is insufficient public awareness, recent studies show that public awareness about ASD is insufficient [20]. Other reasons include the fear of stigmatisation of the family, exposure to insensitive reactions due to misinformation of the society [19], [21]. Therefore, delayed speech and communication problems that are more regarded by families take an important place among the reason of referral to a practitioner [22].

In our study, a higher rate of responses given by the physicians with a professional experience ≤ 15 years about clinical features of ASD compared to those with a professional experience > 15 is a noteworthy finding. In a study by Sabuncuoglu et al., with family physicians, ASD scale scores were higher in physicians with longer duration of the profession. This was attributed to increased professional experience [23]. This study was conducted with family physicians residents in the education process with a median professional experience of 2-4 years and does not reflect family physicians working in the field. Similarly to our study, in their study with first-line physicians who had a mean professional experience of 14 years, Rahbar et al., found higher knowledge level about autism in the physicians with a professional experience < 5 years [24]. These results may be attributed to those physicians with longer duration in practice less follow the renewed criteria, less participate in educational activities, and experience more exhaustion.

There are data in the literature about low ASD awareness among first-line physicians [24], [25]. Also in a study from Turkey, ASD awareness of family practice residents was not found sufficient [17]. In another recent study, awareness of childhood autism of residents belonging to the non-neuropsychiatric disciplines has been moderate [26]. In our study, median conformity of ASD criteria stated by the participants to DSM-5 criteria was found as 60%, 54% of the physicians stated one or more symptoms unrelated to ASD, and 70.8% have not referred any patient suspected to have ASD, showing that autism awareness was not at the desired level also in experienced physicians working in the field. The significantly higher rate of features reported by the physicians trained for autism and those have referred patients with the presumed diagnosis of ASD within the last 6 months, supporting the necessity of ASD training in family physicians.

Symptoms begin between 0 and 3 years of life in ASD [27]. It is known that the diagnosis can be established in 2 years of age, and the American Pediatric Academy recommends screening at 2 years old [28]. However, the mean age of diagnosis was found as 5.7 in a study [29]. According to the American Centers for Disease Control and Prevention, the median age of diagnosis is 52 months [30]. Even it has been reported that more than 50% of children with ASD were diagnosed after 8 years of age [27]. In our study, about half of the physicians reported the age of the first presentation as 4 years and older. It has been shown that early diagnosis and early behavioural and social interventions in ASD significantly improved communication and social skills of these children [7], [15], [31], [32]. Therefore, awareness of family physicians on this issue was found to be insufficient.

In conclusion, our study indicated that a substantial part of family physicians was not trained

on ASD, and have not referred patients with the presumed diagnosis of ASD within the last 6 months. Conformity rate of the age of ASD diagnosis and clinical features related to ASD is not satisfactory. Awareness was significantly higher in the physicians trained in autism. Annual training programs covering early findings, clinical features, comorbidities associated with autism genetic basis and treatment program of ASD should be implements for family physicians. Also, the annual screening of children in the first 3 years may increase the rate of early diagnosis of autism.

Study Limitations: As the most important limitation, our study was cross-sectional and conducted with family physicians working in a single province, and some participants were relatively small. Questions of the questionnaire were prepared as open-ended by the researcher considering DSM-5 criteria to determine person specific answers, and this may cause limitation in the standardisation of some answers. Also, the number of questions was limited to provide privacy and not extend survey duration. The author proposes that questionnaires should be conducted, including more descriptive, accompanying comorbidities (such as epilepsy) about ASD. This study is a preliminary study. Further studies with larger series of participants including post-training comparisons are needed on this issue.

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Clinical Science

Retrospective Analysis of Skin Toxicity in Patients under Anti-EGFR Tyrosine Kinase Inhibitors: Our Experience in Lung Cancer

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Abstract

BACKGROUND: Tyrosine kinase inhibitors (TKIs) have been introduced for the treatment of lung cancer, improving progression-free survival, objective response rate, and quality of life. However, TKIs can lead to cutaneous toxicities, including papulopustular rash, xerosis, paronychia with/without pyogenic granulomas, scalp disorders, facial hair and/or eyelash growth.

AIM: In this study, we describe retrospectively all cases of mucocutaneous side effects in patients with lung cancer under TKIs referring to our outpatient for the skin care of oncological patients.

METHODS: We included patients referring from January 2016 to January 2018 affected by lung cancer and under TKIs. We collected data about the clinical exam, clinical photography, dermoscopy, histology and direct microscopic examination for each patient and we performed retrospectively descriptive analyses to assess whether a specific TKIs is linked significantly to particular cutaneous toxicity.

RESULTS: The majority of skin toxicities were due to afatinib, and the most common skin reaction was rash. We selected 60 patients with skin reactions, treated by TKIs for lung cancer. The majority of skin toxicities were due to afatinib (47/102 adverse reactions) and erlotinib (39/102). The most common skin reaction was rash (63% of patients), followed by xerosis (30%) and granulomas (30%). There was no significant relationship between a specific type of cutaneous reaction and specific EGFRi except for granulomas, developed more frequently in patients under afatinib ($p < 0.05$).

CONCLUSION: Most of our patients (63%) developed a cutaneous rash under TKIs. Most commonly afatinib was the drug involved, although it wasn't the most used EGFRi. Moreover, we noticed a significant correlation between afatinib therapy and appearance of granulomas.

Introduction

According to WHO data, lung cancer is the most common cause of cancer mortality (1.69 million deaths) [1].

The introduction of new therapeutic agents, with a different mechanism of action respect to traditional chemotherapy, led to a dramatic shift in patients' management [2]. In the last decades, several chemotherapeutic agents including target therapy have been introduced in the guidelines, impressively improving the survival rate of patients with lung cancer. Nowadays tyrosine kinase inhibitors (TKIs) have transformed the treatment of lung cancer, improving progression-free survival, objective response rate, and quality of life [3]. To date, three generations of TKIs are available: the first one includes gefitinib, erlotinib and icotinib; the second one afatinib, neratinib and dacomitinib; and the third one osimertinib, rociletinib and olmutinib [2].

However, TKIs can lead to several side effects. Skin toxicities are the most common and earliest reported. [4] Among them, papulopustular rash, xerosis, paronychia with/without pyogenic granulomas, scalp disorders, facial hair and/or eyelash growth frequently occur [5].

Studies suggest that cutaneous specificity for TKIs -associated adverse reactions might be due to the strong expression of EGFR [6] and to the multiple regulatory functions of EGFR/ligand system in the epidermis [7].

Currently, no studies exclusively on cutaneous toxicity of EGFRi in patients affected by lung cancer are available in recent literature.

Our study aims to describe retrospectively all cases of mucocutaneous side effects in patients with lung cancer under TKIs referring to our outpatient for the skin care of oncological patients.

Material and Methods

From January 2016 to January 2018, 263 patients referred to our outpatient for cutaneous side effects from oncological therapy. Seventy-six patients had lung cancer, and 60 of them were treated by TKIs (25 erlotinib, 22 afatinib, 10 gefitinib and 3 osimertinib). We excluded patients already suffering from mucocutaneous symptoms at the beginning of chemotherapy. All data were collected from the computerised database of our department.

We collected data about the clinical exam, clinical photography, dermoscopy, histology and direct microscopic examination results for each patient.

The analysed data also included personal and clinical characteristics such as gender, age, type of lung cancer, therapeutic agent, site and clinical presentation of skin reaction.

We performed retrospective descriptive analyses, and we classified data by patients' clinical characteristics, types of lung cancer, treatment and adverse reactions. The study was conducted by ethical guidelines and providing informed consent from the subjects enrolled.

Statistical analysis

We carried out statistical analysis to assess whether a specific EGFRi is linked significantly to particular cutaneous toxicity. We excluded patients treated with osimertinib because of the insufficient sample size.

Categorical variables were reported as absolute number and percentage and compared using the exact chi-square test. A two-tailed P-value < 0.05 was considered significant. Data were analysed using SAS version 9.4 (SAS Inc, Cary, NC).

Results

We selected 60 treated by TKIs of 76 patients with lung cancer and skin reactions.

A group of 31/60 were males (51.67%), and 29/60 (48.33%) were females. Patients aged from 41 to 80 years (mean age 64.60 ± 10.85 DS). The lung cancer type diagnosed was: 41/60 (68.33%) adenocarcinoma, 6/60 (10.00%) squamous cell carcinoma, 6/60 (10.00%) Small Cell Lung cancer (SCLC) and in 7/60 (11.67%) patients the lung cancer type was not specified nor included in the previous categories.

The most frequent TKIs were erlotinib (25/60, 41.67%) followed by afatinib (22/60, 36.66%) Table 1.

Table 1: Characteristics of patients included in our analysis

Sex	Mean age (years ± DS)	Cancer type	Treatment
31 M (51.67%)	64.60 ± 10.85	41 adenocarcinoma	25 erlotinib
29 F (48.33%)		6 squamous cell carcinoma	22 afatinib
		6 SCLC	11 gefitinib
		7 other or not specified	2 osimertinib

The majority of skin toxicities were due to, in order of frequency: afatinib (47 reported adverse reactions), erlotinib (39), gefitinib (13) and osimertinib (3). The most common skin reaction was rash (63% of patients), followed by xerosis (30%), granuloma (30%), mucositis (18%), psoriasis (8%), fingertips fissures (7%), itching (5%). Alopecia (5%), hand-foot syndrome (2%), and trichomegaly (2%) (Figure 1).

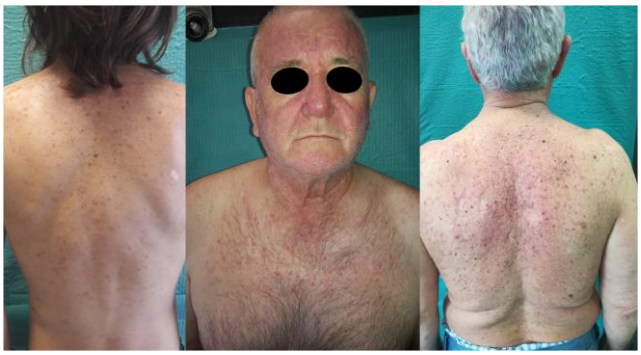

Figure 1: Papulo-pustular rash under EGFRi in its typical localization (trunk and head)

Data and frequency distribution are reported in Table 2.

Table 2: Frequency of adverse cutaneous events related to TKIs' administration

	Erlotinib 25 patients	Afatinib 22 patients	Gefitinib 11 patients	Osimertinib 2 patients	Total ADR/reaction	% ADR/patient
Rash	14 (56%)	17 (77%)	6 (55%)	1 (50%)	38	63%
Xerosis	7 (28%)	7 (32%)	4 (36%)	0 (0%)	18	30%
Granuloma	6 (24%)	11 (50%)	1 (9%)	0 (0%)	18	30%
Psoriasis	3 (12%)	2 (9%)	0 (0%)	0 (0%)	5	8%
Mucositis	2 (8%)	7 (32%)	1 (9%)	1 (50%)	11	18%
Pruritus	1 (4%)	1 (5%)	0 (0%)	1 (50%)	3	5%
Fingertips fissures	2 (8%)	2 (9%)	0 (0%)	0 (0%)	4	7%
Alopecia	3 (12%)	0 (0%)	0 (0%)	0 (0%)	3	5%
Hand-foot syndrome	0 (0%)	0 (0%)	1 (9%)	0 (0%)	1	2%
Trigomegaly	1 (4%)	0 (0%)	0 (0%)	0 (0%)	1	2%
Total ADR/drug	39	47	13	3		

Statistical data revealed that there was no significant relationship between a specific type of cutaneous reaction and specific EGFRi except for granulomas, appearing significantly more frequently in patients under afatinib ($p < 0.05$).

Statistical data are reported on Table 3.

Table 3: Statistical analysis

	Rash	Xerosis	Granuloma	Psoriasis	Mucositis	Pruritus	Fingertips fissures	Alopecia	Hand-Foot Syndrome	Trichomegaly
Erlotinib 25 patients	14 56%	7 28%	6 24%	3 12%	2 8%	1 4%	2 8%	3 12%	0 0%	1 4%
Afatinib 22 patients	17 77%	7 32%	11 50%	2 9%	7 32%	1 5%	2 9%	0 0%	0 0%	0 0%
Gefitinib 11 patients	6 55%	4 36%	1 9%	0 0%	1 9%	0 0%	0 0%	0 0%	1 9%	0 0%
p-value	0,268	0,932	0,037	0,620	0,076	0,999	0,679	0,214	0,190	0,999

Discussion

In our study, the rash was the most common dermatological side effects reported (38 patients), followed by xerosis (18), granuloma (18), mucositis (11), psoriasis (5). Our data showed that only a few patients complaint of fingertips fissures (4), pruritus (3), alopecia (3). Only one patient developed hand-foot syndrome and another one trichomegaly.

The majority of skin toxicities were due to afatinib (47 reported adverse reactions), erlotinib (39), gefitinib (13) and osimertinib (3).

Afatinib was the drug causing more adverse reactions although it wasn't the most used therapy in our patient's sample, confirming *Derrick Chen-Wee Aw et al.* review data [8].

In our experience, no statistical difference linking a type of cutaneous reaction and specific EGFRi were observed except for granulomas.

The majority of anti-cancer drugs can induce rash because they act on rapidly growing cells and hence the skin, but also hair follicles and nail matrix. TKIs may interfere in the epidermal structure, antimicrobial and inflammatory response, leading to dysfunction of normal epidermal barrier and dysregulated cytokines patterns [4].

A papulopustular eruption is the most frequent side effect of anti-EGFR drugs reported in the literature. The eruption may be asymptomatic or accompanied by pruritus, and it tends to improve over time despite the continuation of therapy. It is generally distributed in the seborrheic areas, where EGFR is more expressed [9].

The incidence of rash from TKIs observed in our study is similar to those of several clinical studies: it is more frequent in first or second generation TKIs (44.73% of rash due to afatinib, 36.84% in erlotinib, 15.78% in gefitinib), when compared with third-generation TKI treatment (2.63% in osimertinib) [9], [10].

As already reported by Derrick Chen-Wee Aw et al., our study confirms that afatinib causes rash more frequently than erlotinib, gefitinib and osimertinib [8], [11]. We only had two patients under osimertinib therapy, and one of them developed a rash (50% of total patients), but we cannot conclude that osimertinib causes rash as frequently as first or second generation TKIs since our data were conducted on a few numbers of patients.

In our study, we observed xerosis in 18 patients (7 afatinib and erlotinib, 4 gefitinib). Comparing to Derrick Chen-Wee Aw et al., we didn't find xerosis in the two patients treated with osimertinib, but we cannot consider our percentage statistically significative for the limited number of patients. The rate of body surface area involved can be variable like also the time of onset that can variate from 15 days to 60 days [12].

Xerosis, also known as xeroderma or dry skin, can occur independently or associated with other adverse reactions, particularly pruritus [13].

In our study, pruritus was found in three patients (1 afatinib, 1 erlotinib and 1 osimertinib), but no patient treated with gefitinib complaint of itching. Our results disagree partially with published literature since we didn't observe gefitinib-induced pruritus [8].

A unique common site of xerosis is the fingertips, especially in patients treated with EGFR inhibitors. Dry fingertips commonly prove in pulpitis with painful fissures [9]. In literature, the incidence is 18-25% and the onset time is around 30-60 days [12]. In our study, fingertips xerosis with fissures was seen in 4 cases, two treated with afatinib and two with erlotinib, in agreement with already reported severe cases of pulpitis sicca and painful rhagades [8].

From 4% through 56.8% of patients under TKI can present nails changes, including paronychia, painful fissures, swelling, and noninfectious granuloma [12], [14]. In our experience, we have reported granulomas in 18 patients, and we found that, together with xerosis, the periungual involvement is the second most frequent adverse reaction in patients under TKIs. Our statistical data show that afatinib causes more frequently granulomas.

Psoriasis, both with diffuse or localised involvement of the skin, has been often reported in literature during anti-cancer treatment.

We observed psoriasis in two patients treated with afatinib and in three treated with erlotinib; all of these patients were affected in the scalp area. In literature, there are some contrasting data: there are case reports that describe the positive effect of EGFR inhibitors in psoriasis. Overbeck presented cases of patients with psoriasis treated with tyrosine kinase inhibitors; instead, Zorzou observed that psoriasis

recurred after treatment with anti-EGFR [15], [16].

Mucositis has also been reported with TKIs, more frequently with the second generation of TKIs than the first one. Incidence of mucositis induced by afatinib varies from 29 to 64%, while mucositis induced by erlotinib and gefitinib ranges between 8 and 20% and 19 to 24%, respectively [17]. We observed 11 cases (7 afatinib, 2 erlotinib, 1 gefitinib and osimertinib) and our results confirm that mucositis is more frequently reported with second-generation TKIs than the others.

TKIs can induce hair changes such as hair loss (scarring or non-scarring alopecia), scalp inflammation or hirsutism including hair rigidity and curling, trichomegaly and facial hypertrichosis [8]. In literature, it was reported that TKIs cause androgen-like frontal alopecia with progressive growth of facial hair and eyelashes, more evident in female patients [18], [9]. In our study, alopecia has been reported in three female patients treated with erlotinib, and one of them also showed trichomegaly of the malar region.

Hand-foot syndrome (HFS) or Erythrodysesthesia by TKi has been rarely reported in the literature [19], [20]. In our study, this reaction was found in only one patient treated with gefitinib and not exposed to precedent therapy, contrary to 'recall reactions' which Razis et al., consider in patients previously treated with liposomal doxorubicin and then with gefitinib [19].

Pigmentation disorders (hypo- and hyperpigmentation) [21] photosensitivity and also telangiectasia [22] have been reported, but, hereinbefore, we didn't find these dermatologic toxicities in our patient sample.

Limitations of the study

The main limitation of our study was the limited sample size that leads us to exclude osimertinib in the statistical analysis. Moreover, we cannot conclude completely that there is no association between type of EGFRi and skin reactions, but it's a good start point of view for future guidelines. Future studies should include a higher number of patients treated with different EGFRi.

We can conclude that 63% of our patients presented rash, most commonly those treated by afatinib, although it wasn't the most used EGFRi. Moreover, we noticed a significant correlation only between afatinib therapy and appearance of granulomas.

It is already known that skin reactions occur with different severity and frequency for each drug.

Cutaneous EGFRi-induced side effects, generally classified as moderate, may become chronic, impacting patient's quality of life and requiring therapy reduction or even interruption [23]. As a consequence, the appearance of an adverse reaction may compromise treatment efficacy and cancer response [24].

So, we assume that the knowledge and the correct management of drug reactions are important to prevent their appearance and to avoid unnecessary interruption of drugs, especially of those giving a higher survival rate to oncological patients.

The management of cutaneous toxicities in lung cancer patients should also include patient and family support, self-esteem maintenance and quality of life improvement for 360-degree patient care.

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Clinical Science

Genital-Sparing Cystectomy versus Standard Urethral-Sparing Cystectomy Followed with Orthotopic Neobladder in Women with Bladder Cancer: Incidence and Causes of Hypercontinence with an Ultrastructure Study of Urethral Smooth Muscles

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Abstract

BACKGROUND: Bladder cancer in women is an indication for radical cystectomy (RC) when the tumour is confined muscle-invasive bladder cancer (MIBC) of T2 N0M0, or high risk progressive non-muscle invasive bladder cancer (NMIBC). Radical cystectomy is either genital-sparing cystectomy (GSC) or standard urethra-sparing cystectomy (USC) that is followed with orthotopic ileal neobladder (ONB). Post-operative chronic retention "Hypercontinence" had been reported in different series following URS or GSC and ONB. In long-term follow-up, we evaluated the functional outcome of women who developed hypercontinence after USC or GSC and ONB.

AIM: An ultrastructure study of female urethral smooth muscle was done to elucidate the underlying causes of hypercontinence.

MATERIAL AND METHODS: Retrospective study was conducted on 71 women who underwent RC and ONB, 45women had undergone USC, and 26 women had GSC, follow-up ranged from 5 to 15 years. Ultrastructure studies were done on 5 urethral biopsy specimens from 5 women who had hypercontinence, and 4 biopsies were from a normal control.

RESULTS: Follow-up showed that women who had undergone USC and ONB, 28.88% developed hypercontinence, where in the series of GSC and ONB three women out of 26 developed hypercontinence (7.80%). Three women who had hypercontinence following USC and ONB, they developed stones in the ileal pouch. Ultrastructure study of urethral smooth muscles in women who had hypercontinence showed organized collagen fibrils, absent myelin sheath, and non-detected lymphatic vessels. Normal urethra showed collagen fibrils within the interstitial matrix, preserved myelin sheath of nerve fibres, the presence of lymphatic vessels in the matrix.

CONCLUSION: The present study shoes that GSC with ONB leads to the minimal incidence of hypercontinence (7.80%), while standard USC lead to higher incidence (28.88%). Ultrastructure changes of the female urethra who had hypercontinence were fibrotic changes, loss of myelin sheath and minimal vascularity, their findings explains the underlying cause of hypercontinence and support the technique of GSC rather than the standard USC.

Introduction

Urothelial carcinoma of the bladder manifest as MIBC or NMIBC, an early MIBC T_2 N_0 M_0 and NMIBC which is progressive and not responding to BCG therapy are an indication for RC [1], [2]. Orthotopic ileal neobladder reconstruction after RC became a standard technique in female patients; the 5 years survival was reported to be 62-83% with mean follow-up ranging from 29-103 months [3]. Functional outcome of RC and ONB in female patients showed that the incidence of hypercontinence were (24%), these women had an excellent disease-specific survival which indicated that ONB is a safe and effective urinary diversion in women [4]. An ideal functional result of ONB is achieved when the neobladder gained normal voiding pattern which is four to six micturition daily with 3 to 4 hours interval and voiding urine volume of 250 to 500 ml at a low pressure [5]. Chronic urinary retention in women after ONB is defined as hypercontinence which is the persistent inability to completely empty the neobladder that lead to high volume post-voiding of residual urine

greater than 150 ml urine, in that case, the patient is instructed to do regular clean intermittent catheterisation (CIC) four to five times daily. The incidence of hypercontinence had been observed in different series that ranged between 12% to 58%; Stenzel et al., (12%), [6], Lee et al., (21%) [7], Granberg et al., (35%) [8], Stein et al., (39%) [3], Anderson et al., (31%) [9], Jentzmik et al., (58%) [10]. The high incidence of hypercontinence had been suggested to be due to lax of support of pelvic structures similar to pelvic organ prolapsed; the procedure of sacrocolopexy was advocated after cystectomy by suturing the anterior vaginal wall and vaginal apex to the anterior longitudinal ligament of the sacrum [11]. Treatment of pelvic floor disorders was recommended in women who developed hypercontinence following USC and ONB [12]. Unadjusted analysis showed that hypercontinence was not associated with any variable [13]. During cystectomy, the preservation of the uterus and attempted unilateral or bilateral nerve sparing, resulted in better functional outcome post-operatively that was attributed to the preservation of the urethral innervations [14]. Urinary function and sexual function appeared to be better among those patients undergone GSC and ONB [15]. In GSC the uterus, ovaries, fallopian tubes and vagina are preserved, the ileal neobladder pouch was fixed to the anterior longitudinal sacral ligament, follow- up showed good quality of life, sexual function, with reported low incidence of hypercontinence 7.80% [16]. Standard USC without nerve sparing attempt had led to a higher incidence of hypercontinence of 62.5% [17]. Ultrastructue characteristics of urethral smooth muscle in women with urinary incontinence were the finding of a smaller dense portion of sarcolemma denoting intrinsic sphincter deficiency [18].

The objective of the present study was to find out in long term follow-up the incidence of hypercontinence in the standard USC and GSC and to define by transmission electron microscopy the changes and pattern of female urethral smooth muscle in women developed hypercontinence.

Material and Methods

Patients

Institutional Review Board approval was obtained in the course of approval of PhD thesis on orthotopic ileal neobladder in female patients with bladder cancer who are eligible for RC and ONB for the treatment of neoplastic disease. Eligibility was confined disease of MIBC and/or progressive NMIB or refractory to treatment with BCG. Standard USC was performed in 45 women, GSC was performed in 26 women, both procedures were followed with orthotopic detubularised U shaped ileal neobladder according to the procedure of CameyII [19]. Operative technique of GSC aimed at preserving the uterus, fallopian tubes, ovaries, vagina, broad lateral ligaments, and attempted unilateral or bilateral nerve-sparing [16]. Uretero-ileal anastomosis in the detubularised U-shaped ileal neobladder was done using a dipping technique [20]. The ONB following GSC was fixed on both lateral sides to the lateral pelvic wall and anterior, presacral ligament to prevent anterior angulations of the ileal-urethral junction. Patients were followed every 3 months in the first year, every 6 months for 2 years, and annually after that, follow-up was ranging from 5 to 15 years. Patients who had hypercontinence were defined as their need for daily catheterisation to evacuate the neobladder. Women who had hypercontinence undergone diagnostic urethrocystoscopy, a urethral biopsy was taken from 5 patients for ultrastructure study. Urethral biopsy of normally continent women was obtained from cystectomy specimen of 4 women who undergone RC and ileal conduit diversion; urethral biopsy was taken from the cystectomy specimens. The 9 urethral biopsy specimens were examined by transmission electron microscopy. Clinical and functional data with follow-up for 5-15 years were obtained and analysed.

Transmission electron microscopy

The urethral biopsy specimens were immersed fixed in phosphate buffer containing 4% paraformaldehyde and 3% glutaraldehyde, pH 7.4, 4°C for 2 h at 4°C and rinsed in phosphate buffer. Tissue was immersed in 1% osmium tetroxide for 2 h at 4°C, rinsed in distilled water and incubated with uranyl acetate overnight. Tissue was dehydrated in a graded series of ethanol and embedded in Epon epoxy resin. Ultrathin sections were cut with an ultra microtome and immersed in uranyl acetate and then in lead citrate. Specimens were observed with a transmission electron microscope (FEI/Philips EM208S Transmission Electron Microscope (FEI Electron Optics BV, Eindhoven, Netherlands) The Smooth muscle morphologic characteristics were assessed from a systematic random sample of electron micrographs of the 5 biopsies from women with hypercontinence. A further 4 urethral biopsy specimens from continent women were similarly analyzed to confirm the findings of the initial study.

Results

Patients

A total of 71 women with mean age 52 years (range 23-72) and follow-up for 5-15 years were included in the study. Forty-five women underwent USC, 26 women had undergone GSC, and both

procedures were followed with detubularised U-shaped ileal neobladder urinary diversion. Genital-sparing cystectomy was done as it was requested by women patients desiring future fertility and normal sexual life. The 45 women undergone USC thirteen of them 28.88% developed hypercontinence and were on regular CIC four times daily, 4 women had recurrent urinary tract infection, and 3 developed big stone in the ileal pouch, the stones were removed surgically (Figure 1). The 26 Women who had undergone genital-sparing cystectomy and ONB, three of them 7.80% developed hypercontinence and they were on daily CIC.

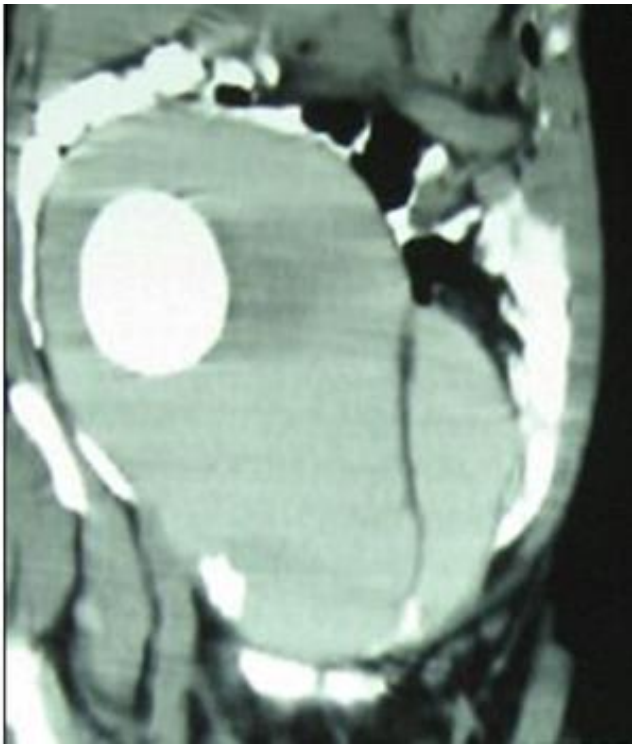

Figure 1: Image of computerised tomography of the urinary tract showing the stone in the ileal pouch in a woman having hypercontinence following standard urethral-sparing cystectomy and orthotopic neobladder

Transmission electron microscopy

Ultrastructural studies of the five urethral biopsy of women with hypercontinence following standard USC and ONB showed excess connective tissues with scattered myelinated nerve fibrils, few muscle fibrils where the scattered sarcolemma was smaller in comparison to normal control, the fibroblasts were characterized by lack of basal membrane (Figure 2d and 2e).

Ultrastructure of four women who had normal urethra and normal voiding function showed abundant smooth muscles, organised fibrils within interstitium matrix, myelin sheath of nerve fibrils consists of many regular membrane layers, and the vessels were in good integrity (Figure 2a, 2b, and 2c).

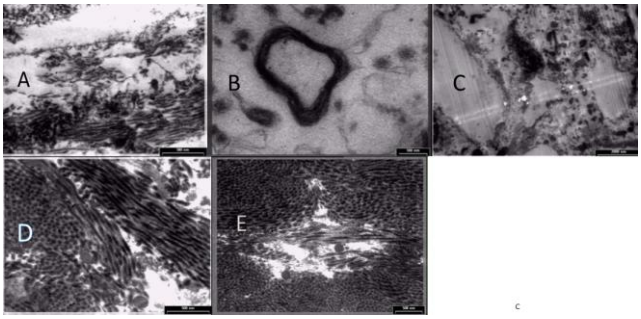

Figure 2: representative images of ultrastructure pattern of urethral smooth muscles in women with hypercontinence and normal urethra; A), B), and C) showing the pattern of normal urethral smooth muscles A) showing collagen fibrils within the interstitial matrix. B) Shows myelin sheath of nerve fibril that is consists of many regular membrane layers; C) Shows lymphatic like vessels in the matrix; D), and E) showing urethral ultra structure of women with hypercontinence, following standard urethral-sparing cystectomy and orthotopic neobladder showing areas of connective tissue with scattered myelinated nerve fibrils, and thin processes of fibroblasts that were characterized by lack of basal membrane

Discussion

Orthotopic ileal neobladder after RC in women has developed after many clinical series included standard cystectomy with urethral-sparing and genital-sparing cystectomy. Concerns remain about the problem of hypercontinence following RC and ONB. Here, we report difference incidences of hypercontinence following 2 operative techniques of RC, that was 28.88% among 45 whom undergone USC, this relatively low incidence is in accordance with results in other series that reported the incidence varied from12% to 24% [4], [6], [7], in other series they reported high incidence of 35% [8], 39% [3], and 31% [9], this wide variation in incidence of hypercontinence would be attributed to operative techniques, in our series the operative procedures were with intend of unilateral or bilateral preservation of neurovascular bundle. Fixation of neobladder to the lateral pelvic wall and anterior sacral ligament avoided angulations of the ileo-urethral angle to minimise the post-operative incidence of hypercontinence, that operative step is similar to the procedure of sacrocolopexy that had minimised the incidence of hypercontinence post operatively [11], [12]. Women whom underwent GSC the incidence of hypercontinence was 7.80%, these satisfactory results were due to the preservation of female genital organ, additionally preservation of ovaries maintained hormonal balance in premenopausal women and assessed in integrity of pelvic floor, the results of the present series of GSC with ONB are consistent with previous studies [15], [16]. Female urethral sphincter is located in the distal two-thirds of the urethra, branches from the pudendal nerve that supply the sphincter course deep to the endopelvic fascia and enter the urethra on lateral sides. During cystectomy,

careful dissection by avoiding distal dissection to bladder neck and preservation of endopelvic fascia would protect the urethral smooth muscles from neural and vascular denervation that will lead to post-operative incontinence or hypercontinencre. We studied by transmission electron microscopy the ultrastructural changed in smooth muscles of the urethra of women having hypercontinence following standard USC, we found that there were excess connective tissues with scattered myelinated nerve fibrils, few muscle fibrils where the scattered sarcolemma was smaller in comparison to normal control, the fibroblasts were characterized by a lack of basal membrane (Figure 2). Our finding indicated an insult during the dissection that leads to vascular and neural denervation of the smooth muscles and had lead to fibrosis, our results are in accordance with the findings in women with incontinence who had been studied by transmission electron microscopy and revealed that The electron-dense portion of the sarcolemma was smaller in urethral biopsy specimens taken from patients with intrinsic sphincter deficiency than in those from control subjects [18].

In conclusion, genital-sparing cystectomy in women with bladder cancer followed with orthotopic ileal neobladder provided good quality of life with a low incidence of post-operative chronic urinary retention, compared to the relatively higher incidence in standard technique; these findings are attributed to the preservation of neurovascular innervation to urethral smooth muscle in the GSC procedure. Ultrastructure analysis of urethral smooth muscles in women having hypercontinence following USC showed loss of myelin sheath, excess collagen fibrils, minimal muscle fibrils, which denoted affection of neurovascular supply. We recommended genital-sparing cystectomy when feasible.

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Clinical Science

Evaluation of Knee Joint after Open Reduction and Internal Fixation Surgery of Posterior Cruciate Ligament in Patients with Avulsion Fracture

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Keywords: PCL avulsion fracture; Lysholm knee score

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Abstract

BACKGROUND: The posterior cruciate ligament is one of the important tissues and structures sustaining the knee joint, and its rupture or detachment may lead to joint instability or destruction.

AIM: The present study aimed at investigating the Open Reduction and Internal Fixation surgery of posterior cruciate ligament and comparing it to the normal knee of the same side.

METHODS: In this study, 25 patients with avulsion fracture at the PCL joint were treated with open surgery and screw fixation. The patients were followed up by Lysholm knee score for at least 12 months after surgery.

RESULTS: All patients were male with an average age of 25 years over the years 2010-2018. The common mechanism of injury in these patients was motorcycle-car accident. In the study with Lysholm knee score, 21 patients (80%) obtained the good score of 60-90 while 20% of patients were placed in the fair group (30-59). The average score was 86.

CONCLUSION: The obtained score of knee function questionnaire in this study had no significant difference from other similar studies, and most patients achieved a good and acceptable score after the surgery. There was no knee instability and functional impairment in the patients compared to the normal knee. Considering the clinical results after the fixation of the PCL avulsion fracture causing a significant improvement in patients, the surgery could be considered as an acceptable and effective method for treating such impairment and fracture.

Introduction

The knee joint plays an important role in the function of various lower limbs. Posterior Cruciate Ligament (PCL) prevents posterior displacement of the tibia about the femur and causes knee posture stability and knee strength. It acts as a posterior knee stabiliser and has a restrictive role in front of the tibia to the posterior [1], [2]. The posterior cruciate ligament is one of the important tissues and structures sustaining the knee joint, and its rupture or detachment may lead to joint instability or destruction [3]. The PCL is very tight, so more impairments occur at the attachment to tibia that the trunk. tibial avulsion is a special kind of the PCL impairments. In fact, PCL avulsions often occur from the tibia side. The tibial avulsion of PCL can cause knee instability and lead to knee degeneration in the long term. The cut piece can be fixed by screw or stitch either during open surgery or by arthroscopy. The larger pieces can be fixed by a screw and a posterior approach. In addition, several methods proposed for stitch fixation [3]. With a detailed overview of the previous studies, it can be found that the surgeries for the PCL avulsion often show a good functional success and good objective [4], [5].

The present study aimed at investigating the Open Reduction and Internal Fixation surgery of posterior cruciate ligament and comparing it to the normal knee of the same side.

The normal recovery rate of knee and the effect of fixation surgeries on the daily routine of these individuals is a topic that can be used for identifying

the defects of these surgeries and resolving them. In this study, the knee function of individuals was examined from two subjective and objective approaches. Lysholm Knee Scale is a standard scale that can estimate the success rate of surgery and postoperative complications based on the patient's symptoms (can be completed and filled by the patient or therapist).

The present study aimed at examining the function of the knee joint after the reconstruction surgery of Posterior Cruciate Ligament Avulsion and comparing it to the normal knee joint in the patients went under surgery in Shahid Beheshti Hospitals during 2010-2018 by using the Lysholm Knee Scale.

Methods

Summary of the used methodology and techniques

In this study, 25 patients undergoing PCL fixation at least 12 months ago were included. The inclusion criteria included the fixation surgery for PCL avulsion at least 12 months ago and completion of treatment, follow-up, physiotherapy course, having no other ligament problems in the knee during or before the surgery, having a problem in one side and having a normal knee with no ligament problem. However, the exclusion criteria included leaving the study unfinished and incomplete for any reason. All participants in the study presented their written consent on participation in the study.

Surgical technique

The patients with avulsion fracture at the tibial side of PCL diagnosed by radiographic and three-dimensional CT.SCAN went under general anaesthesia or spinal anaesthesia according to the anaesthesia physician after being prepared in the operating room (within a maximum of one week of the trauma). Clinical examination was performed under anaesthesia before placement of the patient in the prone position and the results were recorded. In all patients, the tourniquet was closed, and the fracture site was opened from the middle of the medial gastrocnemius and semitendinosus by the posterior approach. Then, the open reduction and fixation were conducted by a distal thread screw. After closing the wound and doing the dressing, the long leg splint was used in the knee flexion of 15-20 degrees and physiotherapy for strengthening the quadriceps femoris muscle was taught to the patient. After 4 weeks, the splint is removed, and the knee brace was given, and simultaneously the muscle physiotherapy started for the knee [3], [7].

Each subject in the study was followed up during 2, 6, 12 weeks and 6 and 12 months after the surgery through knee radiography, physical examination, and completion of the Lysholm knee scale. In this scale, the parameters such as limping, need or lack of need to support while standing and bearing weight, the ability of stair climbing, the ability to bend the knee gradually in the standing position, the knee stability and instability, swelling, and pain were evaluated. Also, some comments were left based on the obtained score on the success of surgery [6]. Physical examination was associated with scoring including posterior drawer and sag test and pain while moving the knee patios move. The collected data were analysed by statistical tests. Significant level was considered as $P < 0.05$.

Calculation of the sample size and sampling method:

Considering the descriptive and prospective study of all patients who went under the fixation surgery of Posterior Cruciate Ligament avulsion in Shahid Beheshti Hospital in Yasuj from the beginning of 2010 to 2018, 25 patients having the inclusion criteria were included in the study.

Inclusion criteria: The patients with isolated PCL avulsion fracture having no other impairments in their affected limbs and a history of surgery or knee degenerative disease with normal opposite lower limbs and no motion limitation.

Exclusion criteria: the patients having multiple fractures in the affected or opposite limb, having no continuous postoperative follow-ups, and refused from continuing the study. The patients with the degenerative disease, motion limitation in the knee, or knee surgery were excluded.

The statistical methods of result analysis

The quantitative data were displayed in terms of mean and standard deviation while the qualitative data were shown as frequency.

Results

In a descriptive study from 2010 to the end of 2018, the patients who referred to Shahid Beheshti Hospital with a diagnosis of posterior cruciate ligament avulsion fracture and were eligible for inclusion in the study went under the open reduction internal fixation (ORIF). Some 20 patients were excluded from the study for a variety of reasons such as the lack of regular referral or lack of medical file, and finally, 30 patients were evaluated. All patients were examined by an orthopaedic surgeon at the clinic under the supervision of the relevant instructor. The average patient's last visit was about 10 months

after the surgery. The average age of patients during the surgery was 28 years (at least 18 years and maximum 50 years).

Table 1: Frequency of patients by age group

		Frequency	Percent	Valid Percent	Cumulative Percent
	< 20 y/o	1	4	4	4
	20-30 y/o	18	72	72	76
Valid	30-40 y/o	4	16	16	92
	> 40 y/o	2	8	8	100
	Total	25	100	100.0	
Missing	System	0	0	0	
Total		25	100.0		

All the patients suffering from the disease were male. Among these patients, 15 subjects (60%) had right involvement, and 10 subjects (40%) had left involvement.

Table 2: Limping rate among the patients

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	No limp	20	88	88	88
	Slight or periodical	3	12	12	100.0
	Total	25	100	100.0	
Missing	System	0	0		
Total		25	100.0		

Based on Lysholm criteria for assessing the performance of the patients who went under the surgery, those with a lysholm score of less than 65 were placed in the poor group, those with a score of 65-83 were in the fair group, those with a score of 84-94 were in the good group, and those with a score of 95-100 were in the excellent group.

Table 3: Knee locking rate among the patients

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	No locking	13	50.0	53.3	53.3
	Catching sensation, no locking	11	40.6	43.3	96.7
	Locks occasionally	1	3.1	3.3	100.0
	Total	30	93.8	100.0	

After the evaluations, the following results were obtained from the Lysholm criteria among the patients who went under the surgery.

Table 4: Stair climbing rate among the patients

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	No problem	2	8	8	8
	Slight problem	23	92	92	100.0
	Total	25	100	100.0	

After filling in the questionnaire and obtaining a biography, the patients underwent a general physical examination and knee-specific examination.

Table 5: Squatting rate among the patients

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	No problem	2	8	8	8
	Slight	21	84	84	92
	Cannot beyond at 90 '	2	8	8	100.0
	Total	25	100	100.0	

The knee examinations included the diagnostic tests for knee ligament injuries such as posterior and anterior drawer tests, Varus and Valgus stress tests, Luchman tests, and other examinations of knee stability.

Table 6: Giving way rate among the patients

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Never	25	100	100	100
	Only during vigorous activities	-	0	0	0
	Total	25	100	100.0	

Pain: pain is one of the most important components of Lysholm.

Table 7: Swelling rate among the patients

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	No swelling	5	20	20	20
	After vigorous activities	20	80	80	100.0
	Total	25	100	100.0	

After performing the physical ex-amination and grading the Lyingholm criterion, these two results were compared to each other and analysed as follows.

Table 8: Pain rate among the patients

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	No pain	1	4	4	4
	Slight	21	84	84	88
	Marked pain in vigorous activities	3	12	12	100.0
	Total	25	100	100	

A significant relationship was found between the results of the questionnaire and physical examination ($P_{value<0/05}$).

Table 9: Lysholm knee scale

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Good	21	84	84	84
	Fair	4	16	16	100.0
	Total	25	100	100.0	

Discussion

The posterior cruciate ligament is vital for normal knee function and is aimed at restoring the normal movement of the joint and returning the patient to previous activity to prevent the secondary arthrosis [3].

Table 10: Lysholm scale

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	69.00	3	12	12	12
	71.00	3	12	12	24
	79.00	1	4	4	28
	84.00	5	12	12	40
	85.00	2	8	8	48
	86.00	9	36	36	84
	90.00	3	12	12	96
	91.00	1	4	4	100
	Total	25	100	100	

The main objective of this study was evaluating the function of the knee of the patients with posterior cruciate ligament avulsion fracture went under the open surgery by distal thread screw No. 4 and comparing it to the normal knee of the same patient.

Table 11: Posterior drawer test

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Normal	4	16	16	16
	< 5 mm post translation	18	72	72	88
	5 mm-10 mm	3	12	12	100.0
	Total	25	100	100.0	

Since the most common cause of posterior cruciate ligament avulsion fracture is the motorcycle accidents and because women are less involved in these accidents, the gender distribution of this type of fracture was justified in this study [7]. In the study of Lysholm, 21 subjects (84%) gained a good score, and 4 subjects (36%) were relatively good (Tables 11 and 12). The posterior cruciate ligament avulsion fracture from the junction to tibia includes a subgroup of posterior cruciate ligament impairment.

Table 12: Comparison of clinical examination and Lysholm knee scale

		Lysholm score		Total
		good	fair	
Posterior drawer test	Normal	0	0	4
	< 5 mm post translation	12	3	15
	5 mm-10 mm	2	4	6
Total		18		25

$P_{value=0/04}$.

The patients with posterior cruciate ligament avulsion fracture undergo non-surgical treatment if they have a small fragment while surgical treatment and fracture fixation will be used in the patients with large or displacements of more than 10 mm in width [8], [9]. The present study indicated no or very little instability after surgery and bone healing as well as the long-term functional capacity were satisfactory. Some 25 patients who referred to the orthopaedic unit of Shahid Beheshti Hospitals in Yasuj due to a knee injury during 2010-2018 were examined, and their posterior cruciate ligament avulsion fracture was diagnosed. These subjects were in the age range of 18 to 50 years old with an average age of 28.5 and a mean of 26. In the study of M Ali et al., in Bangladesh, the age range of patients was estimated from 19 to 35 years and an average age of 27 years [4]. In the study of Sergio Rocha Piedade et al in Brazil, the age range of patients was between 15 and 53 years old and the average age was 30 years [11] indicating that the average age of the patients in this study was significantly lower than the average age of these patients. This fact can be attributed to the prevalence of motorcycle use among young people as well as the prevalence of car accidents in this age range.

The degree of trauma to the affected limb showed that the incidence of posterior cruciate ligament avulsion fracture in the right limb was more than the left limb (60% vs 40%). Another study conducted in Tennessee, the USA showed the trauma to the posterior cruciate ligament in the left limb more than the right limb (72% vs 28%) [12]. The statistics showed that 96.7% of the cause of posterior cruciate ligament rupture was due to trauma caused by accident or overturning of the vehicle which was reported as 100% in the study of Farzad Omidi et al. in Mashhad and 76% in the study of Sergio Rocha Piedade [8], [11]. According to the findings of this study, the incidence of limping among the patients was 12% while 88% of patients did not experience such a complication which was roughly equivalent to the study of Greyory et al., in the USA reporting this complication in 12% of patients [13]. The examination of patients for posterior drawer test showed that 88% of the tests were normal and close to normal, which was reported by Greyory and Lipscomb as 46% and 86% respectively [13], [12].

The present study showed that t 12% of patients had severe pain when walking, and 88% of patients did not feel pain or had no significant pain in their knee that had gone under surgery. About 8% of the patients could not sit squatting, and the rest of the patients had no significant sign of being in this position. Also, the statistics showed that 8% of patients suffered from knee swelling and no one patient felt knee giving way while walking. In the study of Dandy and Pusey which was performed on the patients with posterior cruciate ligament trauma who had not undergone surgery and treated by long leg casting, 70% had early onset of pain while walking, 55% while squatting, 20% knee swelling and 95% knee giving way [10]. Lysholm knee scale is a standard scale that can be used to estimate the success rate of surgery and postoperative complications based on the patient's symptoms (can be completed and filled by patient and therapist). In this scale, the parameters such as limping, need or lack of need to support while standing and bearing weight, the ability of stair climbing, the ability to bend the knee gradually in the standing position, the knee stability and instability, swelling, and pain were evaluated. In the conducted survey, 84% of the patients had acceptable scores the majority of whom scored 86 points. The Lysholm score reported in Greyory et al., [13] was 91.2 while the score reported in Maiani et al., in Italy was 8 ± 94, which was not significantly different from the present study [14]. In the conducted study, 100% of the patients did not complain about knee locking while moving and 43.3% of them had knee cramp without locking while no one person (4%) had knee locking at the time of walking.

Furthermore, 88% of patients had mild pain while climbing the stairs and the others climbed the stairs without any problems. In addition, 100% of the patients were able to move without the need for a cane or crutch. In the study of Katchuyi et al., in Mashhad, 58% of patients had no problems with postoperative problems which were not significantly different from the present study [9].

Positive posterior drawer test and the results of the questionnaire (relatively good and close to normal) were one of the considerable issues in this study and other similar studies, such as the study by Rezazadeh et al., [15] or Sergio Rocha et al., in Brazil [11]. Such results were not expected by assuming that the present study investigated the posterior cruciate ligament avulsion considering the preliminary design of the study. For example, the patients who had positive or near-normal posterior drawer test results, in addition to the score of the relatively good, some of them also had a good score while it was expected to have fairly good and poor scores. Various reasons can be identified based on the results of this study and similar studies including the hidden trauma to the posterior knee capsule such as posterior knee ligament trauma, a disorder in distal junctions of semimembranosus muscle and some mild traumas in the meniscus. By considering these factors in future studies, the error probability can be minimised. The Lysholm knee score in the present study was not significantly different from other studies [11], [13], [15], [16], and most patients achieved a good and acceptable score after the surgery. The patients in this study had no problem with knee joint instability and disabling functional disorder compared to their normal knee. However, they had little disability in knee movements, especially in their exercises and activities which were more than usual.

In conclusion, considering the clinical results after the fixation of the posterior cruciate ligament avulsion fracture, which is significant, improved the patient's function, the surgical procedure can be considered as an acceptable and effective method for the injury and fracture. The clinical results obtained from the physical examination of the knee and the score of the questionnaire indicated that the trauma to the posterior cruciate ligament avulsion fracture is not merely a bone fracture and must be considered as a bone ligament trauma.

The relatively short-term follow-up of patients was one of the limitations of this study, and different results may be obtained in the long-term follow-ups. However, this issue was acceptable in a follow-up of at least 6 months because of this prospective study. The relatively low number of patients can affect statistical strength. However, the number of available patients was the same.

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Evaluation of Rationality of Geriatric Patients' Prescription Based On Beers Criteria in a Tertiary Care Hospital in India

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Abstract

AIM: Irrational prescribing for geriatric patients has become an important public health problem worldwide. Because India is one of the most populated countries having a great proportion of old people in the world, studies on the prevalence of inappropriate prescriptions can be very beneficial to increase the knowledge of health care providers and to reduce the occurrence of adverse drug events among this population.

METHODS: A group of 482 inpatients above 64 years old were enrolled in a prospective study. Chart review method was used. The data were collected from patients' prescription and medicine charts. Each prescription was checked individually for the inappropriate drug by using the AGS 2015 Updated Beers Criteria for Potentially Inappropriate Medication Use in Older Adults. Suggestions were given to the physicians for inappropriate medications.

RESULTS: The prevalence of potentially inappropriate medication is found to be 11.66% (n = 56). Out of 56 inappropriate medications, the most frequently inappropriate medication is Digoxin (25%) followed by Sprinolactone 19.64%. This study founds age, some medication, length of stay and number of diagnosis as predictors for getting a PIM. Feedback of the physicians varies based on the suggestions.

CONCLUSION: This study concludes that the prevalence of PIMs among geriatrics patients of ≥ 65 years old is 11.66%. Some predictors have been identified for getting a PIM. This study shows that physicians' feedback is dependent on the suggestions being given.

Introduction

Inappropriate medication (IM) is the use of medicines that cause more risk than benefit, particularly when safer drugs can be used instead of them [1]. The prevalence of irrational prescribing in older patients is high which can lead to increased risk of adverse drug events, morbidity, mortality and healthcare problems. Therefore, the irrational prescription is a major safety issue, and by the ageing of the population, it is likely to become even more prevalent in the future [2].

It is a very difficult practice to prescribe rationally to elderly patients because available information on rational drug prescription is based on data of younger individuals while the characteristics of them are very different from that of old people [4]. The pharmacokinetic [4] and pharmacodynamic [5] characteristics of older people change over time. Due to the potentially serious consequences of inappropriate prescribing, researchers have designed various tools for measuring inappropriate prescription [6]. Beer's Criteria is one of those guidelines which emphasises on avoiding prescription of medications that are not necessary, which consequently helps to manage the problems of polypharmacy, drug interactions, and adverse drug reactions [8].

Prevalence of inappropriate medication is high in general, but it is variable in different parts of the world [7]. An electronic search of the PUBMED

database for articles published between 1991 and 2006 showed that prescription of potentially inappropriate medications to older people is highly prevalent in the United States and Europe and its proportion ranged from 12% in community-dwelling elderly to 40% in patients of nursing home [8], [9], [10], [11], [12], [13], [14], [15], [16].

Studies have investigated the prevalence of potentially inappropriate medications among elderly patients in different countries but the findings of the studies have not shown the same rate of prevalence of inappropriate medications because each country has a specific clinical practice setting [17], [18], [19], [20], [21], [22], [23], [24], [25].

Every year, out of three adults ≥ 65 years, one has one or more adverse reactions to a medication or medications. This is why it is important for researchers to identify the use of drugs that are associated with more risks than benefits in older people [18]. India has approximately 16% of the world population, so reasonably it will have a large number of elderly patients [19]. Because India is one of the most populated countries having a great proportion of old people in the world, studies on the prevalence of inappropriate prescriptions can be very beneficial to increase the knowledge of the healthcare providers and to reduce the occurrence of adverse drug events and morbidity and mortality among this population.

In this study we aimed at determining the prevalence of PIMs prescribed for elderly inpatients in the Indian setting, identifying the most commonly prescribed inappropriate medications, investigating predictors of PIMs which can act as an alert system for reducing the chance of prescribing inappropriate medication. Another point that we aimed to investigate is physicians' response to suggestions given by the pharmacist about PIMs. This can be considered as a method to assess the mentality of the physicians towards the suggestions given by the clinical pharmacists as clinical pharmacists are not well-accepted by most of the physicians in clinical settings. Such research findings can be used to improve the rapport amongst clinical pharmacist and physicians, which can finally lead to better clinical outcomes.

Material and Methods

A prospective study was carried out in an inpatient setting of a tertiary hospital in Bangalore, India after obtaining approval of the Institutional Review Board. The duration of the study was six months from September 2016 to February 2017.

Four hundred and eighty-two patients ≥ 65 were enrolled from six wards: Male medical ward, Female medical ward, Orthopedic ward, Gynecology ward, ICU and Surgery ward. Patients who had incomplete information in their files were excluded from the study.

A self-developed form was used for collecting information about the patients. The forms were completed at the time of admission and updated daily till the date of discharge of the patients. Chart review method was used. The data were collected from patients' prescription and medicine charts of the patients.

Each prescription was checked individually for inappropriate drug prescribing by using the AGS 2015 Updated Beers Criteria for Potentially Inappropriate Medication Use in Older Adults. For each medication order, the name, dosage, the frequency of dosing and nature of prescription (scheduled or given on an as-needed basis) were collected. Prescriptions for creams, ointments and drops were not included. A prescription was considered to be inappropriate if it had one or more drugs included in Beers list of inappropriateness. Suggestions were given to the physicians for inappropriate medications, and responses of the physicians were recorded.

Statistical analysis

Data were analysed using Statistical Package for Social Sciences (SPSS) software version 16. All the data were presented as frequency and percentage. Prevalence was used for assessing the number of inappropriate medication use. The odds ratio was applied for determining predictors of inappropriate medication use. Value of $P < 0.05$ was considered statistically significant.

Results

The analysis of data for appropriateness of drug therapy was performed, and the results showed that 11.66% (n = 56) out of the 480 patients received at least 1 inappropriate medication (Table 1).

Table 1: Prevalence of inappropriate medicine use by Beers Criteria 2015 (n = 56)

List of medications that should be avoided based on Beers criteria 2015 independent of diagnosis		
Name of drug	Strength of recommendation	Number of patients
Digoxin	Strong	14 (25%)
Spironolactone	Strong	11 (19.64%)
Indomethacin	Strong	5 (8.92%)
Nitrofurantoin	Strong	2 (3.57%)
nifedipine	Strong	3 (5.35%)
Diazepam	Strong	5 (8.92%)
Promethazine	strong	2 (3.57%)
Amiodarone	strong	2 (3.57%)
Amitriptyline	Strong	3 (5.35%)
Metoclopramide	strong	2 (3.57%)
Dicyclomine	strong	3 (5.35%)
Potentially inappropriate medication use due to drug-disease or drug-syndrome interactions		
Ranitidine	Strong	3 (5.35%)
Fluoxetine	strong	1 (1.78%)

Certain risk factors were found to be associated with PIM. These risk factors increased the

likelihood of inappropriate medications. Age was the demographic factor influencing the chance of PIM; the number of medications prescribed, some diagnosis and length of hospitalisation were the clinical variables exerting influence on the chance of getting PIMs in patients (Table 2).

Table 2: Number of patients in each group, Prevalence of PIM and Predictors of PIM using Beers Criteria 2015

Variable		Total (n)	Patients with IMU	Patients with AMU	Prevalence (%)	Odds Ratio (CI 95%)	P value
All		480	56	424	11.66		
Age	65-74	265	40	225	15.09	1 (reference)	
	75-84	152	12	140	7.89	2.074 (1.052- 4.088)	0.035
	85 ≤	63	4	59	6.34	2.622 (0.902-7.622)	0.076
Gender	Male	218	29	189	13.3	1 (reference)	
	female	262	27	235	10.3	1.335 (0.764-2.333)	0.309
No of medication	≤ 6	305	47	258	15.4	1 (reference)	0.0013
	6 >	175	9	166	5.14	3.36 (1.6-7.03)	
Length of stay	≤ 5	198	32	166	16.16	1 (reference)	
	6-10	207	20	187	9.66	1.802 (0.992-3.272)	0.052
	11 ≥	75	4	71	5.33	3.421 (1.166-10.035)	0.025
No of diagnosis	1	54	14	40	25.92	1 (reference)	
	2	150	26	124	17.33	1.669 (0.795-3.502)	0.175
	≥ 3	276	16	260	5.79	5.687 (2.579-12.541)	P < 0.0001

The prevalence of inappropriate medications was reported to 32 physicians, and their feedback was obtained (Table 3).

Table 3: Physician's response to various types of suggestions

Suggestions	Accepted	Not accepted	Percentage
Further information is required for taking a clinical decision	20	12	62.5%
ADR monitoring should be done	24	8	75%
Specific laboratory test should be done	19	13	59.375%
Use drugs with caution	25	7	78.125%
The drug should be avoided	6	26	18.75%
Drug dosing should be changed	18	14	56.25%

Discussion

One of the important safety concerns in prescribing practice especially for old people is inappropriate prescription [20]. The inappropriate prescription is a major concern in countries like India which have a lot of population and logically there are a lot of geriatric patients among them that may suffer inappropriate prescription which causes health issues to the patients and increases the financial burden of the treatment for the patient and the society.

Inappropriate prescribing for older patients in other countries has been well-documented with the estimated prevalence ranging from 11% to 43% [21]. Our study showed a low value of PIM in the range of PIMs documented in other countries, but still, it is an issue of concern because 11.66% is considered a high value for inappropriate prescription especially for geriatric patients who are more vulnerable to inappropriate prescription comparing to young patients.

Different factors can lead to the difference in the prevalence of PIP in different countries and among those factors are demographic characteristics of patients, disease status of the patient, the difference in prescribing patterns, physician specialities, sample size and drugs which are marketed in different counties [21].

The results of our study showed the average of 11.66% of prevalence of PIM, 15.09% was for the age group of 65-74, 7.89% for the age group of 75-84 and 6.34% for the patients who were in the age group of ≤ 85 years. This is showing that the prevalence of PIM has decreased with ageing. Our result regarding the decrease of the prevalence of PIM with ageing was supported by one study which had been performed in Brazilian outpatient setting [22].

Based on our results males had a higher rank of getting PIM (13.3%) comparing to females (10.3%), but gender was not found to be a predictor of getting a PIM in our study because the difference between two groups was not statistically significant (P = 0.309).

Considering the number of medications, higher prevalence of PIM was found to be for the group with having ≤ 6 medications (15.4%) followed by the group of having seven or more than seven medications (5.14%).

Length of stay in hospital was the fourth variable which was considered in our study. The results showed the highest prevalence for the group with ≤ 5 days stay in the hospital (16.16%). The next group was found to be the group of 6-10 days stay in the hospital (9.66%), and the lowest prevalence was for the patients with 11 ≤ days stay in the hospital (5.33%).

Based on our findings, patients with three diagnoses had the lowest prevalence of PIM (5.79%) followed by patients with having two diagnoses (17.33%), and the highest prevalence was found to be for the patients of having one diagnosis (25.92%).

In this study, we found digoxin as the most common PIM (25%) followed by spironolactone (19.64%); Indomethacin and Diazepam were the next PIMs (8.92%); Nifedipine, Amitriptyline, Dicyclomine and Ranitidine were the next drugs (5.35%). Nitrofurantoin, Promethazine, Amiodarone, Metoclopramide were next, and finally, Fluoxetine got the lowest rank of PIMs (1.78%). The similarity in the prescription of inappropriate medication can be seen in another study which had been done in India [21].

Predictors of potentially inappropriate medication

The effect of five possible predictors that may increase the chance of getting a PIM was assessed. The predictors we considered were the age of the patient, sex of the patient, length of stay in the hospital, number of drugs prescribed and number of diagnoses.

In our study, age was found to be a predictor of getting a PIM. The odds ratio of getting a PIM for

the age group of 75-84 was found to be 2.074 (p = 0.035), so our study shows that being in the age group of 75-84 is the predictor of getting a PIM.

Our study showed that gender is not a predictor of getting a PIM (p = 0.309). Some medications were found to be a strong predictor of getting a PIM. Having more than 6 medications comparing with having 6 medications or less gives the odds ratio of 3.36 (p = 0.0013) which indicates that being in the second group (medication > 6) increases the chance of getting PIM more than three times.

Based on our data analysis, length of stay in hospital was a predictor for a PIM. Odds ratio of getting a PIM with staying 6-10 days in hospital compared with staying ≤ 5 days was estimated to be 1.802 (p = 0.052) which was not statistically significant; However, staying in the hospital for 11 days or more increases the chance of getting a PIM more than 3 times (odds ratio of 3.421, p = 0.025). Having 3 or more disease was found to be a strong predictor of getting a PIM (odds ratio = 5.687, p < 0.0001)

Feedback of the physicians to suggestions

The percentages of the acceptance of the suggestions given by the researcher to the physicians were not very high. The low acceptance rate can be due to various reasons: first, suggestions were given to the physicians, but alternative options were not given. This might be the reason for low acceptance. The second reason for physicians' resistance to accepting the suggestions may be the fact that the suggestions were given by the fifth-year student of pharmacy, so the physicians might feel that the students' knowledge was very low compared to their knowledge. Therefore, the lack of acceptance might be originated from the lack of acceptance of the students' knowledge level by physicians. Third: clinical pharmacy is a new subject of study in India and clinical pharmacists are not well accepted in Indian setting by the physicians, so Lack of credence on a pharmacist as a clinical expert might lead to this result. Fourth: some physicians do not have faith in explicit criteria when making a clinical decision, and they rely on their professional judgment.

Still, there may be other reasons for the low acceptance percentage of suggestions given to the physicians. This study only estimated the acceptance rate of the physicians and did not analyse the reason behind that. Other studies in future should analyze this issue because it can be very helpful in building up a good rapport between the physicians and pharmacists which finally can end up in better clinical care to patients.

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Complications and Risks of Percutaneous Renal Biopsy

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Abstract

BACKGROUND: Renal biopsy performed in native and transplant kidneys is generally considered a safe procedure.

AIM: In this study, we evaluated renal biopsy complications and risk factors in one nephrology facility.

MATERIAL AND METHODS: We conducted a three-year retrospective study on patients who underwent renal biopsy between January 2014 and December 2016. Strict written biopsy protocol was followed. Clinical and laboratory data were obtained from medical charts. Complications were categorised as minor and major, according to the need for intervention. Minor complications included macrohematuria and/or hematoma that did not require intervention. Major complications included hematuria or hematoma with fall of hematocrit that required a blood transfusion, surgery or caused death. A binary logistic regression model was used to analyse the possible factors associated with complications after the biopsy.

RESULTS: We analysed 345 biopsies; samples were taken from patients aged from 15-81 years, of whom 61% were men. A total of 21 (6%) patients developed a complication, 4.4% minor and 1.7% major complications. There were no nephrectomy or death due to biopsy intervention. Overweight patients, as well as those with higher creatinine, lower hemoglobin, higher blood pressure and biopsy due to AKI had higher chances to develop complications ($p = 0.037$, $p = 0.023$, $p = 0.032$, $p = 0.002$, $p = 0.002$, respectively). The patients' age, gender, kidney dimension, number of passes and uninterrupted aspirin therapy were not found as significant predictors of complications. In the multivariate logistic model, body weight (OR = 1.031, 95%CI = 1.002-1.062), lower hemoglobin (OR = 0.973, 95%CI = 0.951–0.996) and hypertension (OR = 1.025, 95%CI = 1.007-1.044) increased the risk of complications in biopsied patients.

CONCLUSION: Renal biopsy is a safe procedure with a low risk of complications when strict biopsy protocol is observed. Correction of anaemia and blood pressure is to be considered before the biopsy.

Introduction

In spite of the continuous research on new biomarkers and other renal function non-invasive diagnostic tools, percutaneous renal biopsy remains a gold standard procedure for the diagnosis of renal disease [1]. It is performed in native and transplanted kidneys and is generally considered a safe procedure [2], [3]. The use of ultrasound guidance and automated biopsy gun provide a low risk of complications such as pain, bleeding, or a small hematoma. Major complications, including the need for nephrectomy or death, are extremely rare [4]. However, controversies persist regarding the optimal assessment and management of the bleeding risk [5], different pre and after procedure protocols, especially for the solitary native kidney, the optimal duration of observation and the possibility of performing it on an outpatient basis. Potential risk factors for bleeding complications are the female sex, elevated blood pressure, disturbed hemostasis and low haemoglobin level before intervention [6]. The risk of bleeding appears to be lower for transplant than for native kidney biopsy [7]. An outpatient programmed biopsy is considered to be with lower risk because of the better clinical status of the patient. It is also lower for the protocol transplant biopsies. On the other hand, for biopsies in patients with higher creatinine and acute kidney insufficiency, the risk of bleeding is expected to be higher [5].

In this study, we evaluated the kidney biopsy complications and the risk for bleeding in our nephrology facility.

Material and Methods

Study design

A retrospective observational study was performed with biopsied patients, hospitalised in the nephrology academic tertiary clinic.

Study population

We analysed the medical charts of all the patients who underwent renal biopsy (native or transplant), between January 2014 and December 2016. All subjects were 15-81 years of age, free of infection. All of the study procedures were conducted by the written protocols, and informed consent was provided.

Data collection

We collected data for the clinical parameters including gender, age (years), body weight (kg), history of hypertension, acute or chronic renal disease and proteinuria. Hypertension was defined as an average home systolic blood pressure higher than 140 mmHg before medication. Serum data were collected: creatinine (μmol/L), haemoglobin (g/L), platelet count ($x10^9$/L), prothrombin time (seconds) and activated thromboplastin time (seconds). Post-biopsy urinary tract infection was defined as symptoms of frequency, urgency, or pyuria. Hematoma or hydronephrosis was detected by ultrasound. The number of biopsy attempts and the number of obtained tissue cores was noted for each biopsy.

Biopsy protocol

Before biopsy, patients were controlled for systolic blood pressure (not to exceed 180 mmHg). Antiplatelet or antithrombotic agents (e.g. aspirin, GPII/IIIa inhibitors, dipyridamole and nonsteroidal inflammatory drugs) were discontinued at least 5 days before biopsy and the prothrombin time had to be normalised. Pentoxifylline was not to be taken within 1 day before the biopsy. One day before the biopsy, platelet count, prothrombin time and activated partial thromboplastin time had to be normal. A biopsy was not performed in patients with platelets under 100 and abnormal coagulation. The biopsy procedure, its risks and benefits were explained to the patient. The biopsies from the native kidneys were performed under real-time ultrasound guidance in a prone position with a pillow under the abdomen in order to reduce lumbar lordosis. Transplanted patients were placed in supine position. The patients cooperated by holding their breath for a few seconds. A spinal needle was used to locate the capsule of the lower pole and to provide local anesthesia for the biopsy. Two cores of renal tissue measuring one cm in length were obtained. An automated spring-loaded biopsy device and size of the needle 16 G were used. Immediately after biopsy, check for any bleeding hematoma by ultrasound was performed. Patients were instructed to maintain a supine posture in bed for several hours and bed rest overnight was recommended for programmed biopsy admissions. Vital signs were closely monitored after the biopsy. If any gross hematuria, back or abdominal pain, dizziness or nausea were noted, urinalysis, haemoglobin and serum examinations were conducted. Additional imagining investigations including additional sonography were performed when clinically indicated at the discretion of the attending physician. Complications (hematoma, hematuria, hydronephrosis, blood transfusions, haemoglobin decline, angiographic intervention, nephrectomy and other treatments or death) were all recorded. Complications were categorised as minor and major. Minor complications included macrohematuria and/or hematoma that did not require intervention such as blood transfusion or angiography. Major complications included hematuria or hematoma with fall of hematocrit that required a blood transfusion, angiography, surgery or caused hypotension that required intervention.

Statistical methods

Data were expressed as the mean ± standard deviation for continuous variables and as frequency/percentage for categorical variables. Demographic and clinical characteristics of the entire cohort were recorded. A binary logistic regression model was used to analyse the possible factors associated with complications after the biopsy. A P value of less than 0.05 was considered significant. All statistical methods were performed using the SPSS statistical software package, version 17.0

Results

We analysed 345 biopsies performed in 342 patients with the native or transplanted kidney in three consecutive years, with a mean of 115 biopsies per year. Baseline demographics are shown in Table 1. Female patients and those with transplanted kidney were less frequently biopsied (39% and 14%, respectively). In a large percentage of patient's elective biopsy was performed (69%) and in 43

patients the indication was due to acute kidney injury (AKI). The mean systolic blood pressure was under 140 mmHg (136.02 ± 21.72 mmHg), but the history of hypertension was present in a vast majority of the patients (74%). The size of the biopsied kidney varied between 87-155 mm and mean pre-biopsy creatinine level was 263.79 µmol/L. Patients body weight varied between 30-125 kg, 29% were ≥ 80 kg. In 1.4% of the patients, therapy with aspirin was not interrupted due to biopsy. Technical success (a produced specimen) was achieved in 339 (99.12%) patients. Specimen inadequacy occurred in 3 (0.88%), and those biopsies were subsequently repeated. Mean pre-biopsy haemoglobin level was 116 g/L and it dropped after biopsy by less than 10 g/L in 12.6% of patients and by more than 10 g/L in nearly 7% of patients.

Table 1: Demographic and clinical data of the patients

Baseline data	N (%) mean ± SD
Male (%)	211 (61)
Mean age (years)	47.75 ± 15.5
Body weight (Kg) >80 kg	99 (29%)
Pre-biopsy-Hb (g/L)	116.96 ± 22.49
Hb change (g/L)	1.38 ± 6.78[a]
Hb change >10 g/L (%)	24 (6.9) [a]
Platelets (x10^9/L)	242.03 ± 75.29
Systolic BP (mmHg)	136.02 ± 21.72
Creatinine (µmol/L)	263.79 ± 272.11
History of Hypertension (%)	256 (74)
Solitary native kidney (%)	3 (0.86)
Transplanted kidney (%)	49 (14.2)
Indication due to AKI (%)	43 (12.5)
Elective biopsy (%)	241 (69.9)
Indication due to proteinuria (%)	222 (64.3)
Small sized kidney (<100 mm length) (%)	48 (13.9)
Aspirin (%)	5 (1.4)
Passes	2.09 ± 0.34
Cores	2.02 ± 0.3
Second biopsy (%)	3 (0.88)

Hb – haemoglobin, BP - blood pressure, [a]vailable in 296.

A total of 21 (6%) patients developed a complication (Table 2). There were 15 (4.4%) minor and 6 (1.7%) major complications. The patients who developed major complications received blood transfusion therapy. The most common complication was hematuria (4.9%). Large and small hematoma occurred in 4 patients, but only in two blood transfusion was required. One patient was transferred to ICU because of severe hypotension and one to surgery, but there were no nephrectomy or death due to the biopsy intervention. No urinary infection or hydronephrosis occurred.

Table 2: Complications following a kidney biopsy

Complication	n (%)
Hematuria	17 (4.9)
Hematoma (> 5 cm)	2 (0.6)
Hypotension/shock	2 (0.6)
Minor complications	15 (4.4)
Major complications	6 (1.7)

Identification of significant independent predictors of complications was performed by entering factors in the univariate model of logistic regression. Overweight patients, those with higher creatinine, lower hemoglobin, higher blood pressure and biopsy due to AKI had higher chances to develop complications (p = 0.037, p = 0.023, p = 0.032, p = 0.002, p = 0.002, respectively). The patients' age, gender, kidney dimension, number of passes and uninterrupted aspirin therapy were not found as significant predictors of complications. In transplanted, solitary native kidney and programmed biopsies, the risk was also insignificant. In the multivariate logistic model, the higher body weight (p = 0.037), lower hemoglobin (p = 0.02) and hypertension (p = 0.007) aggravated the risk of complications in biopsied patients (Table 3).

Table 3: Binary logistic regression of factors associated with increased risk for major bleeding

Risk factor	Odds Ratio	95% confidential interval	P-value
Body weight	1.031	1.002 to 1.062	0.037
Hemoglobin	0.973	0.951 to 0.996	0.02
Hypertension	1.025	1.007 to 1.044	0.007

Discussion

Chronic kidney disease is a worldwide problem and determination of the diagnosis by biopsy is of great value in the early treatment of those patients. Renal biopsy is usually recognised as a safe procedure in native and transplanted kidneys [6], [8], [9], [10]. Brachemi's review on large clinical trials provided an estimate of the frequency of complications after renal biopsy with required blood transfusions from 0.3% to 10% and death due to biopsy in less than 0.1% (5). In our analysis, complications that required blood transfusions were classified as major complications, and their number was rather low – 6 (1.7%). Also, we had no deaths or nephrectomies. Hematuria was noted as the most frequent complication in many studies, ranging from 1.9% up to 10% [6], [11], [13]. In our study, not only gross hematuria but also the lightest transient ones were recorded. It occurred in 17 (4.9%) patients. Thirteen of those patients did not need any blood transfusion. Symptomatic hematoma occurred in only two cases (0.6%), which was less than that presented in other studies [14], [15].

In the assessment of the factors associated with biopsy complications, our results confirmed the previously recognised traditional bleeding factors. Higher serum creatinine and AKI were significant confounding factors in biopsy complications in a large multicentric study on 2563 patients [16] but were also found in smaller monocentric studies, as ours [8], [11]. In our results, these factors lost statistical significance in the multivariate model. Arterial hypertension was found to double the bleeding risk in an analysis of 462 biopsies [14]. Similar results were published by the Norwegian registry on 8573 biopsies [6]. Still, there were studies which did not confirm this association [8], [9] emphasising the history of hypertension as a factor and not the present blood pressure. In our study group, the vast majority of patients had a history of hypertension (74%), but only current hypertension

increased the bleeding risk as an independent predictor. As previously published, the lower pre-biopsy hemoglobin level [4], [11] was also associated with higher risk of complications in our patients. Hemoglobin dropped after biopsy for more than 10 g/L in 6.9% of the patients, but blood transfusion was needed in only 1.7%. This fact also partly explained the data that the lower the hemoglobin the higher the risk of need for transfusion. The indication for a kidney biopsy from a solitary kidney remains an important decision with regards to safety issues and a potential nephrectomy [5]. Only three solitary native kidneys were biopsied in the analyzed period of three years. None of those patients showed any complication. In the group of transplanted patients (14.2%), only one patient had severe bleeding, but recovered well. This low grade of complications in graft biopsies has been confirmed in many studies comparing native and transplanted kidneys [15], [17], [18].

Considering the coagulability as a major concern for bleeding complications, before biopsy in all our patients, platelet count was within reference values (Table 1). Antiplatelet or antithrombotic agents were to be withheld 5 days before biopsy. In 5 (1.4%) of our cases, aspirin was not interrupted at the discretion of the physician, and no bleeding complications occurred. Recent studies support the strategy of not stopping aspirin before renal biopsy in native [16] and transplanted kidneys [19], demonstrating a similar safety profile of the procedure. Obesity is a growing problem in modern medicine. Conventional biopsy is difficult to perform in obese patients and hence, an alternative approach has been developed [20]. In Lees study on 2563 native renal biopsies, there was no increased risk in obese (BMI > 30) patients [16]. But in our study, the body weight was found associated with a higher risk of bleeding complication.

In all 342 patients, 345 biopsies were performed with a standard 16 Ga needle, with two or three passes. The number of passes did not affect the risk of complications, which is in line with previous studies [14].

In conclusion, renal biopsy is a safe procedure with a low risk of complications. Careful assessment of the risk for complications should be performed before the biopsy. Strict biopsy protocol must be observed. Correction of anaemia and blood pressure is to be considered before the biopsy.

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Spinal-Induced Hypotension in Preeclamptic and Healthy Parturients Undergoing Cesarean Section

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Abstract

BACKGROUND: There is a widespread belief that spinal anaesthesia in patients with preeclampsia might cause severe hypotension and decreased uteroplacental perfusion. This study aimed to evaluate the incidence and severity of spinal induced-hypotension in preeclamptics and healthy parturients.

METHODS: Total of 78 patients (40 healthy and 38 preeclamptic) undergoing a C-Section with spinal anaesthesia were included. Spinal anaesthesia was performed with a mixture of 8-9 mg isobaric 0.5% bupivacaine, 20 mcg fentanyl and 100 mcg morphine (total volume 2.2-2.4 ml). Blood pressures (BP)-SBP, DBP, MAP were recorded non-invasively before performing spinal anaesthesia and at 2.5 minutes after a spinal puncture.

RESULTS: The BP falls (%) from baseline were significantly greater in the healthy parturients compared to those with preeclampsia (25.8% ± 10.1 vs 18.8% ± 17.0 for SBP, 28.5% ± 8.8 vs 22.5% ± 10.4 for DBP, and 31.2% ± 14.2 vs 18.2% ± 12.6% for MAP, $p < 0.05$). The incidence rate of hypotension in the preeclamptics was 25% compared to 53% in healthy parturients ($p < 0.001$). Higher doses of vasopressors both ephedrine (16.5 ± 8.6 vs 6.0 ± 2.0 mg) and phenylephrine (105 ± 25 mg) in the healthy women were required. There was no need for phenylephrine treatment in the preeclamptic group.

CONCLUSION: This study showed that the incidence and severity of spinal-induced hypotension in preeclamptic patients are less than in healthy women. The use of low dose spinal anaesthesia also contributed to this statement.

Introduction

There is a widespread belief that that spinal anaesthesia in patients with preeclampsia might cause severe hypotension and decreased uteroplacental perfusion. However, several studies had shown that the risk of spinal hypotension seen with spinal anaesthesia in preeclampsia is not as effective as it was believed, especially when a low dose of spinal anesthetic was used [1], [2]. In fact, studies show that parturients with severe preeclampsia experience less frequent and less severe hypotension than healthy parturients [3]. The aim of this study was to evaluate the hemodynamic effects of spinal anesthesia in patients with preeclampsia, as compared to healthy parturients undergoing Cesarean delivery.

Patients and Methods

Seventy-eight (78) parturients, 40 healthy (group SA H) and 38 preeclamptic parturients (group

SA PE)-for a period of 2 years (2015-2017) were included in this study after providing informed consent and Ethic committee approval.

Inclusion criteria were parturients defined as preeclamptic, which means: a systolic blood pressure (SBP) of 160 mmHg or higher, or a diastolic blood pressure (DBP) of 100 mmHg or higher, or both, associated with proteinuria > 3 g/24 hours. All the preeclamptic patients were treated with a 4.0 g loading dose of intravenous magnesium sulfate (Mg SO4), followed by an -1.5 g/h infusion for 48 hours as seizure prophylaxis. Methyl-dopa or nifedipine, or both, was given for blood pressure control, but this antihypertensive protocol was not standardised and was left to the choice of the obstetrician or anesthesiologist. Mg therapy was discontinued just before the operation; antihypertensive drugs were excluded for at least 4 h before spinal puncture.

Exclusion criteria were the parturients with severe fetal distress or those in labour, placental abruption, placenta praevia, cord prolapse or less than 30 weeks' gestation, twin pregnancy; signs of hypovolemia, HELLP or coagulopathy (< 85,000), oligoanuria, cerebral or visual disturbances.

Before performing the spinal puncture, once after the first call, preoperative IV fluid administration equal to a maximum of 500 ml 0.9% saline for preeclamptic and 15 mL/kg for the healthy group of 0.9% saline was administered over the 15-20 minutes with the patients turned to the left lateral tilt. After skin disinfection, a 26-27 G Pencan needle was inserted at the L3-L4 or L2-L3 vertebral interspaces. Spinal anaesthesia was performed with a mixture of 8-9 mg isobaric 0.5% bupivacaine, 20 mcg fentanyl and 100 mcg morphine (total volume 2.2-2.4 ml) in the sitting position. Each patient was then placed in the supine position with a left lateral tilt of 15-20 degrees. All of the patients in both groups continued to receive 1.000-1.500 ml of 0.9% saline after the spinal puncture and during the operation. The height of the sensory block was assessed, and after achieving an adequate sensory block (T4 level), the procedure was initiated.

Patients were monitored with non-invasive automated blood pressure cuffs, ECG, pulse oximetry and capnograph.

Heart rate (HR) and blood pressure (BP) were recorded before performing spinal anaesthesia and at 2.5-minute intervals for 10 minutes after the puncture, and then every 5 minutes until the end of the surgery. Hypotension was defined as more than a 20% decline in mean arterial blood pressure (MAP) below the baseline in both groups and decrease of systolic blood pressure (SBP) less than 100 mmHg in healthy parturients.

Hypotension was treated with boluses of 5 mg IV ephedrine, and if it persisted, IV phenylephrine 50 mcg was given following 10 mg ephedrine. The total amounts of IV administered fluid, and the total doses of ephedrine (phenylephrine) were recorded as well. The largest and lowest value of maternal hypotension and HR from the baseline were also recorded and compared.

Data are presented as number, median and range, mean ± SD, or percentage as appropriate.

Fisher's exact test was used for intergroup comparisons of the incidence of hypotension and the upper sensory level and the incidence of changes in HR. Student t-test was used to detect a significant difference for difference of means. A p value of less than 0.05 ($p < 0.05$) was considered to indicate statistical significance and was highly significant if $p < 0.001$. Data was compiled in Microsoft Excel worksheet.

Results

Total of 78 patients, 40 healthy (group SA H) and 38 preeclamptic parturients (group SA PE) were included in this study. No spinal patient was excluded because of inadequate analgesia or another reason. Patient characteristics: a dose of 0.5% bupivacaine (mg), the upper sensory level at 5 min, spinal puncture to uterine-incision period, the Apgar score at 5 min was similar between groups.

Preeclamptic parturients were older than those in the healthy group, included more nulliparous, and their neonates had a younger gestational age, which was the likely reason for the lower Apgar 1-min scores on neonates in this group. However, four (4) neonates had an Apgar 1-min score < 5 in the preeclamptic group, compared to two (2) in the healthy group (Table 1).

Table 1: Maternal, anaesthetic and neonatal characteristics

Variable	Healthy parturients	Preeclamptic parturients	P value
N	*40*	38	
Age (yr)	25.6	29.0	P < 0.05
Gestational age	37.8 ± 1.8	32.8 ± 2.9	P < 0.05
Nulliparous	8	18	P < 0.05
Volume preload (ml)	740 ± 150	450 ± 130	P < 0.05
Upper sensory level at 5 min, median (range)	T4 (T1-T4)	T4 (T2-T4)	P > 0.05
Dose of 0.5% bupivacaine (mg)	*8* ± 1.4	8 ± 0.6	P > 0.05
Ephedrine dose (mg)	16.5 ± 8.6	6.0 ± 2.0	P < 0.05
Phenylephrine (mcg)	105 ± 25	0	P < 0.001
Incidence of hypotension % (n)	53 (21)	25 (9)	P < 0.001
Duration of hypotension (min)	3.5 (2.0-4.6)	1.2 (1.0-2.4)	P < 0.05
Spinal Punct.-Uterine An incision (min)	12.5 ± 8.6	13.8 ± 4.5	P > 0.05
Apgar score 1 min, median (range)	9 (5-10)	8 (2-9)	P < 0.05
Apgar score 5 min, median (range)	10 (8-10)	10 (5-10)	P > 0.05

In the preeclamptic patients, SBP and DBP were consistently higher than the corresponding values among the healthy parturients, and the same trend was happening to MAP, which was at a constantly higher level in preeclamptic (Figure 1).

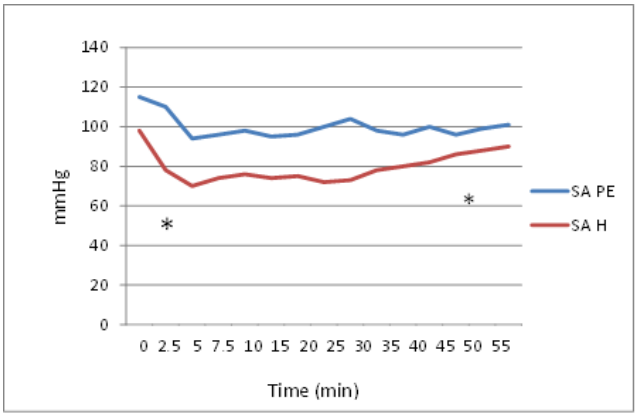

*Figure 1: Change in mean arterial pressure (MAP) after spinal anaesthesia in preeclamptic (SA PE) and healthy parturients (SA H). * Start and end point time for significant differences between mean MAPs in both groups (p < 0.05)*

There was decreased BP after the spinal block in both groups, but the BP falls were significantly greater in the healthy parturients compared to those with preeclamptics: 25.8 ± 10.1 vs 18.8 ± 17.0 for SBP, 28.5 ± 8.8 vs 22.5 ± 10.4 for DBP and 31.2 ± 14.2 vs 18.2 ± 12.6% for MAP (p < 0.05), (Table 2).

Table 2: Changes in blood pressure after spinal anaesthesia

Variable	Healthy parturients N = 40	Preeclamptic parturients N = 38	P value
Hypotension MAP % (n)	53 (21)	25 (9)*	P < 0.001
SBP	128 ±10.0	155 ± 15.0	
Lowest after SA (mmHG)	95 ±16.8	126.0 ± 16.8	
Decrease from baseline %	- 25.8 ±10.1	-18.8 ± 17.0	P < 0.05
DBP	85.8 ± 9.8	100.4 ± 12.8	
Lowest after SA (mmHG)	48 ± 16.8	74.8 ± 10.4	
Decrease from baseline %	-28.5 ± 8.8	-22.5 ± 10.4	P < 0.05
MAP	98.4 ± 15.2	114.8 ± 11.4	
Lowest after SA (mmHG)	70.4 ± 15.0	94.0 ± 12.0	
Decrease from baseline %	- 31.2 ± 14.2	-18.2 ± 12.6	P < 0.05
Heart rate (HR)			
Baseline (beats/min)	102 ± 16.4	94 ± 10.2	P > 0.05
20% increase HR	8 (35)	4 (13.3)	P < 0.05
20% decrease HR	8 (35)	8 (26.6)	P > 0.05

* no decrease of SBP < 100 mmHg in the group of preeclamptic parturients.

The incidence rate of hypotension in the preeclamptics was 25% and was significantly less than that of the healthy parturients (53%), p < 0.001. It should also be taken into account that the preeclamptic parturients were prehydrated with lower volumes of saline (450 versus 740 ml), and secondly, the hypotension under 100 mmHg for SBP was not seen in any parturient from the preeclamptic group.

Furthermore, higher doses of vasopressors, both ephedrine (16.5 ± 8.6 vs 6.0 ± 2.0 mg, p < 0.05) and phenylephrine in the healthy group, were used to correct hypotension. There was no need to use phenylephrine to correct hypotension in the preeclamptic group.

Discussion

The belief that spinal anaesthesia in patients with preeclampsia might produce severe hypotension and decreased uteroplacental perfusion has prevented the widespread use of spinal anaesthesia in these patients. It was traditionally believed that epidural anaesthesia is safer than spinal anaesthesia in preeclamptics because the former was expected to produce a lower risk of clinically significant hypotension, but this method of choice has now been rejected [4], [5]. Concerns that spinal anaesthesia might produce severe hypotension in the preeclamptic population have dissipated as a result of greater familiarity with this technique and less expected complications that follow spinal anaesthesia in this population. Nowadays, spinal anaesthesia has become a priority technique over general and epidural anaesthesia, primarily because of its unique advantages: it's a simple and practical technique, owns rapid onset of action and causes a dense sensory block, less tissue trauma and lower risk of spinal-epidural hematoma. If time allows, it can be used in a setting of acute fetal compromise also.

Also, some studies have been conducted, and reports of the risk of spinal-induced hypotension in preeclamptics are encouraging. In a most rigorous study concerning this issue, a multicenter-controlled trial involving 100 severely preeclamptic parturients, Visalyaputra et al., concluded that differences from spinal-induced hypotension compared to epidural-induced hypotension is not clinically significant [6]. A prospective study by Aya et al., found that the risk of hypotension following spinal anaesthesia in preeclamptic patients was significantly lower than the risk among healthy-term parturients (17% vs 53% in healthy parturients), [7]. Similar to the study by Aya et al., Nikooseresht M. et al., reported that the incidence of hypotension in severely preeclamptics undergoing spinal anaesthesia for C-Section was found to be significantly lower in comparison to the rate among healthy parturients (55% vs 89%). Factors such as the difference in gestational age, the carrying of a smaller fetus, less aortocaval compression, sympathetic hyperactivity, and high vascular tone might have led to this finding [8]. Additionally, some other studies show that parturients with preeclampsia might experience less frequent and less severe hypotension than the healthy ones [9], [10], [11].

The lower incidence of spinal-induced hypotension in preeclamptic patients compared to the healthy ones might be more causative:

1. Preeclamptic pregnancy ends with less gestational maturity carrying lower birth weight neonates (smaller uterine size) compared to a healthy pregnancy. Hence the risk of aortocaval obstruction is lower. For the same reasons, the epidural venous plexuses in preeclamptics are less exaggerated, thus leading to a lower cephalic spread of the local

anaesthetic. Aya et al. suggested that the risk of hypotension following a subarachnoid block in preeclampsia was related to other preeclampsia-associated factors rather than to a small uterine size [9].

2. The vasodilator system in preeclampsia (regulated by the endothelial pathway via endothelial-dependent relaxation of small resistant vessels) has an altered response-thus maintaining a high vascular tone on a constantly higher level, independent of spinal-induced sympathetic blockade, keeping the BP high [6].

3. The circulation of preeclamptic patients contains an increased production of numerous potent vasopressor factors, which also keep BP at a higher level. Also, there is an increased sensitivity of small resistant vessels to the exogenous vasopressor stimulation; this can explain the lower ephedrine dose needed to correct the spinal-hypotension in preeclamptics [12].

Results from our study show that hypotension is greater in healthy parturients as opposed to preeclamptics (53 vs 25%, $p < 0.001$). Spinal-induced hypotension was short-lived (1.2 min) and was easily treated with a low dose of vasopressors. The ephedrine requirement for treatment of spinal-induced hypotension in preeclampsia has been reported to be lower than that required by healthy parturients [12], [13]. Preeclamptics have also been reported to require significantly less phenylephrine to treat hypotension [14]. These results were comparable to our findings in that the total doses of IV ephedrine for treating hypotension were significantly lower for the preeclamptics (6.0 ± 2.0 mg) than for the healthy patients (16.5 ± 8,6 mg, $p < 0.05$). Furthermore, there was no need to treat the preeclamptics with phenylephrine.

Regardless of the previous reasons, we consider that the incidence of spinal anaesthesia induced-hypotension might be related and to the local anaesthetic dose, so a low dose concept should provide a lower incidence of spinal hypotension, but certainly not to the expense of unsatisfactory surgical analgesia [15], [16]. In a pilot study which compared the hemodynamic consequences of two doses of spinal bupivacaine (7.5 mg vs 10 mg) for a C-Section in those with severe preeclampsia, predelivery MAP was lower, and the ephedrine requirements were greater in the 10 mg group [3]. In another study, Roofthoof and Van de Velde had shown that when low dose spinal anaesthesia (6.5 mg bupivacaine) was administered with sufentanil as part of a combined spinal-epidural technique (CSE) in shorter surgeries (less than 60 minutes), the need for epidural supplementation was rare [16].

The originality of this article is that this study includes a concept based on a mixture consisting of low bupivacaine dose (8-9 mg) added to two opioids (lipophilic fentanyl 20 mcg and long-acting hydrophilic morphine 100 mcg) thus providing stable hemodynamics with good surgical anaesthesia and satisfactory postoperative analgesia for the next 24 hours with. Adding (two) opioids to the LA act synergistically, thus strengthening both the analgesic potential of LA and reducing the possibility of LA dose-induced spinal hypotension. The rapid intraoperative analgesic onset of lipophilic fentanyl is well-known, but some authors believe that hydrophilic long-lasting intrathecal morphine could reduce the intraoperative discomfort as well as improve intraoperative analgesia [17], [18]. Other researchers have reached a similar conclusion, and a decrease in intraoperative pain with spinal morphine was seen in some studies [19], [20]. In the event of a short time interval between spinal puncture and the start of a C-Section, Weigl W. et al., also suggest a mixture of two opioids-fentanyl and morphine-addled to LA, thus confirming the previous statements [21].

In conclusion, this study showed that the incidence and severity of spinal-induced hypotension associated with patients undergoing C-Section are less in preeclamptics than in healthy parturients. Like healthy patients, however, preeclamptics may also experience some degree of spinal hypotension, but it is short-lived and easily treated with significantly lower ephedrine dose than in healthy parturients. The concept of low-dosage spinal anaesthesia in preeclamptics can successfully contribute to reducing the spinal-induced hypotension, thus positively influencing both hemodynamics and neonatal wellbeing. However, more patients and further research are needed to find and optimise maternal hemodynamics in preeclamptics undergoing spinal anaesthesia for C-Section.

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Psoriasis Features in Patients with Inflammatory Bowel Disease

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Keywords: Inflammatory bowel disease; Psoriasis; Clinical features

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Abstract

BACKGROUND: Psoriasis and inflammatory bowel diseases (IBD) share common pathways based on immune dysregulation with an important role of tumour necrosis factor-α and Th17 cells, as well as the genetic background. Several studies showed an increased prevalence of psoriasis in IBD patients. However, data regarding psoriasis features in IBD patients are still lacking.

AIM: We aimed to conduct an observational study to assess psoriasis clinical features and its severity in a group of patients with IBD.

METHODS: Dermatological assessment was performed consecutively in 200 IBD patients (123 with CD and 77 with UC) attending the IBD Care Centre of Gastroenterology at the University of Naples Federico II from 2015 to 2016.

RESULTS: A group of 32 from 200 IBD patients (16%) had a familiar history positive for psoriasis, whereas, medical history and dermatologic examination revealed that 18 (9%) IBD patients were affected by psoriasis: 11 out of these 18 subjects (61.2%) had CD, and 7 had UC (38.2%); no significant differences were found between CD and UC groups. Concerning psoriasis severity, the mean psoriasis area severity index score was 3.7.

CONCLUSION: This one-year retrospective study showed that psoriasis and IBD both require the use of immunosuppressive drugs so; we can count on a better treatment outcome for both diseases.

Introduction

Psoriasis and inflammatory bowel diseases (IBD), which typically include Crohn's disease (CD) and ulcerative colitis (UC), are chronic relapsing inflammatory conditions [1]. Association between these diseases is confirmed by a common immune dysregulation [increased pro-inflammatory cytokines such as tumour necrosis factor (TNF)-α and activated Th17 cells] and a shared genetic susceptibility and DNA polymorphisms [2], [3], [4]. However, these diseases also show some differences, such as the efficacy of some agents that highlight discrepancies in their pathophysiology [5], [6]. Despite several studies report an increased frequency of psoriasis in IBD patients, data regarding the clinical features of psoriasis in these patients is limited [7], [8].

We conducted an observational study to assess psoriasis clinical features and its severity in a group of patients with IBD.

Material and Methods

The dermatological assessment was performed consecutively in 200 IBD patients (123 with CD and 77 with UC) attending the IBD Care Centre of Gastroenterology at the University of Naples Federico II from 2015 to 2016. The mean age was 45.7 ± 20.5 years (ranging from 16 to 77 years), of whom 98 were males (49%) and 102 females (51%); the mean duration of IBD was 8.7 ± 4.3 years. Almost 30% (n = 60) of patients were treated with biologic drugs (anti-

TNF-α such as adalimumab and infliximab) for their gastroenterological disease, whereas 70% (n = 140) of subjects received conventional therapies (systemic steroids, azathioprine, methotrexate and aminosalicylates).

Results

A group of 32 from 200 IBD patients (16%) had a familiar history positive for psoriasis, whereas, medical history and dermatologic examination revealed that 18 (9%) IBD patients were affected by psoriasis: 11 out of these 18 subjects (61.2%) had CD, and 7 had UC (38.2%); no significant differences were found between CD and UC groups. As regards to the 18 patients with both psoriasis and IBD, 8 were females and 10 males (mean age 55.3 years, range 25-75) with a mean IBD duration of 6.7 ± 3.6 years. Concerning psoriasis severity, the mean psoriasis area severity index score was 3.7. Mild psoriasis was more frequent compared to moderate-severe psoriasis [16 (88.8%) vs 2 (11.2%); $P < 0.01$ using Student's t-test] and plaque psoriasis was reported as the most common clinical form (n = 17, 94.5%), followed by palmoplantar pustular psoriasis (n = 1, 5.5%). Scalp (9/18; 50.0%), trunk (5/18; 27.7%), extensor surfaces of the limbs (5/18; 27.7%) and genitals (6/18; 33.3%) represented the sites most frequently involved.

Regarding IBD ongoing treatments, 5/18 (27.8%) patients received mesalazine, 3/18 (16.7%) were on azathioprine therapy, 6/18 (33.3%) received adalimumab, and the remaining 4/18 (22.2%) were on infliximab treatment. In patients with both IBD and psoriasis treated with biologic therapy (10/18, 55.5%) the skin disease was not considered a paradoxical reaction to biologics since all subjects had already shown psoriatic skin lesions before starting anti-TNF-α, as well as for their clinical aspect. Topical therapies based on emollients and low potency corticosteroids were able to control psoriatic skin lesions in all patients with both IBD and psoriasis. Anti-TNF-α use resulted more common in patients with both IBD and psoriasis compared to patients with only IBD (55.5% vs 30%) probably for the increased level of systemic inflammation.

Discussion

As reported in previous studies [8], [9], [10], the prevalence of psoriasis in IBD patients was three times that of the general population (9% vs 3%). Furthermore, IBD patients showed a higher frequency of mild psoriasis because of the anti-inflammatory and immunosuppressive activity of the drugs used for IBD, confirming what reported by Eppinga et al., [11]. The aetiology of the coexistence of psoriasis and IBD is still unknown; the correlation among genetic background, immune dysfunction, systemic inflammation, and dysregulation of gut microbiota may represent possible explanations [12]. It has been supposed that this association could be the result of the influence of environmental and immunological factors, such as IL-17 and TNF- α, in genetically predisposed people [11]. Moreover, Lolli et al. speculated that some immunological mechanisms involved in the pathogenesis of psoriasis (i.e. interleukin-23) might play a role in the observed different psoriasis phenotypes in the IBD population [8].

Finally, despite the well-known association between psoriasis and IBD, our observational study showed a very low frequency of moderate and severe psoriasis forms in patients with IBD. Further studies are needed to highlight eventual peculiarities of psoriatic disease in this setting of patients, to set up adequate management strategies.

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Angioplasty with Stenting in Acute Coronary Syndromes with Very Low Contrast Volume Using 6F Diagnostic Catheters and Bench Testing of Catheters

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Abstract

AIM: To safely perform angioplasties in acute coronary syndromes with low contrast volume using Cordis 6F diagnostic catheters and to perform mechanical bench tests on the diagnostic and guide catheters in a radial path model.

METHODS: In 191 patients (242 lesions/268 stents) with acute coronary syndromes angioplasty were performed with cordis 6F diagnostic catheters.

RESULTS: The lesions were present at left anterior descending (121), Left main (5), left circumflex (51), ramus (5) and right coronary artery (60). In 72% of cases, Iodixanol was used. All contrast injections were given by hand. Regular follow-up of the patients was performed at 30 days. The procedures were performed in the femoral route only. Pre-dilatation was performed in 43 cases. Successful revascularization of the target lesion was achieved in all cases. The mean contrast volume used per patient was 28 ml (± 8 ml). Mild reversible contrast-induced nephropathy (CIN) was observed in two patients. Cardiogenic shock was seen in 7 cases, and one death was observed. Pushability and trackability tests showed good force transmission and hysteresis in diagnostic catheters compared to guide catheters.

CONCLUSIONS: Angioplasty with stenting could be performed safely in patients using cordis 6F diagnostic catheters using a low volume of contrast in acute coronary syndromes. Low contrast volume usage would result in a lower incidence of contrast-induced nephropathy and cardiac failures.

Introduction

Angioplasties in acute coronary syndromes (ACS) are challenging, and these procedures are associated with higher morbidity and mortality than elective procedures. Longer procedural times, higher contrast usage, deranged renal parameters and bleeding risk commonly add to the complications in addition to the primary cardiac problems. Hence, minimising these will greatly improve the outcome of the procedure. Contrast-induced nephropathies, as well as contrast related problems, add to complications gambit. Contrast-induced nephropathy is common, and the incidence is about 7 to 10% in primary angioplasties and about 5 to 10% in routine angioplasties [1], [2], [3]. Long-term kidney injuries are frequent in patients with contrast-induced nephropathy [4], [5], [6]. Contrast media are known to have direct cytotoxicity on human kidney cells in vitro evaluation [7].

Some studies [8], [9], [10], [11] have shown higher contrast requirement in radial procedures, or at least it is equal volumes in femoral and radial [11]. Also, the radial routes are known to have increased

radiation dosage to patients and the physicians [12], [13]. Contrast agents are also associated with thrombotic and bleeding manifestations, which was observed in various studies [14], [15], [16], [17], [18]. They are well known to induce platelet degranulation [19]. Also, they can induce renal apoptosis and thereby cause chronic renal failure [20]. This is a report of angioplasty in 191 cases with acute coronary syndromes, which were performed with minimal contrast volume to achieve a higher clinical success of the patients using 6F diagnostic catheters.

Methods

In the initial part of the study, diagnostic catheters were used to perform angioplasty when guide catheters failed to engage in the same patients [21]. Subsequently, when the technique was improved in high-risk patients with acute coronary syndromes, the angioplasty procedures were performed with diagnostic catheters itself.

Angioplasty and stents

In 191 patients' angioplasty with stenting was performed by this technique using 6F diagnostic (Cordis) catheters, and 268 stents were deployed from June 2016 to May 2017. During this period 265 angioplasties were performed in total. The lesions (242) included left anterior descending (121), Left main (5), left circumflex (51), ramus (5) and right coronary artery (60) lesions, and in total 268 stents were used. A variety of coronary stents from various standard companies were used in the procedures. Cardiogenic shock was seen in seven cases. The aim was to reduce contrast-induced nephropathy as well as hemodynamic problems and cardio-renal syndromes. In 72% of cases, Iodixanol was used. All injections were given by hand only. Regular follow-up of the patients was performed at 30 days. All the procedures were performed through the femoral route only. All procedures were performed in Siemens Axiom Artis cardiac catheterisation lab, and the images were acquired at a frame rate of 15 frames/s. Pressure tracings were monitored during the procedure though there was mild dampening the pressure tracings were visible (Figure1).

Pre-dilatation was performed in 43 cases with 57 lesions using 74 balloons (semi-compliant 2 mm x 10 mm and lesser sizes) and in 4 cases, 2.5 x 10 mm and in two patients 3 x 10 mm. In 5 cases short balloons (1.25 x 6 mm or 1.5 x 6 mm) were used with wires to cross the lesions though inflations were not given. For the procedures pre-dominantly floppy wires (Allstar or run through) or balanced middle-weight (BMW) wires were used.

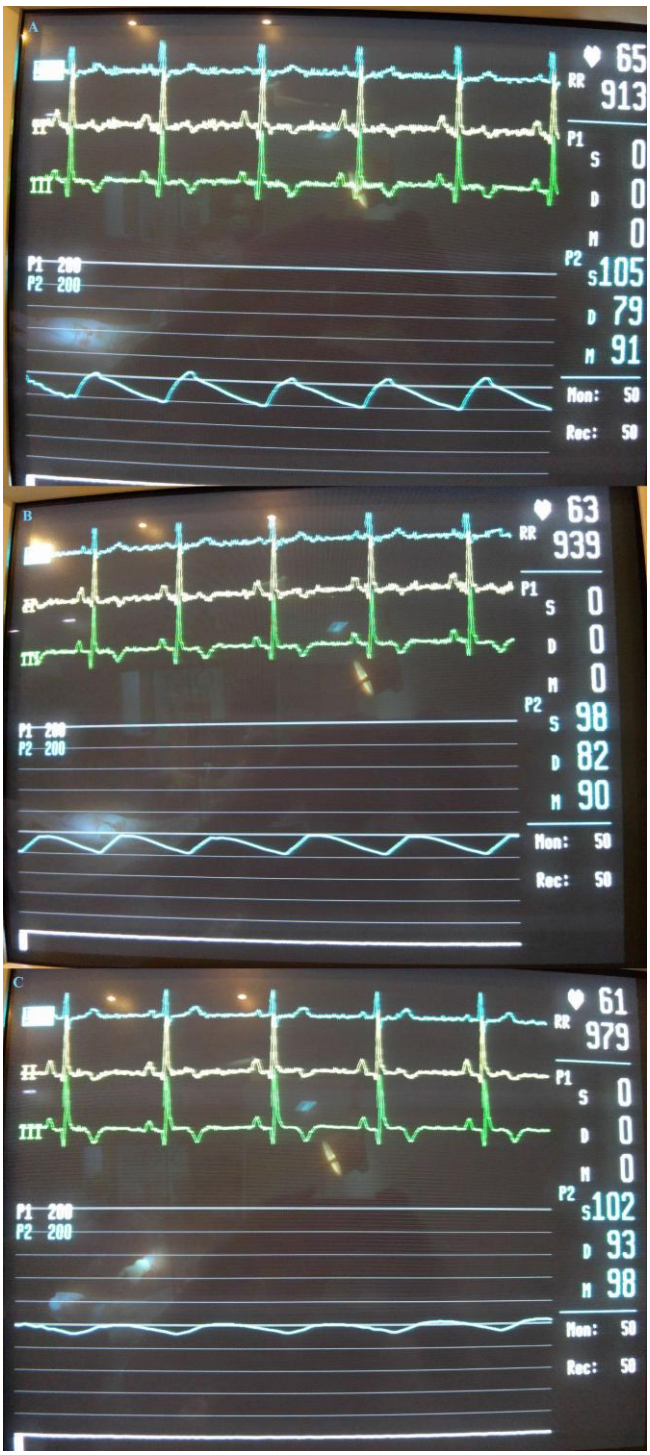

Figure 1: Pressure tracings show the pressure waveforms during angioplasty using a diagnostic catheter. Panel A showed the pressure tracings when a left anterior descending artery lesion was wired. Panel B showed pressure tracings when the stent was being deployed. Panel C showed the pressure tracings from right coronary when a different stent was used for right coronary lesion

Smaller balloon sizes were chosen to minimise the risk of coronary dissections and severe coronary spasms, which would deem further procedure difficult. The stent deployments were usually at 14 to 16 atm (Figure 2).

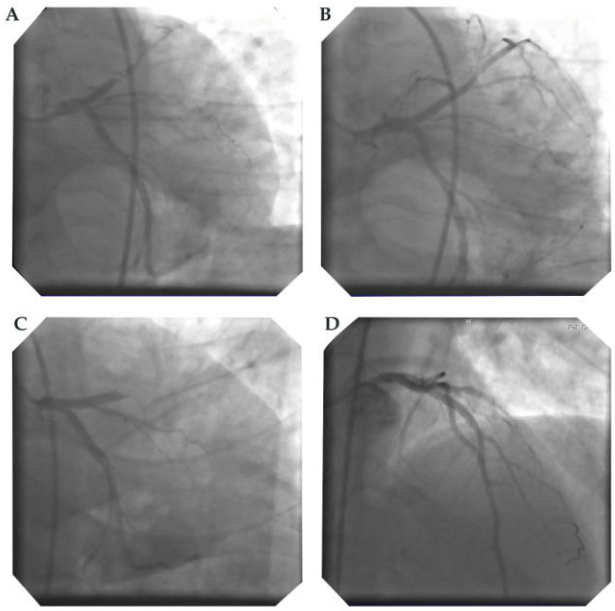

Figure 2: Shows angioplasties and proximal left anterior artery stent deployments. Panels A and B show stenting to proximal LAD with near total occlusion and Panels C and D show stenting to proximal LAD with complete occlusion

Two inflations were given for 5 to 7 seconds with a time interval of about 5 to 10 seconds of deflation, which is the user routine in all the cases. High-pressure deployments (> 16 atm.) were used in only 6 cases. Post-dilatation with the semi-compliant balloons was used in 5 cases only.

In cases with difficulty in wire crossing, balloon-assisted wire crossing was performed, wherein the balloon and wire were taken to the tip, and with gentle manipulation, the wire crosses the lesion. The length of the stents varied from 8mm to 28 mm and the diameter of the stent varied from 2.25 mm to 4.0mm. IVUS and OCT were not performed in these cases. Five patients underwent coronary artery bypass grafting in the past, and stenting was performed in the native vessels (Figure 3). Buddy wires were used in four cases for deployment of the stents.

Medications

Aspirin, clopidogrel, tirofiban and low molecular weight heparin were used in appropriate doses in all patients. Ticagrelor was not used in any of the cases in the first two days of angioplasty, and in four patients at discharge ticagrelor was added after stopping clopidogrel.

Tirofiban was given to all patients, and the dosage was titrated based on the creatinine values. Immediately after stenting a bolus of 10ml tirofiban (Conc. 5 mg/100 ml) was given for one stent forcibly by hand, and if further stents are required extra bolus of 5ml was given through intracoronary route in the diagnostic catheter. When the creatinine levels were > 3 mg/dl, tirofiban was given at 1 to 1.5 ml/hr. Infusion for 14 hours. At normal creatinine values, the standard flow rate of tirofiban was 4 ml/hr. for 18 hours (conc. 5 mg/100 ml). In frail patients, the infusion rate was 3.5 ml/hr. For 18 hours. If there was any minor bleeding Tirofiban was stopped for 1 to 2 hours and restarted at 2.5 to 3 ml/hr. When the creatinine was 2 to 3 mg/dl, the infusion rate was 1.5 to 2 ml/hr. For 12 to 14 hours. The concentration of the tirofiban solution used for bolus and infusion was 5 mg/100ml.

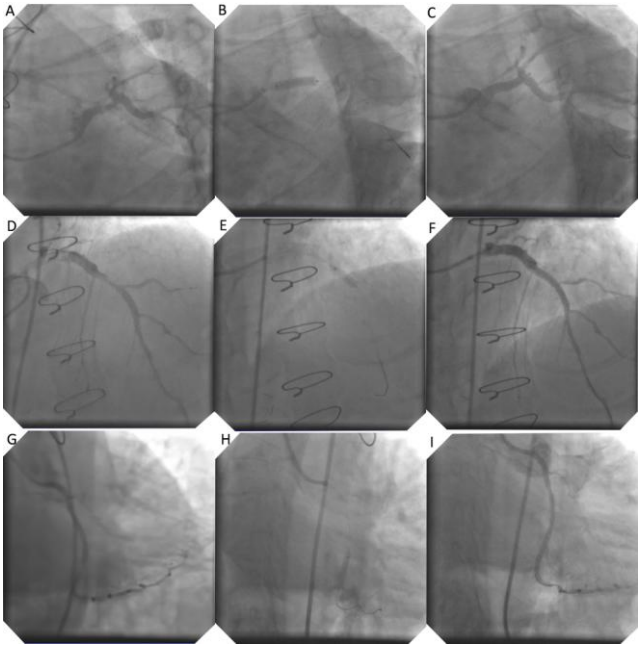

Figure 3: Stenting in patients who underwent coronary artery bypass grafting in the past. Panels A to C show stenting from LMCA to proximal LAD, Panels D to F show stenting in proximal LAD and panels G to I show stenting in proximal LCA

Contrast-induced nephropathy was defined as a rise in creatinine of 0.5 mg/dl from the baseline or increase in creatinine up to 25% from baseline values. N-acetyl cysteine was started orally immediately after completion of the procedure in patients with elevated creatinine. Blood sugars were controlled predominantly by rapid-acting insulin injections and if required insulin infusion. At discharge, the patients were started with adequate doses of (rapid-acting + long-acting, mix) insulin along with diabetic medications. All patients were followed up to 30 days after discharge.

Bench testing through the radial path

Bench testing of the diagnostic and guide catheters was performed. A radial path was created based on anatomy with suitable angles and dimensions. The radial path creation is shown in Figure 4. The path starts with 4 mm and gradually increases in width and joins the aortic arch. The insertion sheath was 6F with 10 cm length instead of the standard 7cm. Details of indexation of the

catheters for pushability and trackability parameters are shown in supplementary figures 1. The average length of the radial path is about 81 cm, and that of the femoral path is 56 cm.

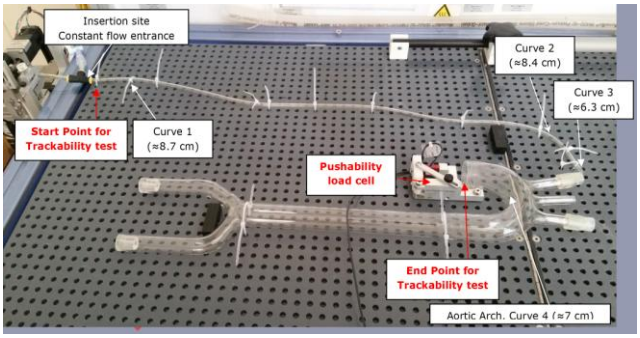

Figure 4: Radial and Femoral paths used in the bench testing evaluation

Results

The baseline characteristics, contrast requirement, and CIN

Table 1 summarises the baseline and patient characteristics. All parameters were approximated to the nearest integer. Successful revascularisation of the target lesion was achieved in all cases. The mean contrast requirement in this retrospective series per patient was 28ml (± 8 ml), and one death was seen. Contrast-induced nephropathy was observed in two patients. Patient 1 had CIN had risen in creatinine from 1.7 to 2.3 mg/dl and returned to near baseline 1.9 mg/dl. This patient had accelerated hypertension and gastritis and vomiting. However, the urine output was good. Patient 2 had an increase in creatinine from 1.1 to 1.5 mg/dl. Patient 2 was 72 years old and had heart failure and moderate left ventricular dysfunction and was on diuretics.

Table 1: Baseline and the patient characteristics in the study

Parameter	Value (N = 191)
Age, yrs	58 ± 12
Sex, M (%)	79
Diabetes, (%)	82
Hypertension, (%)	46
Primary PCI, n	55
Rescue PCI, n	73
NSTEMI (Post MI or Troponin positive), n	47
Unstable angina (Troponin negative), n	16
Baseline chronic renal failure with Cr > 2.0, n	6
Ticagrelor, n	4
Groin hematoma, n	6
Blood Transfusion, n	2
Antibiotics, n	10
Mortality, n	1
Another minor bleeding	3
Mean Fluoroscopy time, min	5.3 ± 1.6

A reversible insignificant rise in creatinine less than the definition criteria for CIN was seen in 5 more cases. CIN was mild, and none required dialysis. The mean fluoroscopy time in this series of patients was 5.3 ± 1.6 min. Coronary perforations and wire breakages were not seen.

Antibiotics

Need-based higher antibiotics (Imipenem-3 and piperacillin-tazobactam-2 cases) were given in patients associated with sepsis (10 patients). Polymyxin B was used in two patients, and vancomycin in three patients. Oral ciprofloxacin and nitrofurantoin were given to ten patients.

Clinical observations

Cardiogenic shock was seen in 7 cases, which was successfully managed with inotropes, and IABP support was kept as a backup. The details of patients with cardiogenic shock are given in a separate paragraph below (Figure 5).

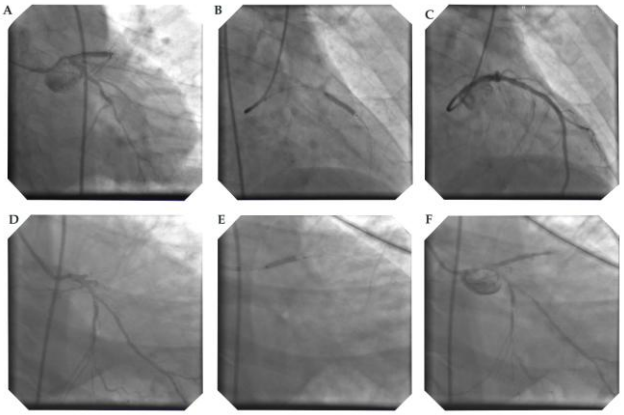

Figure 5: Proximal LAD stenting in patients with cardiogenic shock successfully recovered

Distal vessel spasm was seen in 5 cases, which were managed with a low dose of nitrates. Ventilator support was required in two patients. Mild pulmonary edema/heart failure was seen in 9 cases, which were managed with diuretics and they were managed with oxygen, and in seven cases transiently with non-invasive ventilation. Mild groin hematoma was seen in six cases, and blood transfusion with one unit was required in two patients.

Three patients had high creatinine > 3 mg/dl at baseline (3.1, 3.3 and 3.8 mg/dl). However, after the procedure and N-acetyl cysteine therapy, there was mild fall in creatinine. No mortality was observed in any of these cases.

The radio-opacity of the arteries and the stent was adequate to perform the procedure safely. Reperfusion ventricular tachycardia was seen in three patients, which was managed by DC cardioversion. Ventricular fibrillation was seen in one case, which was successfully reversed with DC cardioversion. Non-sustained ventricular tachycardia was seen in four more cases. Symptomatic transient vasovagal mediated bradycardia and mild hypotension were

seen in 7 cases immediately after stenting and revascularisation, which responded to saline infusion, atropine, and vasopressors. Proximal vessel dissections were not seen by the radiological method in any of the cases. This was observed even after occasional sucking of the diagnostic catheters into the coronaries immediately during withdrawal of stent balloon post-stenting.

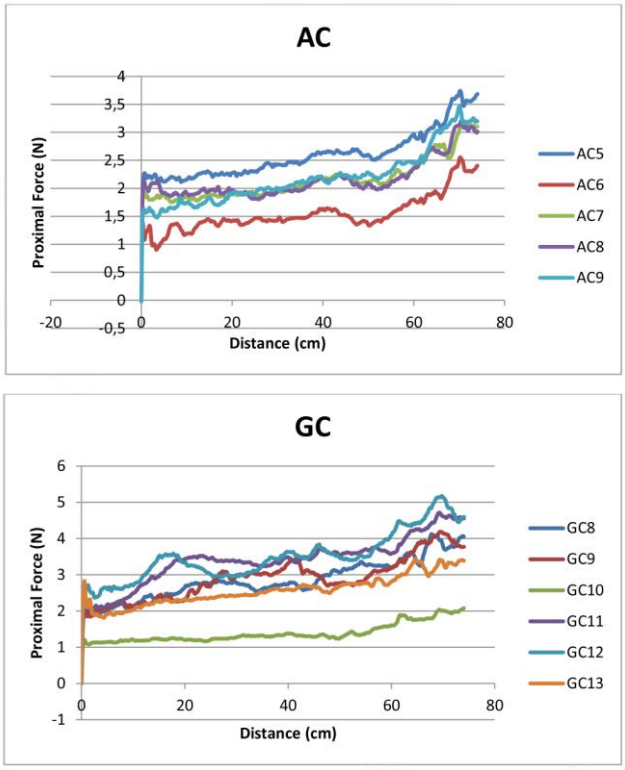

Figure 6: Trackability testing for diagnostic and guide catheters in a radial path

Hydration

Hydration through normal saline was given only when it is required or when transient hypotension was observed. This was seen in 9 cases where fluids were given due to transient hypotension. However, effective oral fluids were started as early as possible after the procedure. In four cases fluids were given > 1 litre, after insertion of the central venous pressure line. In patients with vasovagal or transient fall in blood pressures, fluids were given.

Cardiogenic shock

Cardiogenic shock was seen in 7 patients (Figure 5). Patient 1 had anterior wall infarction with hypotension, tachycardia (heart rate 140/min) and was thrombolysed, Killip 4. Frusemide infusion was started, and angioplasty was performed with 20 ml contrast and stent was placed to proximal LAD. Patient 2 had severe hypotension, tachycardia and anterior wall MI and stent was placed to proximal LAD with 25 ml contrast. The patient had persistent severe hypotension and decreased urine output. Fluid therapy was given with CVP guidance and inotropes. Urine output improved and the patient recovered. Patient 3 had thrombolysis outside and presented with Killip Class 4 and hypotension and tachycardia. Angioplasty with stenting was done to proximal LAD and the patient recovered. The patient also had septicemia, Polymyxin with imipenem was given for seven days, and the patient recovered. He was on a ventilator for one day.

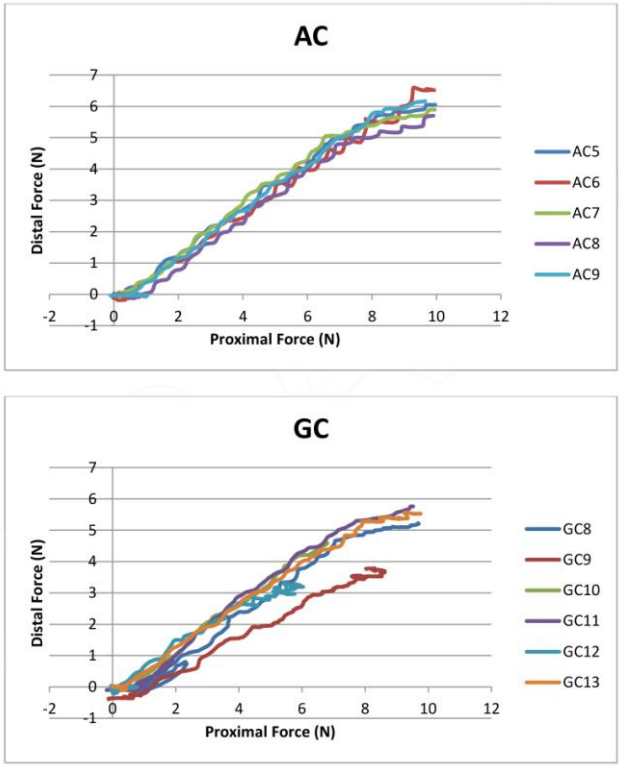

Figure 7: Pushability testing for diagnostic and guide catheters in a radial path

Patient 4 had hypotension and anterior wall MI. After angioplasty, the patient improved. Patient 5 had inferior wall MI and chronic total occlusion of LAD and culprit RCA was stented and patient improved. Patient 6 had old anterior wall MI and chronic total occlusion (CTO) of proximal LAD, and stenting was done to culprit proximal RCA after multiple pre-dilatation, and the patient improved successfully. This patient had ventricular fibrillation immediately after stenting, which was successfully reversed with DC cardioversion. In the last 2 cases, CTO therapy of the LAD was not attempted in the acute setting. Patient 7 was an 82 yr. The old lady who had acute anterior wall myocardial infarction with the massive left ventricular clot and cardiogenic shock. After successful stenting, the patient developed ventricular tachycardia, which was successfully reversed with a shock. This patient lost sensorium due to embolisation of the clot to the brain, and 12 hours later the patient expired.

Radial path pushability and trackability

The possibility parameters of the radial track are shown in figure 6 and Supplementary Figure 2. The diagnostic and guide catheters show similar pushability parameters. The mean ratio of distal to distal force transmission in the femoral approach was 0.28 (± 0.13) for diagnostic, and 0.51 (± 0.11) for guide catheters. The mean ratio of distal/proximal force transmission in the radial track was 0.63 (± 0.07) for angiographic catheters and 0.58 (± 0.06) for guide catheters. The force transmission curve was better with diagnostic catheters. The trackability of the catheters is good up to the 74 cm, and after that the trackability was restricted (Figure 7/supplementary Figure 3). The bench testing in the femoral paths showed a force transmission ratio of 0.28 and 0.51 for diagnostic and guided catheters respectively [21].

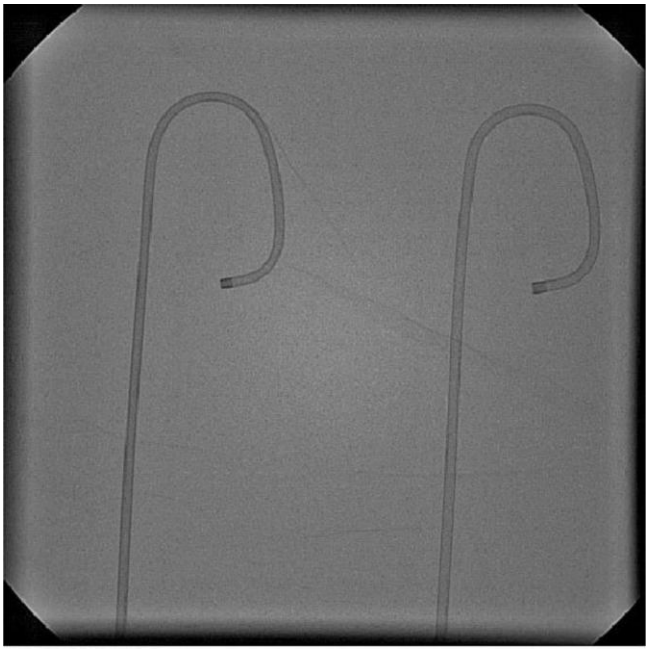

Figure 8: Comparison of the 6F Judkin's left diagnostic (left) and guide catheter (right) by radiological screening. The tapering tip in the diagnostic catheter is seen

Discussion

This is a series of angioplasties performed with diagnostic catheters using limited contrast. Bench evaluation of the catheters showed balanced force-displacement curves, compared to guide catheters. Contrast usage is minimised during the procedure, as the inner lumen of the diagnostic catheters is smaller. All types of coronary stents could be taken through the diagnostic catheters, and stent balloon could be easily withdrawn into the catheters. The bio-vascular scaffold was not used in this series of cases. The mean fluoroscopy time for the procedure was significantly lesser, which was about 5.3 min. In the previous study involving 34 patients in emergency setting [21], the Cordis 6F diagnostic catheters were used for angioplasty and stenting when difficulty in engagement in the coronary ostium was encountered, and the bench testing of catheters was performed in the femoral route. In this study, the 6F diagnostic catheters were used regularly for angioplasty, and bench testing of catheters was conducted through the radial path.

In the past, an IVUS guided study in stable coronary disorders has shown a lesser contrast requirement [22]. However, in acute coronary syndromes, IVUS alone strategy is difficult as the clot is unstable and the available time is limited. Routine hydration to all patients was not given though adequate oral fluids intake was ensured. Hydration is often recommended; however, since the contrast usage was less, it was used only when required [23]. Frequent hydration has its downside that it could worsen heart failure [24], and in the setting of anticoagulation, this can result in acute lung injury/ARDS. In a few patients' intravenous fluids tend to cause rigors.

Clinical implications

The significant reduction in contrast load and fluoroscopy times [25] translate into lesser renal, respiratory injuries and coagulation abnormalities. Even in patients with mild CIN, and oliguria was not seen. While renal injury by contrast, is well known, the worsening of congestive heart failure in the setting of anticoagulation can cause acute lung injuries. These lung injuries could cause ARDS or significant oxygen desaturations which would lead to the development of unnecessary arteriovenous shunts and eventually diversion of about 15 to 20% of the cardiac output [26].

This in the setting of acute coronary syndromes could worsen the ischemia or arrhythmias could result. Also, the contrast media by its action on the platelets can cause coagulation abnormalities which could be thrombotic or even bleeding [14], [15], [17], [18], [19], and it can injure the endothelium [27] resulting in the increase in inflammatory markers [28]. Even infection control is modulated by contrast media volumes [29], and a significant number of cases can have coexisting septicemia. These interactions make patient management difficult. Hence, a reduction in contrast media volume would give good clinical results.

Also, technically, large stents up to 4.5 mm and long stents of about 40 mm size can be used through these catheters. Left main lesions were performed and in left circumflex lesions a slight rotation to align coaxial to the vessel may be required.

Bernoulli's theory, Poiseuille's equation, and Reynolds number

The reduced volume of contrast is well explained by the diameter of the catheter and flow properties. The internal volume of the diagnostic catheter is 1.69 cu mm, and the internal volume of the guide catheter is 2.79 cu mm. The inner lumen of the diagnostic catheter is 1.4 mm, and the inner lumen of the guide catheter is 1.8 mm. When the stent or balloon is inside the coronaries the volume required to visualise the coronaries is still reduced. This is due to more force required to generate the rate of flow, which results in less contrast volume in the lesser space required to opacify the coronaries. This is further explainable by a derivative of Bernoulli's Theorem [30], which is the Poiseuille's equation [31]. Also, when the lumen of the catheter is narrow the volume of contrast wastage at the distal tip is reduced. This is due to better control of Reynold's number or the laminar flow properties at the head of the contrast stream in thinner diagnostic catheters than in large lumen guide catheters. Reynold's number is directly proportional to the diameter of the vessel [32]. This results in more turbulent flow at the tip and higher wastage of contrast in guide catheters. The coefficient of friction is higher in diagnostic catheters than in large lumen guide catheters especially when coated with hydrophilic material. All these reasons explain the usage of low contrast volume in angioplasties performed with diagnostic catheters.

Contrast-induced nephropathy is a major concern during these procedures, and it significantly influences the clinical outcome. Also, pulmonary oedema or congestive cardiac failures are often worsened by contrast volume and pulmonary haemorrhages, the need for ventilation and the duration of the ventilation if required are influenced by renal failure.

Couette flow and dynamic catheter sizes on bending

The diameter of the catheter sizes though fixed in the resting stages dynamically changes with the bending of the catheter [33]. The diagnostic has a tapering tip, near 90° angles at rest and on engagement. The guide has a wider angle (about 110°), which increases engagement (Figure 8). The dynamic diameter is directly proportional to the small bending, and the catheter diameter is less in diagnostic after the engagement. The guide retains the catheter thickness as it has wider bending radius.

Couette flow represents the flow of fluid between two surfaces, and it can be variably differentiated and applied in different flow patterns with or without gradients in the flow [34]. The blood flow velocity and volume in the coronaries during angioplasty is dependent on the catheter engagement angle, Sine (90° Vs. 110°); the effective radius [(effective R^2 = R^2 LMCA – R^2 catheter)], i.e. the difference radius of LMCA and radius of distal end of the catheter; and inversely proportional to the distal tip-length of the catheter after the distal end.

Radial vs Femoral Observations

This lesser ratio of distal to proximal force in the femoral is desirable for better torque control (δπ) of the catheter, which is inversely proportional to time (δt). The trackability of the catheters was good in the radial track with a unique restriction at 74 cm out of the total 81 cm. This correlates anatomically with the brachiocephalic trunk joining the aortic arch. Also, the angular momentum and velocity applying the right-hand thumb rule were directed towards the left shoulder. In the femoral path, which is shorter, the trackability of the catheters was good up to the end of the path, which is near the coronary ostium. These parameters indirectly correlate with the duration of the procedure and contrast usage.

Radial vs Femoral procedures – Angular momentum and velocity

The lesser trackability of catheters in the radial path is due to angular momentum, and angular velocities are directed away from the main axis of torque at the subclavian to brachiocephalic bend. The vector for these parameters follows the right- hand thumb rule, which is directed away from the main axis [35]. This would clinically translate to a slightly lesser control of catheters in the radial path. Large forces or torque are transmitted through the radial compared to the femoral due to thinner radial path compared to femoral. Hence, manual control of catheters tends to be slightly difficult in the radial path.

Frictional energy loss

The frictional energy loss is directly proportional to the lumen diameter of the catheter. In the diagnostic catheter since the lumen is smaller the loss of energy is more, and this limits the flow of contrast. Some latest catheters especially the newer generation guide catheters show improvements in tribology, frequently with hydrophilic coatings. This reduces the frictional energy loss. However, this could lead to additional contrast usage.

5F Guide catheters vs 6F Diagnostic catheters

The 5F guide catheters and the 6F diagnostic catheters have the same lumen dimensions. However, the 6F diagnostic catheters have thick wall compared to 5F guide. Bench testing showed less force to displacement in the 5F guide catheters in the pushability, and distal tip flexion test [21], and the 3-

bend test showed large hysteresis (plasticity) [21]. The higher wall thickness in the 6F diagnostic catheters facilitates direct stenting by providing a better backup force (F = ma). Hence, by comparing these parameters, the 6F diagnostic catheters show better performance.

Acute coronary syndromes vs stable coronary artery disease

Angioplasties in acute coronary syndromes unlike routine angioplasty behave differently in mechanics and hemodynamics, and also in the biological and inflammatory response. The outcome is worsened by long procedure times and high contrast overload. As the ventricle is unstable, arrhythmias, cardio-renal syndromes with transient hypotension frequently complicate the procedure. Also, higher contrast load could release inflammatory markers like IL6, TNF-alpha, MCP-1, which could worsen the scenario, especially in cardiogenic shock [28]. Previous studies have shown elevated inflammatory markers in cardiogenic shock and their association with CIN and poor clinical outcomes.

Buddy wires, covered stents and IVUS

Using this technique two wires can be used in the diagnostic catheter during the procedure with a single stent on one wire. The second wire can be used as a buddy wire or in another vessel for stabilisation. Thin or low profile covered stents (5F) are feasible by this technique. Though IVUS was not performed routinely in these cases, it was performed in 2 cases using the latest 3F IVUS catheters in the diagnostic catheters (supplement Figure 4). The OCT catheters (2.7F) have lesser calibre compared to IVUS catheters (3F) and therefore are compatible with this technique.

Air bubble

During the procedure occasionally, a small air bubble could be formed at the proximal valve of the Touhy Borst (Y-connector). This air bubble can be expelled simply by a gentle flush of saline (2-3 ml) through the manifold after keeping the proximal valve of the Touhy open.

Limitations

Bifurcation stenting was not performed in this series. However, in primary angioplasties, provisional stenting is the treatment of choice. OCT and IVUS were not performed in this series, though these imaging catheters could be well taken inside these diagnostic catheters if required as they are 3F or less in diameter. Also, thrombo-suction was not performed in any of these cases. If the thrombus load is high and thrombosuction is contemplated, guide catheters could be used. Of late, this disadvantage could be overcome with the availability of 5F aspiration catheters with an outer luminal diameter of 1.09 mm, which is easily compatible with 6F diagnostic catheters.

Also, in appropriate cases if the cardiologist is not comfortable or when the procedure is likely to be prolonged or long calcific lesions are encountered, guide catheters could be chosen. However, the simplicity and stability of the diagnostic catheters, the improvised speed of the procedure, lesser fluoroscopy and radiation times, and the favorable lower contrast volume with good clinical outcomes in this series may not be underestimated.

The Artist Psychology and KISSS principle

It is a psychology for the artists to make any artistic procedures meticulous, difficult to perform with perfection [36]. This often compromises the simplicity of the procedure. This series shows lesser pre-dilatations, use of femoral route, direct stenting of lesions and lesser contrast usage with good clinical outcomes when used in appropriate cases. This is also following KISSS principle (Keep it simple, swift and safe), which is often highlighted in bifurcation lesion therapy [37].

During this period the author performed the angioplasties with an expert nursing assistant only. There was no training program during this period. The procedural technique and the decision making to restrict acquiring images, and further therapy with medications were at the discretion of the author. These could have influenced the results.

In conclusion, angioplasty with stenting can be performed safely in patients using cordis 6F diagnostic catheters using low volume of contrast in acute coronary syndromes. Large studies are required to validate the results.

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Case Report

Surgical Outcomes After Fixation of Acromioclavicular Joint Dislocation with Hook Plate and Coracoacromial Ligament Transfer Technique

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Keywords: Acromioclavicular joint; Hook plate; Coracoacromial ligament

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Abstract

BACKGROUND: Treatment of acute and chronic acromioclavicular joint dislocations is still controversial. We aimed evaluation of surgical outcomes after using the combined technique with a hook plate and transposition of the coracoacromial ligament in the treatment of acromioclavicular dislocation.

CASE PRESENTATION: During two years 4 patients (2 acute and 2 chronic cases) were operated with this technique. Three male and one female with an average 37 (26-43) years old were: three on the right and one of the left side. Rockwood classification was used. The evaluation was done according to Constant score - preoperatively, 3 months after the operation and 3 months after the titanium plate was removed.

CONCLUSION: Evaluation of the effectiveness using this combined technique show excellent result in all four patients. No surgical site infection and the favourable cosmetic result were present.

Introduction

Treatment of acute and chronic acromioclavicular joint dislocations is still controversial. Surgical treatment is indicated for fixation of complete acromioclavicular joint dislocation what means rupture of acromioclavicular and coracoclavicular ligaments [1]. Acromioclavicular (AC) joint injuries often occur in men in their third or fourth decade of life due to fall from a height, fall on an outstretched arm and sports injuries. Most of the surgical techniques involve reconstruction of the coracoclavicular ligament and transfer of the coracoacromial ligaments to improve surgical outcomes [2]. A lot of modification of the surgical techniques are described to enhance the mechanical stability of the acromioclavicular joint.

Case Presentation

Between 2015 and 2017 we operate four acromioclavicular joint dislocation gr. V according to the Rockwood classification system (Figure 1).

Three of them were male, and one was female. Mean age was 37 years old (36-39).

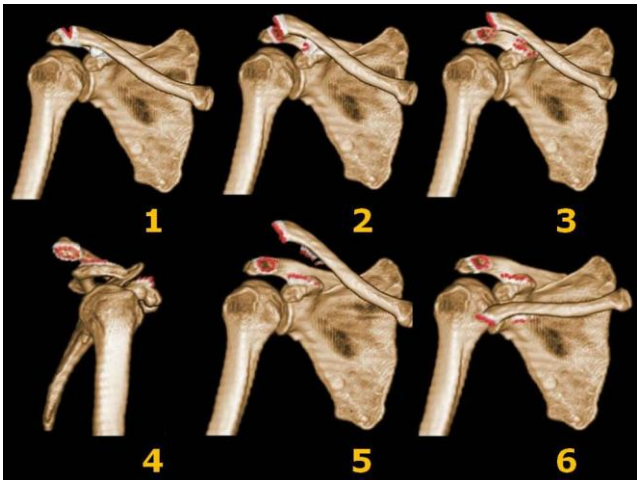


Figure 1: Rockwood classification image

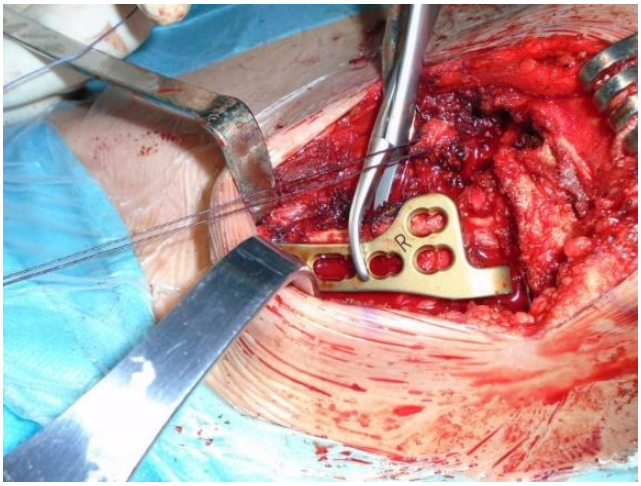
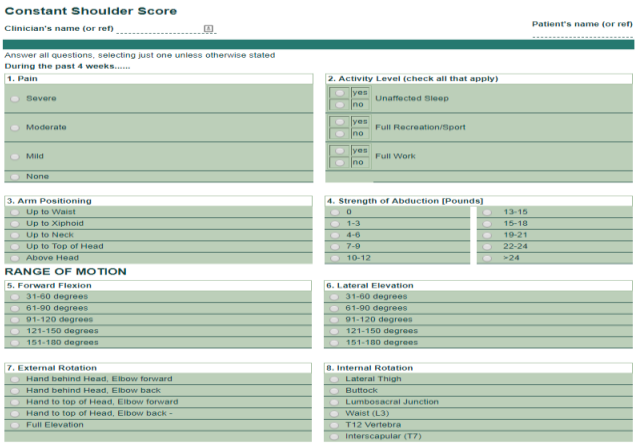


Figure 3: Intraoperative view image

Two of the dislocations were acute treated – until three weeks from the injury, and two of them were chronic more than one month from the injury.

Hook plate was removed between 6-9 months to all patients. Mean follow up was an average of 12 months (11-15 m). The evaluation was done preoperatively, 3 months postoperatively and three months after removing the hook plate. The constant score was used for the evaluation of the surgical outcomes (Figure 2).


Constant Shoulder Score

Clinician's name (or ref) .....

Patient's name (or ref) .....

Answer all questions, selecting just one unless otherwise stated

During the past 4 weeks.....

1. Pain
- Severe
- Moderate
- Mild
- None

2. Activity Level (check all that apply)
- Unaffected Sleep (yes / no)
- Full Recreation/Sport (yes / no)
- Full Work (yes / no)

3. Arm Positioning
- Up to Waist
- Up to Xiphoid
- Up to Neck
- Up to Top of Head
- Above Head

4. Strength of Abduction [Pounds]
- 0
- 1-3
- 4-6
- 7-9
- 10-12
- 13-15
- 15-18
- 19-21
- 22-24
- >24

RANGE OF MOTION

5. Forward Flexion
- 31-60 degrees
- 61-90 degrees
- 91-120 degrees
- 121-150 degrees
- 151-180 degrees

6. Lateral Elevation
- 31-60 degrees
- 61-90 degrees
- 91-120 degrees
- 121-150 degrees
- 151-180 degrees

7. External Rotation
- Hand behind Head, Elbow forward
- Hand behind Head, Elbow back
- Hand to top of Head, Elbow forward
- Hand to top of Head, Elbow back -
- Full Elevation

8. Internal Rotation
- Lateral Thigh
- Buttock
- Lumbosacral Junction
- Waist (L3)
- T12 Vertebra
- Interscapular (T7)

Figure 2: Constant score evaluation form

Surgical techniques - Coracoacromial ligament transposition and hook plate

Patients were operating in the beach-chair position, using transversal incision over the acromioclavicular joint. We use the modifications of the original Weaver-Dunn procedure which include distal resection of the clavicle, transfer of the detached coracoacromial ligament with the addition of a hook plate to improve clinical results and surgical outcomes from the fixation (Figure 3, and 4).

The objective evaluation involves the range of shoulder motion, and subjective assessment includes patient satisfaction and pain score.

We use Constant score for evaluation of surgical outcomes, and we noted average result 91.5 (88-96) at three months after removal of the hook plate (Table 1).

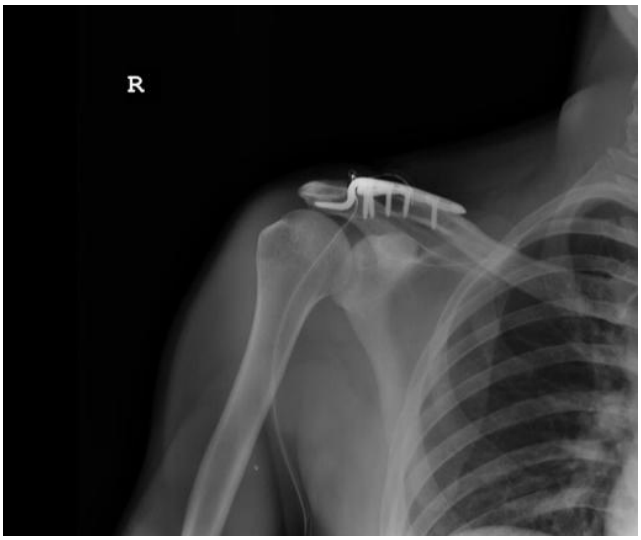


Figure 4: Postoperative x-ray

All of the patients were satisfied with the clinical results, with favourable cosmetic appearance and with an excellent range of motion (Figure 5).

Table 1: Demographic results and surgical outcomes

Sex	acute\chr	mechanism	age	side	type	Constant score		
						Preoperative	3 postop	after removal
1st male	acute	fall from height	43	right	type V	45	87	92
2nd female	chronic	fall on shoulder	38	sin	type V	67	85	88
3rd male	acute	sport injury	40	right	type V	53	87	96
4th male	chronic	fall on arm	27	right	type V	72	85	90

We had one patient with impingement syndrome so, we remove the plate sixth month postoperatively, and one patient develops superficial wound infection which response to wound debridement and oral antibiotic administration.

Figure 5: Clinical results

Discussion

The original method for the surgical treatment of acromioclavicular dislocation is the transfer of the coracoacromial ligament to the end of clavicle [1], [3], [4].

Von Heideken et al. presented excellent surgical outcomes treating acromioclavicular joint dislocations type V using hook plate [2].

The main difference between the modifications of the original surgical technique Weaver–Dunn operation depends from placing the coracoacromial fixation subcoracoid using suture loops [5] or putting the anchors directly to the coracoid, [6]. Another described modification of what we were using-consists of the addition of a hook plate [7]. A comparative study was published by Rolf et al., between two groups of patients, one treated in the acute period and the second one in the chronic phase after unsuccessful conservative treatment. In both groups, the modified Phemister surgical technique was used. According to the results, surgical outcomes were significantly better in the group of patients which was operated in the acute period [9]. Similar results were noted from Mignani et al., comparing results between acute and delayed surgical treatment of the acromioclavicular dislocation. They use the same technique with resection of the distal clavicle and fixation with k-wires. Difference between the clinical results of the two groups was not statistically significant for acute treated injuries [10].

In conclusion, we can accept that this modification of the original Weaver-Dunn procedure with the addition of a hook plate to enhance the mechanical stability of the fixation could be an effective method for treating unstable injuries in acromioclavicular joint.

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Case Report

Ovarian Cancer Immature Teratoma Type in Pregnancy: Management and Feto-Maternal Outcomes

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Keywords: Pregnancy; BEP chemotherapy; Premature ovarian failure (POF); Surgical staging; Immature teratoma

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Abstract

BACKGROUND: Immature teratoma is malignant ovarian germ cell tumours (MOGCTs). The case in pregnancy is very rare which less than 1% of all ovarian teratoma cases. The aim is to reach optimal and comprehensive management for immature ovarian teratoma in pregnancy to gain the healthiest maternal and fetal outcomes.

CASE PRESENTATION: Thirty-one years old female G2P1A0, 8 weeks 1-day pregnancy, with left ovarian solid tumour 15 x 15 x 15 cm in size. At gestational age (GA) of 19 weeks 5 days, the size of the tumour was increasing rapidly to 30 x 30 x 30 cm. Alfa-fetoprotein raised to 699.9 IU/mL and LDH 579 U/L. The patient had gone primary conservative left oophorectomy, omentectomy, and ascites fluid cytology with histopathological conclusion grade II immature teratoma of left ovary containing the immature neuroepithelial and fat component: magnetic resonance imaging (MRI) at 25 weeks 3 days GA, no spreading. Amniocentesis performed at 27 weeks 2 days GA, the fetus had normal 46 chromosomes and sex XX without major structural abnormality. The patient had BEP chemotherapy start at 27 weeks 2 days GA. Patient in labour at 40 weeks 2 days GA. The female baby had spontaneous delivery with 2700 grams in body weight without congenital abnormality. Complete surgical staging performed at 58th days postpartum and histopathological result there was no malignant cell anymore, but post-chemotherapy ovarian atrophy feature had found on the contralateral ovary. The patient showed psychosocial problem including post-chemotherapy depression and premature ovarian failure (POF). Immunohistochemistry (IHC) ER and PR of teratoma tissue showed immature component had ER (-) and PR (+). Follow up of the baby was in good condition.

CONCLUSION: BEP chemotherapy become regimen choice for this case with fetal outcomes was good, but there was a POF sign on the mother. Survival of patient on this case is 62%, free recurrence survival post-BEP 84% and progressivity post complete surgical staging 8% without delay the chemotherapy.

Introduction

Malignant Ovarian Germ cell tumours (MOGCTs) is ovarian primordial cells origin, the most common is dysgerminoma, the second is immature teratoma, and the third is yolk sac tumour. The incidence of MOGCTs in pregnancy is very rare, 1 in 12,500-25,000 of all pregnancy [1].

Immature teratoma is teratoma which contains embryonal neuroectodermal tissue component. The incidence of immature teratoma in pregnancy is considered as < 1% of all ovarian teratoma cases which occur in the first three decades of life and reproductive period. The incidence of immature teratoma in 2017 at Sanglah Hospital Denpasar – Bali was very rare, from 5 cases just 1 case in pregnancy.

The incidence of immature teratoma in pregnancy is very rare which cause a rare consensus about its management strategy. Pregnancy which occurs at the same time with immature teratoma type ovarian cancer will arise vice-versa effects both directly or indirectly for the pregnancy itself, fetus, and the progressivity of immature teratoma. The multidisciplinary approach involves oncologist, pathologist, and perinatologist is needed for early diagnosis, definitive operation procedure, chemotherapy choice, management of delivery, perinatology management, long-term follow up of

maternal and fetal chemotherapy effects, and the necessity of complete surgical staging relaparotomy and evaluation the possibility of metastasis.

Case Illustration

Thirty-one-year-old female, G2P1A0, 8 weeks 1 day GA, with solid ovarian tumour 15 x 15 x 15 cm in size. At 19 weeks 5-day GA, the tumour size became 30 x 30 x 30 cm, solid, rough surface, mobile, and painful. Tumour marker was raising, AFP 699.9 IU/mL and LDH 579 U/L. The patient had conservative primary surgery (left oophorectomy, omentectomy, and ascites fluid cytology). Midline incision performed, Durante operation evaluation with minimal uterus manipulation and seen solid mass 40 x 40 x 40 cm in size, rough surface, left ovary origin, omental attach then adhesiolysis done, rupture, and successfully removed. Internal abdominal organ evaluation: uterus corresponding 18-20 weeks GA, right and left Fallopian tube, right ovary, peritoneum, omentum, and liver normal. Histopathological concluded grade II immature teratoma of the left ovary with the neuroepithelial and fat immature component.

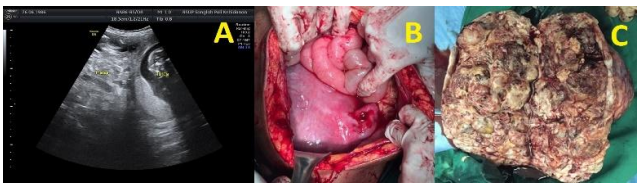

Figure 1: Ultrasonography and Macroscopy of Immature Teratoma Durante Operation. A. Left adnexal mass hypohyperechoic with size that cannot reach by the probe at 19 weeks 5 day GA; B. Uterus size corresponding with 18-20 weeks GA, left Fallopian tube normal, post-oophorectomy; C. Lobulated solid mass with multiseptate cystic inside it.

Patient management post-primary conservative surgery was contrasted MRI at 25 weeks 3 day GA with the result there was no spreading. Chromosome analysis (amniocentesis) at 27 weeks 2 day GA with the result the number of chromosome 46, XX, and not seem abnormality. It was decided to perform 4 cycles BEP chemotherapy administration, started at 27 weeks 2 day GA and stopped 2 weeks before labour (37 weeks 6 day GA) with regular fetomaternal USG evaluation (fetal scanning and fetal well being) every 2 weeks post chemotherapy, not found abnormality, and regular non stress test (NST) at GA > 34 weeks show reactive result.

The pregnancy continued until at term; the female baby was born at 40 weeks 2 day GA, 2700 gram, spontaneous *pervaginam*, APGAR score 7-8, not seen a congenital abnormality. Placental metastasis can occur even though it is very unlikely; the incidence has reported since 1866 just less than 80 cases [2]. It will be better if the placenta is keep being evaluated pathologically, to exclude metastasis. The evidence of fetal metastasis has reported to date just 11 cases [2]. In this case, the placenta was seemed complete born, histopathological examination of the placenta and the amniotic membrane was performed, not seen immature teratoma cell invasion. The postpartum condition of the mother was good and followed up of the baby condition not found the sign of transient myelosuppression, IUGR (the baby born with weight 10%-25% at percentile), no congenital abnormality, no abdominal mass, and the level of AFP in the normal range.

Supporting investigation at 1 week postpartum not found any signs of transient myelosuppression that is leucopenia (WBC count < 5000/mm^3), neutropenia (absolute neutrophil count < 1500/mm^3), anaemia, thrombocytopenia (PLT count < 15.000/mm^3). The baby has WBC count 17,230/mm^3; hemoglobin 21.41 g/dL; Neutrophil 3,240/mm^3; platelets 276,600/mm^3. Alpha-fetoprotein of the patient > 5000 IU/mL which still in normal range (normal level for 2 weeks postpartum: 33.113 IU/mL), it is suggested for routine AFP follow up every month for 2 years. The patient was not suggested to breastfeeding at the chemotherapy period. The patient was not suggested to breastfeeding at the chemotherapy period.

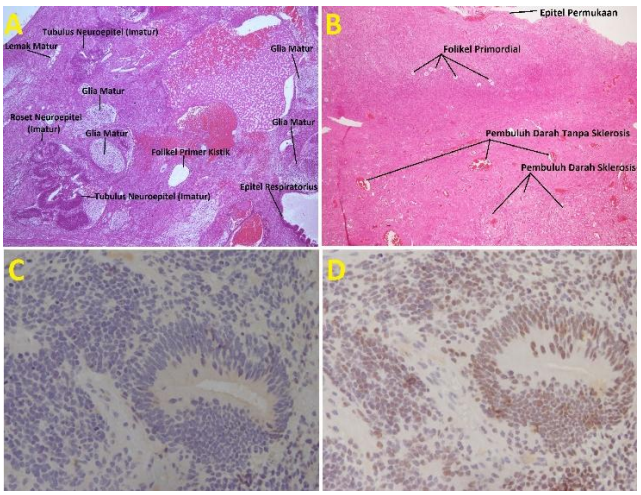

Figure 2: Histopathology of Left Ovarian Immature Teratoma and Atrophy of Right Ovary. A. Immature neuroepithelial component (40 X magnification); B. Cortex contain primordial follicle depletion which some of it did not contain oocyte and blood vessels sclerosis (40X magnification); C. IHC ER-negative and D. IHC PR positive on neuroepithelial component (100 X magnification)

Relaparotomy complete surgical staging including TAH, salpingectomy *sinistra*, SOD, lymphadenectomy pelvic bilateral and paraaortic, omentectomy, also peritoneal biopsy (bilateral paracolic-prevesical-Douglas cavity) performed at 58th days post*partum*. The result of histopathological examination there was no malignant cell and metastasis.

The patient shows the psychological signs of POF including frightening and worry, irritability feeling,

easy to mad, hard to concentrate, behaviour changes, depression, libido disturbance, and FSH level 50.01 mU/L at post relaparotomy complete surgical staging. The result of histopathological examination of right ovary (contralateral) show ovarian atrophy. Immunohistochemistry to detect (estrogen receptor) ER and progesterone receptor (PR) were performed on left ovarian tumour (immature teratoma), with conclusion ER (-) and PR (+), so hormonal replacement therapy (HRT) can be given to managing POF signs with the possibility of tumour to relapse due to hormonal administration is low.

Discussion

The increasing of serum tumour marker during pregnancy often associated with the physiology of normal pregnancy. Tumour markers for diagnosis of the solid ovarian tumour are alpha-fetoprotein (AFP), CEA, LDH, CA19-9, and β-HCG. Immature teratoma often associated with the increasing of AFP and LDH [3]. Imaging for diagnosis of ovarian cancer including USG which have sensitivity 90%, specificity 87%, positive predictive value (PPV) 69% and negative predictive value (NPV) 97% in define of malignancy suspected ovarian mass [4]. Magnetic Resonance Imaging *(*MRI) performed if the diagnosis of USG is uncertain or to evaluate the spreading outside of the ovary. The using of MRI with contrast gadolinium-based is saved during second and third trimester; it proved to have no mutagenic or teratogenic effects [5].

Alpha-fetoprotein is increased in part of a patient with pure immature teratoma which from embryogenic cells, but β-HCG is not increased [6]. In this patient the AFP level 699.9 IU/mL (normal level of AFP at second trimester 22-93 IU/mL), LDH: 579 U/L (normal level at second trimester: 240-480), CEA: 12.43 ng/mL, CA 19-9 216 U/mL. During pregnancy, AFP is produced by the fetal yolk sac and then continued by the liver and gastrointestinal tract, the high level of AFP is suspected for the existence of neural defect abnormalities such as spina bifida, anencephaly, oesophageal defect, the failure of babies abdominal closing, and trisomy 21. Amniocentesis most accurate performed at 16 to 18 weeks of gestational age [7], [8]. The patient had an amniocentesis at 27 weeks 2 days of gestational age for indication of the raising of AFP and searching congenital and also the chromosomal abnormality of the baby. If there is lethal anomaly abnormality, the pregnancy suggested being terminated. The result of amniocentesis analysis was not found chromosomal abnormality, the number chromosome was 46 with fetal sex chromosome XX and was not seen any major structural abnormality, and the result of fetal scanning ultrasonography after 22 weeks gestational age was not found major abnormality and the level of AFP gradually decrease after primary tumour removal until postpartum periods. The increasing of AFP level of the patient highly suspected from the primary tumour (germ cell).

Estrogen and progesterone are increased during pregnancy. High level of estrogen receptor (ER) is often found in ovarian cancer cells. Meanwhile, a tumour with positive progesterone receptor (PR) has a higher survival rate compared with negative PR tumour [8].

The effects of ovarian cancer to pregnancy can be direct or indirect — the direct factor which affected directly such the metastasis to placenta and fetus. Metastasis to the placenta is very rare; it incidence since 1866 have reported less than 80 cases and proved fetal metastasis until now just have been 11 cases [2]. The indirect effects are mediated by operative procedure and chemotherapy effects [3], [5], [8], [9].

Conservative Laparotomy Operation

The side effect of surgery for the baby including exposure of anaesthetic agent, intrapartum and postpartum complication. The effect of anaesthetic agent administration that is hypoxia, hypotension, hypoglycemia, fever, pain, infection or thrombosis can cause serious side effects for the health of the baby. During surgery, it is necessary to minimalise the manipulation and retraction of the uterus to avoid decreasing of uteroplacental blood flow and separation of the placenta. Postpartum period needs adequate analgesic administration for pain can induce premature contraction [10].

The result of several studies, adnexal mass removal during pregnancy is safe enough for the mother and the baby [5]. The second trimester of pregnancy is ‘safety period’ or the best moment for performing surgery intervention of adnexal mass because at this period the dependency of hormonal secretion from corpus luteum during pregnancy is decreased so that the risk of spontaneous abortion is low [2], [5], [11]. There was a tocolytic administration consensus, the tocolytic agent can be given before or immediately after surgery and to be continued 24 to 48-hour post-surgery [12]. Routine tocolytic administration still controversial, but the administration can be considered if there are signs of preterm labour [7], [13]. Tocolytic has benefit on ovarian tumour surgery, from 28 cases is proved that 86% of it had not found any uterine contraction and Mathevet, et. al., (2003) reported that 48 laparoscopy cases at first trimester (n = 17), second trimester (n = 27), and third trimester (n = 4), tocolytic administration just for the indication of ovarian torsion, rupture of cyst, or persistent mass removal. The result showed minimal risk to the mother and the baby with the surgical technique and supporting expert experiences as

consideration. On the cases without tocolytic before or after surgery was not found the signs of preterm labour postoperative. It is necessary to perform minimal manipulation of uterine to prevent contraction.

Adnexal mass detected at trimester I or low malignancy suspected is managed by conservative (observation). But, septal mass, solid, papillary, nodular or persistent until 16 weeks of GA, surgery delay until trimester II (16-18 weeks GA) due to spontaneous abortion risk can reach 10%. Other surgery indications are acute abdomen: pain, rupture, and torsion [13]. Retrospective study show conservative management have high morbidity and mortality due to cystic fluid spill caused by spontaneous rupture [14], [15].

Corticosteroid administration for fetal lung maturation performed a minimum of 48 hours before the operation when it did at 24-34 weeks GA [7]. Adnexal mass detected after 35 weeks GA removed at the same time with *section Caesarea* [5].

Primary conservative surgery at an early stage is unilateral *oophorectomy* or *cystectomy*, *omentectomy,* and peritoneal fluid cytology. In advanced stage (II-IV), the pregnancy considered to be terminated before 24 weeks GA followed by adnexal mass removing and chemotherapy. If the advanced stage tumour found at more than 24 weeks GA, it just performed a biopsy, and following chemotherapy cytoreduction delay until labour [16], [17]. Relaparotomy complete surgical staging or cytoreduction performed after 3-6 weeks post*partum*.

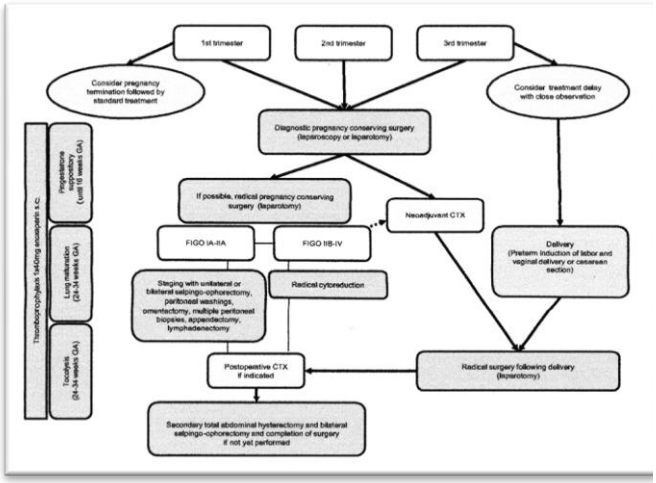

Figure 3: Surgery Management Algorithm of Ovarian Cancer in Pregnancy [18]

Chemotherapy

The Society of Obstetricians and Gynecologist Canada (SOGC) recommended chemotherapy at trimester II with the period of teratogenesis at organogenesis as consideration, so the best time is ≥ 12-14 weeks or trimester III (until 35-37 weeks GA) [19]. Chemotherapy is given on the same dosage as a woman without pregnancy and it without significant side effects for the fetus [20]. Chemotherapy administration at trimester I raise 10-20% the incidence of congenital malformation, fetal death, and spontaneous abortion [4], [5], [19]. Meanwhile, at trimester II and III, it raises the risk of IUGR (7%), low birth weight, preterm labour (5%), and stillbirth (5%). Chemotherapy after 35 weeks GA can raise the risk of neonatal neutropenia. At birth and first weeks of life can be found transient myelosuppression signs which can promote severe infection. At long term follow up can be found neurological development and psychological effects on the child.

The first choice chemotherapy combination for MOGCTs is 4 cycle every 3 weeks of *bleomycin-etoposide-cisplatin* (BEP). *Bleomycin-etoposide-cisplatin* has a recurrence-free survival rate of 84% [5]. The first side effects of BEP at trimester II: found 1 case cerebral atrophy accompanied by ventriculomegaly, 4 cases polyhydramnios and IUGR, nephrotoxic suspected induced by cisplatin and neonatal alopecia [20]. The second, liver damage which caused decreasing synthesis of plasma procoagulant proteins and anticoagulant including fibrinopeptide A protein, fibrinolytic, factor VIII and thrombocyte activator that can be activated disseminated intravascular coagulation/DIC, thrombosis, and also bleeding. The third, indirectly destroy primordial follicle reserve, oocyte, and ovarian stroma caused premature ovarian failure (POF). Fertility profile of female BEP patients: primordial follicle decrease and stromal fibrosis in cortical atrophy with 42% of a patient developing POF [15].

Table 1: Cancer Therapy Choice Corresponding to Gestational Age [19]

	Surgery	Chemotherapy	Radiotherapy[a]
First trimester	Possible	Contraindicated	Possible with adequate shielding
Second trimester	Possible, consider intraoperative fetal heart rate monitoring ≥24–26 weeks	Possible ≥12–14 weeks	Possible with adequate shielding
Third trimester	Possible, consider intraoperative fetal heart rate monitoring	Possible, aim for 3-week interval between 3-weekly chemotherapy and delivery	Contraindicated[b]

The Mode of Delivery

The optimal time to labour is after 35-37 weeks and 3 weeks post-chemotherapy to avoid accumulation of chemotherapy and to allow recovery from possible bone marrow suppression of both mother and baby [8]. Mode of delivery preference was spontaneous vaginal delivery rather than a caesarean section. The benefits of spontaneous delivery are related to less blood loss, less operative risk and reduced infection risk. Mother under chemotherapy is not recommended to breastfeed due to several chemotherapy regimens excreted through breast milk which could result in neonatal pancytopenia [19].

Prognosis

A grade of immaturity predicted metastasis potency and prognosis. Five years survival rate is 82%, 62%, and 30% for grade 1, 2, and 3 respectively

in patients with grades 1, 2, and 3 treated with optimum chemotherapy, which it recurrence is 36% on 6 years follow up, and 50% of patient succeed reach term pregnancy after performing primary surgical. The progressivity of patients performing complete surgical staging 8 % without delay the chemotherapy [3]. Long term outcome of a study done in Mexico on 84 children who got affected by chemotherapy intrauterine due to their mother had cancer, not found congenital abnormality, development of neurology, cardiology, psychology and also disturbance in their marks at school [21].

In conclusion, the incidence of ovarian cancer in pregnancy is very rare. The choice of BEP chemotherapy, the time of operation and chemotherapy, regular follow up of fetal well-being will determine prognosis. Fetal outcome is good, but occur the signs of POF on the mother. Patients survival 62%, post-BEP free survival rate 84%, and progressivity post complete surgical staging 8% without delay chemotherapy.

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Case Report

Atypical Manifestations in Classic Kaposi Sarcoma: Case Series of Two Patients HIV - Negative

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Keywords: Eye; Classic Kaposi sarcoma; Chemotherapy; Vinblastine

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Abstract

BACKGROUND: Kaposi's sarcoma (KS) is a tumour of endothelial, blood and lymphatic cells, caused by an infection with human herpesvirus-8 (HHV-8). The skin lesions of KS, especially of the classical or Mediterranean variant (CKS), are represented by red-purple macules, plaques and nodules, localised mainly on the extremities.

CASE REPORT: This case series intend to describe multifocal atypical kaposian manifestations in two HIV negative subjects, affected by CKS, treated with successful chemotherapy.

CONCLUSIONS: Although atypical manifestations are extremely rare events, we suggest an accurate, objective examination because a prompt diagnosis can lead to a vital intervention in the patient's health and sometimes to the identification of the disease itself.

Introduction

Kaposi sarcoma (KS) is a malignant neoplasm with multifocal diffusion, originating from endothelial cells, associated with human herpesvirus-8 (HHV-8). HHV-8 infection remains latent, and most people do not develop KS until their immune system is affected by the disease, such as human immunodeficiency virus (HIV), or by drugs are given after an organ transplant [1].

According to the literature, there is still no standard treatment of CKS. The prognosis and choice of treatment depend on the type of KS, the global assessment of the patient, in particular, the patient's immune system, whether cancer has just been diagnosed or has recurred [2].

Herein we present two cases of an HIV-negative Mediterranean male with disseminated skin KS treated with different therapy, obtaining a remission of clinical manifestations.

Case 1

A 78-year-old Italian man attended our attention in January 2018 for a 2-years history of red lesions on his lower legs, treated incorrectly for chronic stasis dermatitis. His medical history was relevant for compensated type 2 diabetes mellitus and hypertension. Clinical examination showed multiple blue and purplish red nodules varying in size from 1-3 cm, affecting the lower legs and forefoot, abdomen, neck, oral mucosa, tongue and genital region (Figure 1). A sample of the skin biopsy showed proliferation of

fusiform cells and endothelial cells with extravasation of red blood cells and intervening slit-like spaces. Endothelial cells were positive HHV-8 by immunohistochemistry.

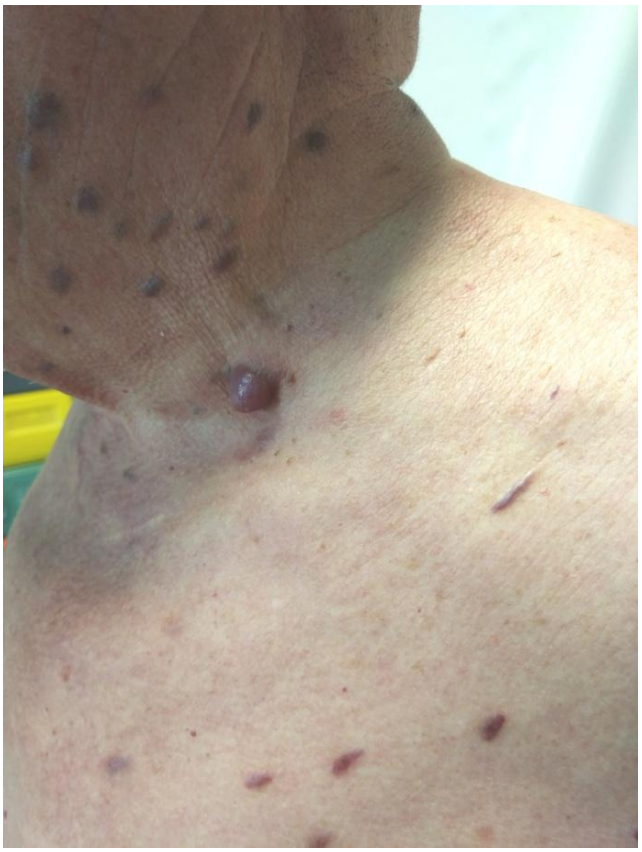

Figure 1: Multiple blue and purplish red nodules varying in size from 1-3 cm affecting the neck

These findings were consistent with Kaposi Sarcoma diagnosis, nodular stage. Laboratory investigations showed HIV test negativity (antibodies anti HIV1/2-antigen p24), White Blood Cells 10.57 x 10^3/uL (normal range 4.8-10.8), Red Blood Cells 4.16 x 10^6/uL (4.2-5.6), Platelets 234 x 10^3/uL (130-400); Hb 15 g/dL (12-17.5), liver function [ALT 21 U/L (0 – 55); AST 22 U/L (0 – 34)] and renal function [creatinine 0.7 mg/dL (0.7-1.2)] tests within the normal limit. Chest X-ray, abdominal and lymph node ultrasound and low gastrointestinal endoscopy were normal. Faecal occult blood test and video-laryngoscopy were performed, and no abnormalities were documented. Considering widespread skin involvement (> 25 lesions) and oral lesions, he was put on monthly chemotherapy with vinblastine dose of 10 mg. White blood count was normal, and patient-reported only nausea. The clinical examination nine months after starting therapy showed clinical improvement with reduction of nodules dimension (Figure 2). After one year, no recurrence was observed.

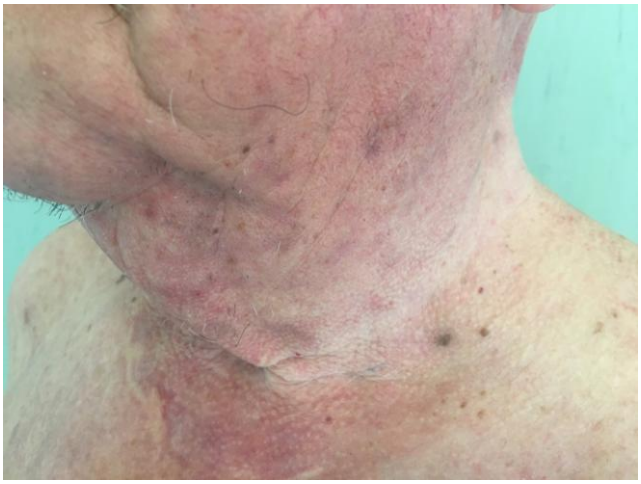

Figure 2: Clinical improvement with reduction/absence of nodules dimension

Case 2

An 82-year-old Italian man HCV-positive (HCV antibodies positive) and HIV negative (antibodies anti-HIV1/2-antigen p24), with a 5-year history of KS, presented to our attention in March 2015. Clinical examination showed a red-brownish nodular lesion on the left nostril, about 1 cm in diameter, without systemic symptoms. Excisional biopsy and histological examination were performed with confirmation of KS'diagnosis: the lesion presents angiomatous areas surrounded by spindle cells (HHV-8 positive by immunohistochemistry), perivascular inflammatory infiltrate with T and B cells. Almost simultaneously, nodular lesions confluent into plaques in the lower limbs appeared, for which cycles of chemotherapy with vinblastine (10 mg) began one time a month, with improvement of manifestations without side effects (white blood cells [7.87 x 10^3/uL (4.8-10.8)], transaminases [ALT 11 U/L (0 – 55); AST 18 U/L (0 – 34)]).

In August 2017, chemotherapy was discontinued due to an acute myocardial infarction. In November of the same year, for the improvement of the cardiac clinical conditions and previous oncological counselling, therapy with vinblastine resumed under strict cardiological control. After 7 months, the patient reported the appearance of two adjacent intraorbital nodules, the medial area of the left tear chamber, of a brownish red colour from the diameter of about 5 mm, indicative of kaposian lesions (Figure 3).

Being the lesion particularly proliferative and not responsive to vinblastine, we proceeded with the removal of the same to restore the visual features and avoid the further increase.

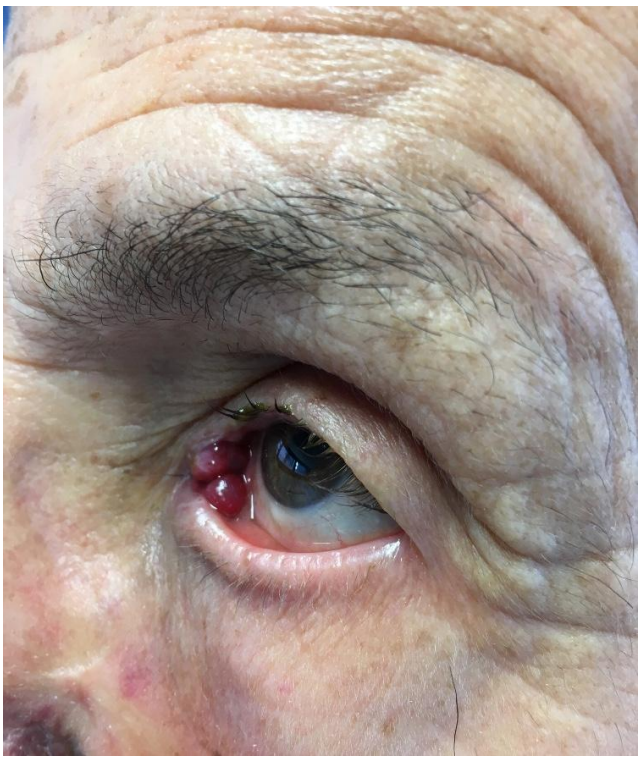

Figure 3: Two adjacent intraorbital nodules, the medial area of the left tear chamber, of a brownish red colour from the diameter of about 5 mm

The operation, performed at the eye clinic (May 2018), and the subsequent histological examination confirmed KS. Currently, the patient practices cycles of liposomal doxorubicin, finding a containment of the manifestations. After six months, there are no other ocular manifestations (Figure 4).

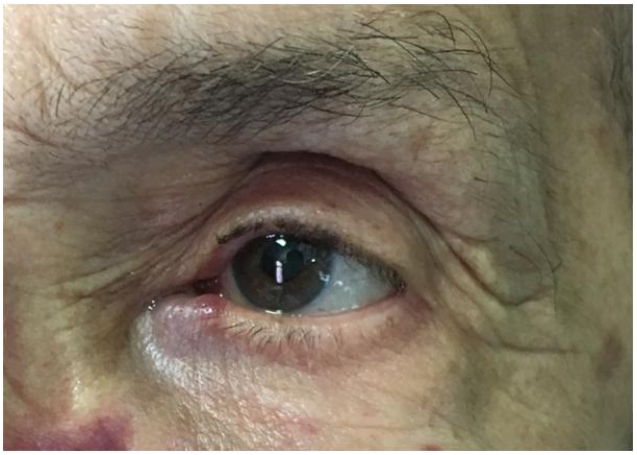

Figure 4: Complete resolution after surgical treatment

Discussion

KS is an angioproliferating skin cancer can involving mucosa and viscera, like a respiratory and gastrointestinal system [3].

KS is one of the most common sarcomas in patients with AIDS, but in its classical form (CKS) it is found widespread especially in the Mediterranean area [4]. The epidemiology of KS partially reflects the seroprevalence of HHV-8 infection. HHV-8 infection has a low incidence, less than 5%, in Asia, Northern Europe, Australia and America, while it increases in Mediterranean countries (with diffusion in southern Italy) and in Africa sub-Saharan, with the preferential non-sexual transmission as determined by the high incidence of seropositive children [5]. The CKS prevails in the Mediterranean basin. In America, the percentage is higher in AIDS patients. The highly active antiretroviral therapy in the treatment of HIV disease showed a reduction of AIDS-associated sarcoma Kaposi [6].

Head and neck involvement in HIV-negative patients is uncommon, reported in less than 5% of classical forms. Classical skin lesions generally consist of angioedematous plaques and nodules located on the limbs, on the contrary, HIV-related form is characterised by disseminated cutaneous manifestations, with oral and craniofacial involvement in about 95% of patients [7], [8]. However, in our cases, chemotherapy with vinblastine seems to be effective, with control of atypical manifestations without evidence of skin recurrence or visceral involvement. Ocular involvement, although atypical, is more frequent in the HIV-related form where it may even represent the first clinical sign [9]. Our experience proves the importance of accurately identifying the ocular involvement of Kaposi's sarcoma, which can be confused with simple angiomatous lesions. Although it is an extremely rare event, a prompt diagnosis can lead to a vital intervention in the patient's health and sometimes to the identification of the disease itself. Our cases show that the cutaneous manifestations of CKS do not always follow the trend of the Mediterranean variant, can take the typical manifestations of an HIV sarcoma preserving responsiveness to the therapy with vinblastine.

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Case Report

Steroid Induced Cataract in Langerhans Cell Histiocytosis Patient

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Keywords: Steroid; Cataract; Histiocytosis

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Abstract

BACKGROUND: Cataract is an opacification of the lens. Pediatric cataracts can be congenital or acquired. Acquired cataract including the one caused by corticosteroid used. It occurred as bilateral posterior subcapsular cataracts and tended to be progressive. Treatment of choice is lens extraction with or without intraocular lens (IOL).

CASE PRESENTATION: We present a case of posterior subcapsular cataract that occurs in a patient with Langerhans cell histiocytosis that was using corticosteroid therapy.

CONCLUSION: The routine ophthalmologic examination should be performed in children who received treatment with corticosteroids in the long term so that with early detection it can be given early treatment.

Introduction

Cataract is the opacification of the lens that can occur as a congenital or acquired disease. Cataracts can affect all ages, including children. Cataracts in children can be a stand-alone disease or as part of a systemic disorder, congenital or acquired, unilateral or bilateral. Epidemiological studies and clinical observations have successfully identified the risk factors responsible for the formation of cataracts including exposure to ultraviolet light, myopia, diabetes and long-term use of corticosteroids. Pathophysiology of cataract formation in corticosteroids usage hasn't certainly known yet, but several mechanisms that alleged to be responsible described in some theories including osmotic theory, the oxidative theory, modification of proteins, and metabolic disorders [1].

Cataracts that are formed in the long-term use of corticosteroids are usually formed as posterior subcapsular cataracts, which mean the opacity formed on the polar part of the posterior cortex of the posterior lens capsule [1], [2], [3]. This type of cataract usually found on adults rather than children occurs bilaterally, and progressively. Symptoms often complained including reduced visual acuity and disturbingly glary vision [4].

There are 1.5 million children with corrected visual acuity below 20/40 in the world, and 1 million of these children are living in Asia. The prevalence of children with cataracts recorded is 1-15 cases out of 10,000 children in developing countries [2]. It is estimated that 200,000 children are blind due to bilateral cataract [5], [6]. Cataracts in children may cause visual impairment. The number of visual impairments caused by cataracts is more than any other preventable cause of blindness. Children with cataract that affect visual acuity but not treated properly could face a lifetime of blindness, with a

miserable quality of life and socio-economic, they would be a burden for themselves, family, and the environment [7].

Currently, the most effective cataract therapy for children is lens removal surgery. Cataract surgery in children is a complex procedure. The eye's anatomy and physiology aspects in children that are still growing are quite different than in adults. Most times it would also take a long process of post-surgery follow-ups. The timing of the surgery, IOL measurement and installation, surgical techniques, and post-operative care are some things that should be considered carefully [6].

Langerhans cell histiocytosis (LCH) is a proliferation of Langerhans cells, which are the member of the dendritic cell of bone marrow and characterised by abnormal accumulation of dendritic cells, lymphocytes, macrophages and eosinophils in various organ systems [8], [9]. The aetiology and pathogenesis of LCH have yet to be known clearly. Several hypotheses explained the involvement of somatic mutations, chromosomal instability, human herpesvirus-6 (HHV6) infection, dysregulation of cytokine and apoptosis [10], [11].

The prevalence of LCH is 1 in 50,000 children with incidents of 1.08 in 200,000 children per year. LCH can occur in all age groups, but about 50% of LCH cases were diagnosed at the age of 1-15 years and the highest incidence rate found are in the age group of 1-3 years [12], [13], [14]. The clinical manifestations of LCH may involve various organ systems with the most common area affected is skin and bones. The diagnosis is confirmed with histopathology, immunohistochemistry and electron microscopy examination [13].

LCH therapy is implemented based on the course of disease and organ system involvement. Patients with localised skin lesions may not require special treatment with spontaneous resolution in some cases reported. Systemic therapy is given to patients with multi-systemic or extensive LCH, with choices such as vinblastine 6 mg/m^2 intravenous every week for 24 weeks and methylprednisolone 30 mg/kg/day administered intravenously for three days followed by a lowered dose. Another option of regimen includes etoposide 150 mg/m^2/day intravenously for three days that must be repeated every 3 weeks until a total of 8 cycles of 24 weeks is reached, combined with methylprednisolone 30 mg/kg/day administered intravenously for three days followed by lowered dose.

The evaluation of the treatment will be carried out on the sixth week, in patients that do not respond to the therapy performed, cytostatic replacement is used, with the combination of mercaptopurine and prednisone or methotrexate and prednisone. The prognosis of patients with LCH are quite varied, depending on the response to initial therapy, age at onset of the disease, organs involved, and organ dysfunction involved [8], [9], [10], [13], [14].

We are presenting a case of posterior subcapsular cataract that occurs in a patient with Langerhans cell histiocytosis that was under a corticosteroid therapy.

Case Illustration

A 7 years-old boy came to the ophthalmology clinic complaining of progressive blurred vision on both eyes since three months ago. The condition was disturbing the patient's activity, especially when he was at school. The patient also complained about glary feeling that felt annoying when he was doing activity outdoors. His birth history and family history were unremarkable.

The patient was diagnosed with LCH since he was 7 months-old. The disease was preceded by a lump in the bottom of his right eye. He was then brought to a hospital where he underwent biopsy examination. Another lump grew on the bottom of his left eye two months later. A paediatrician performed a biopsy of the lump, and he was later diagnosed with juvenile xanthogranuloma. He received chemotherapy for one year but then lumps reappeared on his neck, arms, and legs. Another biopsy revealed LCH, and he was scheduled for chemotherapy.

Figure 1: Physical finding when the patient first came to the ophthalmology clinic

Eye examination (Figure 1) revealed the visual acuity is of the right eye (OD) was 4/60 which was unimproved with a pinhole vision. His inferior eyelid retracted because of the biopsy scar. The conjunctiva, anterior chamber, cornea, and iris exams were unremarkable. The lens appeared cloudy at the posterior subcapsular, with a clear vitreous, positive fundus reflex, but difficult to observe the details (Figure 2).

Left eye (OS) examinations showed visual acuity of 1/60 which was also unimproved with pinhole vision. The inferior eyelid was also retracted due to biopsy scar. The conjunctiva, anterior chamber, cornea, and iris exams were unremarkable. The lens appeared cloudy at the posterior subcapsular, with a

clear vitreous, positive fundus reflex, but difficult to observe the details.

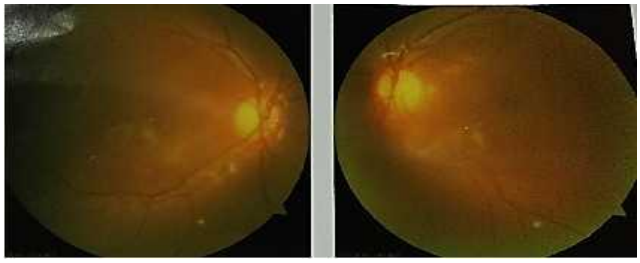

Figure 2: Fundus exam findings

Both eyes could move normally to all directions. Eye pressure was 14,00 mmHg on the right eye and 12.00 mm Hg on the left eye. Exophthalmometer examination using Hertel found a base of 78 with values on the right eye and the left eye were 19-20 mm. Ultrasound examination was performed to determine the length of axial length, which were obtained that the axial length on the right eye and the left eye 23.22 mm and 23.59 mm (Figure 3).

Figure 3: Ultrasound findings

The patient was diagnosed with right and left eye complicated cataract caused by steroid usage. Biometric inspection indicates the size of the lens for the right eye and the left eye were 15.00 and 14.50 diopters, respectively.

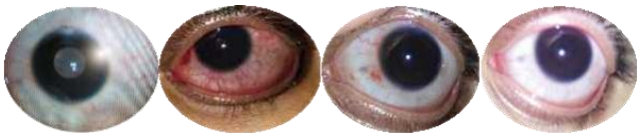

Figure 4: Left eye photos. From left to right: during the surgery, one day after the surgery, one week after the surgery, and one month after the surgery

He underwent surgery for the lens extraction and IOL mounting on the left eye. One day after surgery the left eye showed improved visual acuity of 6/30 with unimproved with a pinhole. Eyelid oedema, conjunctival vascular injection (CVI), pericorneal vascular injection (PCVI), and subconjunctival bleeding (SCB) were present on the left eye. IOL was installed properly in the central, with clear vitreous and positive fundus reflex. He was discharged from the hospital with antibiotic and Lpred eye drops.

Two months later, he underwent a second surgery for cataract extraction and IOL mounting of the right eye. One day after the surgery, examination on the right eye revealed visual acuity was 6/15 (unimproved by pinhole), with the presence of oedema of the eyelid, CVI, PCVI, and SCB. IOL was installed properly in the central, with clear vitreous and positive fundus reflex. At this point, the OS visual acuity was 6/10 with an unimproved with a pinhole. Eye pressure was 12.00 mmHg for both eyes. He was then discharged from the hospital with antibiotic and Lpred eye drops.

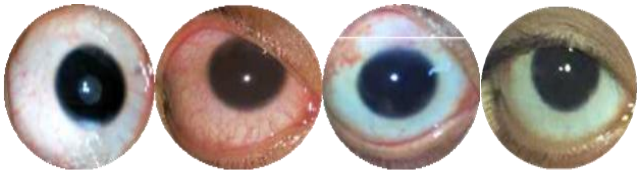

Figure 5: Right eye photos. From left to right: during the surgery, one day after the surgery, one week after the surgery, and one month after the surgery

One month later, eye examination showed visual acuity on the right eye with was 6/10 (improved to 6/7.5 with pinhole), that was corrected with the spherical lens of -1.00 to 6/7.5. The left eye's visual acuity was 6/7.5 (unimproved with pinhole). He was discharged with the provision of eyeglasses with best correction lens.

Discussion

Cataracts are opacities of the lens. Lens opacities in children can occur congenitally or acquired, and one of the reasons is the use of corticosteroids in the long term. Cataracts in children may be formed unilaterally or bilaterally, stand-alone or as part of a systemic condition, and can be either stable or progressive.

The patient presented in this case came with a sharp decrease in vision in both eyes for three months, which worsen over time. This proved to be very disturbing, especially during the learning process at school. He had a history of suffering from LCH and received chemotherapy for 4 years using a protocol where it included the use of methylprednisolone and prednisone. Patients' family had no history of cataracts in childhood. Ophthalmology examination on the patient showed cloudiness in the polar part of the posterior capsule in both eyes and was advancing rapidly, with a decrease in visual acuity continued to deteriorate in a matter of months.

While the treatment of cataracts in children includes surgery, some important things must be considered beforehand. The younger the child, the sooner surgery is required to prevent amblyopia due

to visual deprivation. In older children, surgery is performed when the vision of children is less than or equal to 20/40. The second thing to consider is the installation of IOL depending on the age of the patient and lateralisation of the cataract, where the IOL implant should be performed on children aged 1-2 years or more, because of the magnitude of change and refractive errors are still possible [6].

Postoperative management with medication of steroid and antibiotics are important, whereas, in children who underwent IOL installation, steroids administration should be more aggressive [6], [7], [8]. This patient received antibiotics, and steroid eye dropped immediately after surgery and continued until 6 weeks postoperatively.

Treatment and prevention of amblyopia in children with cataract should be done immediately after the surgery. Patients with bilateral aphakia should be given corrective lenses a week after surgery. In patients who are older and underwent IOL replacement directly after cataract surgery, refractive correction should be done one month postoperatively [5], [6].

In conclusion, cataracts are opacification of the lens that can occur in children, one of which can be caused by the use of corticosteroids in the long term. Diagnosis and appropriate treatment can save the patient from complications that may occur. Long-term follow-up is necessary given the ongoing development in children. Chemotherapy addition in children to provide the desired effect is not uncommon but also can result in adverse side effects. Routine examination in children who received chemotherapy should be done to detect early adverse effects that may occur. In this case, the routine ophthalmologic examination should be performed in children who received treatment with corticosteroids in the long term so that with early detection it can be given early treatment.

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The patient's parent provided us with a written consent for possible future publication regarding this case.

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Case Report

Cerebral Venous Sinus Thrombosis with an Intracranial Haemorrhage: A Case Report

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Keywords: Cerebral Venous Sinus Thrombosis

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Abstract

BACKGROUND: Cerebral venous sinus thrombosis (CVST) is a rare, life-threatening disorder. It has an annual incidence of approximately two to four per million people per year. Nearly 70–80% of all cases of CVST are located in the superior sagittal sinus (SSS). CVST presents a diagnostic challenge due to different presentations.

CASE PRESENTATION: We describe the case of a young pregnant female who presented to the emergency room with an acute headache attributed to multifactorial causes.

CONCLUSION: This report highlights the importance of including CVST in the differential diagnosis when treating a pregnant female with headaches. Although the symptoms of CVST are varied, the most common occlusion is in the SSS. In such cases, the patient may present with signs and symptoms that include headaches, intracranial hypertension and papilloedemas.

Introduction

Cerebral venous sinus thrombosis (CVST) is a rare, life-threatening disorder. It has an annual incidence of approximately two to four per million people per year [1]. Nearly 70-80% of all cases of CVST are located in the superior sagittal sinus (SSS) [2].

CVST tends to be multifactorial in aetiology, with estimates that up to 65% of patients with CVST have more than one risk factor [3], [4]. According to the literature, CVST shows a 3:1 ratio of females to males [4]. The increased prevalence among females may be due to the use of oral contraceptives, pregnancy and postpartum [4]. Pre-disposing risk factors are found in 80% of cases of (CVST) [5].

Case Report

A 24-year-old primigravida female at 6 weeks of gestational age was admitted to the intensive care unit with the onset of a generalised tonic-clonic seizure. In addition to the seizure, the patient had slurred speech, with rolling of the eyes and salivation. The seizure lasted for more than 5 min, and the patient then lost consciousness for more than 30 min.

The patient had a known history of migraines, chronic sinusitis and coeliac disease for the last 3 years. The patient had been attending a family medicine clinic. She had developed a headache in the last 5 days. The headache was unilateral in the right temporal region and extended to the supraorbital and orbital areas. It was throbbing in nature, continuous, with photophobia and phonophobia. The severity of the headache increased in response to bending, and it was associated with nausea, vomiting and dizziness. The patient reported no pain in the neck or mid-region of her back and no fever, chest pain, palpitations, cough, shortness of breath or rhinorrhoea. She had mild lower abdominal pain, with vaginal bleeding. There was no dysuria, urinary frequency or urgency. The patient had a history of loss of appetite of long duration but showed no change in weight. She had been in a road traffic accident 1 year earlier and had fractured her jaw and had lower vertebral prolapse. In terms of the patient's family history, her mother had allergic rhinitis, sinusitis, migraines and myeloproliferative neoplasms. Her sister also had migraines.

On examination, the patient was conscious, oriented and haemodynamically stable, with a positive Kernig sign and negative Brudzinski sign, with normal neurological findings. She had tenderness of the right frontal sinus and nasal septum deviation to the left side, with discharge.

The laboratory findings were unremarkable. A computed tomography (CT) venogram showed superior sagittal sinus (SSS) thrombosis, with a right frontal lobe haemorrhage insult, Figure 1.

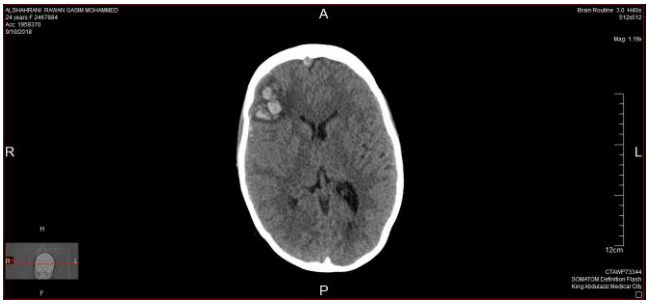

Figure 1: A CT venogram showed SSS thrombosis, with a right frontal lobe haemorrhagic insult and mild surrounding vasogenic oedema, in addition to a mass effect over the adjacent sulci and right lateral ventricle

The patient was stabilised and received an anticoagulant. She subsequently made a good recovery and was discharged. The patient was prescribed low-molecular-weight heparin (LMWH) and levetiracetam. Two months later, levetiracetam was discontinued, and the patient completed her pregnancy. The LMWH treatment was continued

Discussion

CVST is an under-diagnosed condition. Patients with CVST may present with various signs and symptoms, which lead to delays in the diagnosis. The median delay from the time of presentation to diagnosis is 7 d [6]. Our patient had a history of migraine headaches, with frequent visits to her family doctor. Before presentation to the emergency department, she had developed a headache, which had lasted for 5 d. This may have explained the delay in the diagnosis of CVST. The early stages of CVST may be characterised by cortical vein thrombosis, without sinus thrombosis. The latter may develop only later due to the progression of the thrombotic process.

Although the clinical syndrome of CVST is not well defined, it is thought to be characterised by the rapid onset of focal deficits and/or seizures [7]. The most common symptom of CVST is a headache. It is estimated that up to 80–90% of CVST patients first present with either focal-, diffuse- or migraine-type headaches [8], [9]. The findings in the present case were by the literature.

The varied presentation of CVST makes it a diagnostic challenge. Focal deficits, such as hemiparesis and hemisensory disturbances, together with seizures, an impaired level of consciousness and papilloedemas, occur in one-third to three-quarters of cases [9]. Most patients present with symptoms that have evolved over days or weeks [7], [10]. Investigations should focus on establishing the diagnosis and searching for underlying causes. CT venography and contrast-enhanced magnetic resonance venography are highly sensitive. A CT scan can be used to exclude other conditions, such as intracerebral haemorrhages or abscesses [11]. Invasive cerebral angiography may be performed if the results of contrast-enhanced magnetic resonance venography are inconclusive. The initial management of CVST should include stabilisation and anticoagulation, even in the presence of an existing haemorrhagic venous infarct, as anticoagulation is the mainstay of treatment [12]. More than 80% of CVST patients recover with this treatment modality [13]. Our patient showed a marked clinical and radiological improvement following the administration of an anticoagulant.

The prognosis of CVST is generally good. However, the prognosis may be poor in elderly patients and patients with sepsis, malignancies and deep cortical venous thrombosis, as well as in the presence of a coma [5]. More than 80% of patients, as in the present case, have a good neurological outcome. On the other hand, a delay in diagnosis can result in death.

In conclusion, CVST is a relatively rare condition, which is challenging to diagnose. This report highlights the importance of including CVST in

the differential diagnosis when treating a pregnant female with headaches. Although the symptoms of CVST are varied, the most common occlusion is in the SSS. In such cases, the patient may present with signs and symptoms that include headaches, intracranial hypertension and papilloedemas. The use of imaging can help aid the diagnosis, with magnetic resonance or CT venography being important in detecting the occlusion. The mainstay of treatment for CVST is anticoagulants, with LMWH prescribed for a minimum of 6 months. A pregnant patient may require LMWH during pregnancy until the time of delivery.

Consent

Written informed consent was obtained from the patient for publication of this case report and accompanying image.

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Dental Science

Antibacterial Activity of *Lumbricus Rubellus* Earthworm Extract Against *Porphyromonas Gingivalis* as the Bacterial Cause of Periodontitis

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Keywords: L rubellus earthworm extract; Inhibition zone diameter; P gingivalis bacteria

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Abstract

AIM: The purpose of this study was to determine the antibacterial activity of *Lumbricus rubellus* earthworms through inhibitory zone diameter to the growth of the bacterium *Phorphyromonas gingivalis* as the cause of periodontitis.

METHODS: This was an experimental study with randomised posttest-only control group design. The study was conducted at the Microbiology Research Center laboratory at the Faculty of Dentistry, Airlangga University, Indonesia. The study was conducted in vitro, the sample size was calculated using the Federer formula as many as four agar plates containing bacteria *Phorphyromonas gingivalis*, with each plate given five different treatments: control (ethanol), *Lumbricus rubellus* earthworm extract (ECT) with concentrations of 50%, 25%, 12.5%, and 6.25% respectively. The data in the form of inhibition zone diameter (measured in millimetres) obtained were tested using One-Way ANOVA.

RESULTS: The mean diameter of the inhibitory zone extract of *Lumbricus rubellus* earthworm on the growth of Phorphyromonas gingivalis bacteria in the treatment group had significant differences ($p < 0.05$). The mean inhibition zones between controls and the ECT treatment group (ECT 50%, ECT 25%, ECT 12.5%) were statistically different ($p < 0.05$), in contrast with ECT 6.25% ($p > 0.05$) which did not show significant difference with the control group ($p > 0.05$).

CONCLUSION: *Lumbricus rubellus* earthworm extract with a concentration of 50% has the largest diameter of the inhibitory zone on the growth of the *Phorphyromonas gingivalis* bacteria. The 6.25% earthworm extract showed no antibacterial activity against the growth of *Phorphyromonas gingivalis* bacteria.

Introduction

Periodontal disease is an inflammation that involves the gingiva and alveolar bone. It is one of the most widespread diseases in society, with the most common forms of the disease are gingivitis and periodontitis [1]. Periodontitis is defined as inflammation or infection of the tooth-supporting tissues including gingiva, alveolar bone, periodontal ligament, and cementum. Periodontitis may develop from untreated gingivitis. The disease will spread from the gums towards the bone below the teeth, causing more damage to the periodontal tissue [2]. Microbiological factors are one of the causes of periodontitis. Periodontitis occurs due to unbalanced conditions between host and bacteria, caused by a decrease in host conditions and increased plaque biofilm and bacterial virulence [3]. Specific *Phorphyromonas gingivalis* microorganisms are often detected in patients with periodontitis. These bacteria can be detected in periodontally healthy subjects in the subgingival sulcus region as they can be part of the normal flora of many individuals. These bacteria do not ferment carbohydrates. Their lives depend on amino acid fermentation as energy production. The absolute requirement for the growth of this bacterium is iron. It is a Gram-negative bacterium in the form of

an obligatory anaerobic, non-motile, asaccharolytic stem, which forms pigmented black colonies on a blood agar plate [4].

The degree of periodontal damage depends on the balance between the damaging and protective inflammatory mediators. The primary goal of periodontitis therapy is to maintain the integrity of the teeth by achieving and maintaining healthy periodontium function. It consists of motivation and oral hygiene instructions and mechanical supra and subgingival plaque removal and calculus deposits, plaque correction as the main factor and risk factor (e.g., smokers). Debridement on the root surface of the tooth using scaling and root planning is relatively commonly used in periodontal therapy. Certain patients sometimes do not respond well to conventional mechanical treatment; for various reasons the use of antimicrobials as an adjuvant may be beneficial to patients. Antimicrobials are chemotherapeutic agents that reduce the number of bacteria present in certain surface organisms or by cutting all bacteria. A systematic review by Mathur et al. was conducted to update scientific evidence about antimicrobial properties in addition to subgingival debridement in the treatment of chronic periodontitis [5]. Local application of chlorhexidine and metronidazole locally shows minimal effects; this systematic review shows that scientific evidence supports the use of antimicrobials, especially when carrying out conventional therapy. Antimicrobials are the basis for the treatment of microbial infections, but irrational use is a significant factor in the resistance of microorganisms to antimicrobials [6]. The purpose of irrational antimicrobials will cause unexpected resistance and side effects. *Lumbricus rubellus* earthworm is one of the natural ingredients which has the ability as an antimicrobial.

Lumbricus rubellus contains an antimicrobial peptide (AMP) called Lumbricin-1 which functions as a natural defence against pathogenic microbes [7]. Subsequent research was conducted by Rinanda et al., who compared the antimicrobial activity of deep spectrum *Lumbricus rubellus* powder to several microbial resistant drugs such as *Multidrug-Resistant* (MDR) immune to *Pseudomonas aeruginosa*, *Methicillin-Resistant Staphylococcus* aureus (MRSA) and Fluconazole resistant to *Candida albicans*. Statistical analysis showed that *Lumbricus rubellus* powder in the concentration tested had significant antimicrobial activity in a broad spectrum of microbial resistant bacteria [8]. Research on anti-microbial activity was also carried out by Istiqomah *and* her colleagues regarding the inhibition of *Lumbricus rubbellus* worm granule extract against pathogenic bacteria *Escherichia coli, Salmonella Pullorum, Pseudomonas aeruginosa* and *Staphylococcus aureus* in vitro. The study used two types of *Lumbricus rubellus* (ECT) worm extract, dry worm extract (ECT-k) and granular worm extract (ECT-g). The results showed no antibacterial activity from ECT-g to E coli. The diameter of the 24-hour inhibition zone showed that *S aureus* was the most sensitive bacteria to ECT and ECT-k and *S pullorum* most sensitive to ECT-g [9].

Based on the various antimicrobial activities exhibited by studies mentioned above, the purpose of this study was to observe the antibacterial activity of *Lumbricus rubellus* earthworm extract through the inhibitory zone diameter of the bacterium *Phorphyromonas gingivalis* as a cause of periodontitis.

Material and Methods

This study was an experimental study in vitro with a randomised posttest-only control group design, conducted at the Microbiology Research Center laboratory at the Faculty of Dentistry, Airlangga University, Indonesia. The sample size was calculated by Federer formula as many as four agar plate for *Phorphyromonas gingivalis* bacteria replication. This study was designed using 5 different treatment groups: control (ethanol), extracts of *Lumbricus rubellus* earthworm (ECT) with different concentrations: 50%, 25%, 12.5%, and 6.25% [10] . The data in the form of inhibition zone diameter (millimetres) obtained were tested with One-way ANOVA. The technical procedures were as follow:

1. Earthworm flour production

The Earthworm flour was obtained from *Bali Organic Association (BOA)* worm farming, Denpasar, Bali, Indonesia. Earthworms were separated from the culture media and fasted for 6 hours. The dirt from the digestive worms was removed and washed with water. The worm bodies were soaked with distilled water 6-8 hours, then dried. Soaked worm bodies were being put in the oven with a constant temperature of 40°C for three days, then ground with a blender until it became a flour-like consistency.

2. Earthworm extract production

The earthworm extract was made by the maceration method using 1 kg of earthworm flour dissolved with 3500 ml ethanol. The solution was stirred and soaked for 24 hours, then filtered with *Whatman* paper to separate the filtrate and residue. A total of 1500 ml of filtrate was obtained, then evaporated with a rotary evaporator to obtain 15,570 grams of earthworm extract [11].

3. Culture media for *P. gingivalis* bacteria

The agar media was made using HHI-enriched BHI-A with vitamin K. The components needed to make 100 ml BHI-A were 50 µl hemin solution, 10 µl vitamin K, BHI-A 37 g in 100 ml sterile aquades and 500 µl yeast extract. The media was

divided into four Petri dishes and then be awaited until they became solid. One bacterial use from the ATCC 33277 bacterial stock was inoculated and then incubated at 37°C for 24 hours [12].

4. Preparing the *P gingivalis* bacteria suspension

The bacterial suspension was made by incorporating one use of *P gingivalis* bacteria from BHI-A into a liquid media with total volume of 10 ml containing 0.37 g BHI-B, 1 µl vitamin K, 5 µl hemin and 50 µl yeast extract. The suspension was then incubated for 24 hours, and the concentration was measured to obtain turbidity equivalent to 1.5 x 10^6 CFU/ml [12].

5. Planting the suspension of *P gingivalis* bacteria.

The *P gingivalis* suspension was swabbed on the surface of the agar media. The even distribution was made possible due to the nutrient contents in the suspension. The paper discs containing different concentrations of ECT, 50%, 25%, 12.5% , and 6.25% respectively were placed on the agar surface, then incubated at 37°C for 24 hours [13].

6. The area without visually apparent bacterial growth (clear zone) around each disc was observed. The diameter of the clear zone was measured using a calliper [13].

Results

The results of the research on the inhibitory capability of *Lumbricus rubellus* earthworm extract on the growth of *Phorphyromonas gingivalis* bacteria can be seen in Figure 1, below:

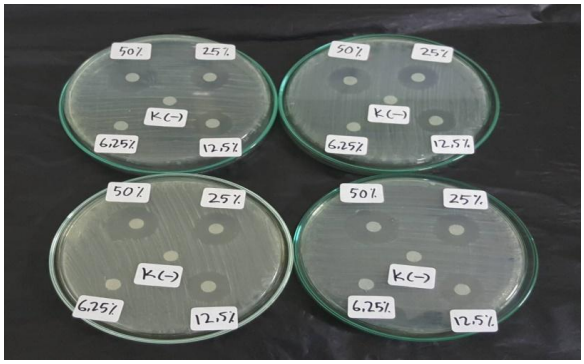

Figure 1: The inhibitory zone of Lumbricus rubellus earthworm extract (in millimetres)

The mean ECT inhibition zone diameter of the *Phorphyromonas gingivalis* bacteria in the treatment group was tested using One-Way ANOVA as in Table 1:

Table 1: The width of *Phorphyromonas gingivalis* Inhibitory Zone in The Treatment Groups

Subject Group	n	Mean ± SD *P. gingivalis* inhibition zone (millimetres)	*P**
Control	4	0.00 ± 0.00	
ECT 50%	4	21.88 ± 0.55	
ECT 25%	4	17.23 ± 0.54	0.001
ECT 12.5%	4	15.50 ± 0.42	
ECT 6.25%	4	0.00 ± 0.00	

Note; Analysis with One-Way ANOVA Test; * significant if $p < 0.05$.

The result from Table 1. shows that the mean diameter of the inhibitory zone of the ECT had significant differences ($p < 0.05$).

Table 2: The difference of *Phorphyromonas gingivalis* Inhibitory Zone Between the Treatment Groups

Group	Mean Diff.	*P**
Control - ECT 50%	21.88	0.001
Control - ECT 25%	17.23	0.001
Control – ECT 12.5%	15.50	0.001
Control – ECT 6,25%	00.00	1.000
ECT 50% - ECT 25%	04.65	0.001
ECT 50% - ECT 12.5%	06.38	0.001
ECT 50% - ECT 6.25%	21.88	0.001
ECT 25% - ECT 12.5%	01.73	0.001
ECT 25% - ECT 6.25%	17.23	0.001
ECT 12.5% - ECT 6.25%	15.50	0.001

Note; Analysis with *Post Hoc Test*; * significant at $p < 0.05$.

Table 2 shows the mean difference of the inhibitory zone between controls and the ECT treatment groups. The mean differences between ECT 50%, ECT 25%, ECT 12.5% groups, and control group were statistically significant ($p < 0.05$), but there was no significant difference between ECT 6.25% and control group ($p > 0.05$).

Discussion

Antibiotics and antimicrobial compounds inhibit the growth of microorganisms such as bacteria, fungi, and yeast. A zone of inhibition is the clear area surrounding a sample of an antimicrobial agent that has been deposited on an agar-based culture. Microbial colonies will grow on the agar surface. If the antimicrobial agent is useful, it will produce a clear zone that is free of bacterial growth. The length (or diameter) of the zone of inhibition is measured with a ruler or compiler [13]. The results from Table 1 showed that there were differences in the mean diameter of inhibition produced by extracts of the *Lumbricus rubellus* earthworm at different concentrations. The earthworm extract with a concentration of 50% has the highest mean inhibitory zone measuring 21.88 millimetres, compared to the 25% and 12.5% concentration which were 17.23 and 15.50 millimetres respectively. At the concentration of 6.25%, the earthworm extract did not cause inhibition for the growth of *Phorphyromonas gingivalis* bacteria, where the average diameter of the inhibition zone was the same as ethanol control which was 0 millimetres. The width of the inhibitory zone appeared to decrease with fewer concentrations of *Lumbricus rubellus*

earthworm extracts. The difference in the diameter of the inhibitory zone of *Lumbricus rubellus* among the treatment groups as portrayed in Table 2 showed that there was a significant difference between the control and *L. rubellus* earthworm extract at the concentration of 50%, 25% and 12.5% ($p < 0.05$). In the *L. rubellus* earthworm extract group at the concentration of 6.25%, when being compared to the control group, there was no statistically significant difference ($p > 0.05$). In the group of *L. rubellus* earthworm extract concentration of 50% compared to the treatment group at the concentration of 25%, 12.5%, and 6.25% there were significant differences ($p < 0.05$). There were significant differences ($p < 0.05$) between the mean inhibitory zone in the treatment groups of *L. rubellus* earthworm extract of 25% concentration and the 12.5% and 6.25% concentration groups. Similarly, there was a significant difference between treatment groups extract of earthworms at the concentration of 12.5% and 6.25% ($p < 0.05$).

The results of this study prove that earthworm extract can inhibit the growth of the bacteria *Phorphyromonas gingivalis*. Earthworm extract has long been used as a food ingredient and treatment for Ayurveda, Traditional Chinese Medicine, Vietnam, Japan, and Korea [14]. Earthworms have unique properties for treatment including anti-inflammatory, anti-oxidant, anti-tumour and anti-bacterial [15]. Cooper et al., found the role of earthworms on bacterial lysis and their implications on other diseases through lysenin and eiseniapore molecules. Binding of lysenin to sphingomyelin at the cellular membranes serves as a useful tool in investigating the function of sphingomyelin in biological membranes and in explaining the bacterial lysis mechanism of the earthworms. This mentioned lysis pathway might explain the earthworm extract's contribution to the defensive immune system against bacteria. Some studies have shown that earthworms are a source of antibacterial agents that earthworms as land occupants have a robust survival mechanism. The defence of earthworms to protect themselves against attacks by pathogenic organisms has various immune mechanisms by producing granular amoebocytes, chloragocytes and hyaline. Granular amoebocytes and hyaline help in the process of phagocytosis, while chloragocytes produce extracellular products that are cytotoxic and antibacterial [14]. Another study determining the capability of *Lumbricus rubellus* earthworm extract as an antibacterial agent calculated the *minimum inhibitory concentration* (MIC) and *minimal bactericidal concentration* (MBC). It was found that earthworm extract had a strong bactericidal effect against *Shigella flexneri*, and was bacteriostatic against *Streptococcus beta hemolytic* bacteria and *Vibrio cholera* [16].

The *Lumbricus rubellus* earthworm also contains an antimicrobial peptide (AMP) called Lumbricin-1 which functions as a natural defence against pathogenic microbes in the environment [7]. This proline-rich peptide is expressed constitutively by adult worms from *L rubellus* and has shown a broad spectrum of antimicrobial peptides to gram-positive and negative bacteria and fungi [17]. Lumbricin-1 has a hydrophobic surface formed by hydrophobic amino acids. Lipid bilayers on bacterial cell membranes have a hydrophilic and hydrophobic surface. The antimicrobial mechanism could be described as the hydrophobic surface of the peptide interacts with the hydrophilic surface of the cell membrane which results in increased permeability of the cell membrane. Therefore lumbricine-1 can enter the hydrophilic lipid layer.

Furthermore, lubricant-1 enters the hydrophobic layer, where lubricant-1 can adjust its shape to the cell membrane surface so that cell membranes cannot distinguish foreign peptides. The latter process results in intracellular instability and growth inhibition [18]. Research by Rinanda et al., which aimed to prove the antimicrobial activity of *Lumbricus rubellus* had broad-spectrum effect against *Multidrug-Resistant* (MDR) bacteria *Pseudomonas aeruginosa*, *Methicillin-Resistant Staphylococcus aureus* (MRSA) and fluconazole-resistant *Candida albicans*, obtained significant results ($p < 0.05$) [8].

Earthworm extract, the especially coelomic fluid has the potential to fight against several pathogenic and non-pathogenic bacteria such as *Escherichia coli, Streptococcus pyogenes*. In particular, coelomic liquid earthworm *Eisenia Andrei* displays antibacterial properties against *Bacillus megaterium*. The G-90 protein, the anti-carcinogenic molecule mentioned earlier, also has proven antibacterial activity against *Strep. Pyogenes, P aeruginosa*. By using new sources of antimicrobial substances such as the G-90, it seems that high activity of certain earthworm products can be tested to restrain or control microbial threats. These findings prove that effective earthworm extract as an antimicrobial agent against various bacteria only shows one of the many potential benefits of earthworms as therapeutic agents [14].

In conclusion, *Lumbricus rubellus* earthworm extract with a concentration of 50% has the largest diameter of the inhibitory zone against the growth of *Phorphyromonas gingivalis* bacteria. The 6.25% earthworm extract showed no antibacterial activity against the growth of *Phorphyromonas gingivalis* bacteria. These findings can be used as a basis for periodontitis therapy by using *Lumbricus rubellus* earthworm extract.

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Author Contributions

All authors had contributed in providing suggestions and thoughts in this research by their respective disciplines, thereby doing this research ran with no significant hindrance and was subsequently achieved in obtaining results following the research objectives.

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Influence of Small, Midi, Medium and Large Fields of View on Accuracy of Linear Measurements in CBCT Imaging: Diagnostic Accuracy Study

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Keywords: Fields of view; Linear Measurements; CBCT; Diagnostic Accuracy

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Abstract

AIM: This study aimed to assess the effect of changing the field of view on the dimensional accuracy of CBCT imaging.

METHODS: The implant-bone models were randomly numbered from 1 to 13 by the principal researcher, and then on each model at the incisors region three positions were selected and marked on the model with a permanent blue marker. Then at each marked position three radio-opaque 'RO' markers "gutta-percha pieces" were glued on the model surfaces as following; two pieces on the facial surface one occlusally (at the alveolar crest) and one apically (at the inferior border of the model) both were on the same vertical line and perpendicular to the horizontal plane, while the third one was placed on the lingual surface opposing the occlusally placed buccal piece. CBCT examinations of each bone model were performed using Cranex3Dx CBCT (Helsinki, Finland) machine. Each model was scanned four times with standardised tube current and voltage of 12.5 mA and 90 kVp respectively at four different FOVs. The FOVs used were as following: Small FOV: 50 x 50 mm with voxel size 200 µm, Midi FOV: 61 x 78 mm with voxel size 300 µm, Medium FOV: 78 x 78 mm with voxel size 300 µm, Large FOV: 78 x 150 mm with voxel size 350 µm. The reference standard in this study was the real linear measurements that were obtained directly on the implant-bone models using high precision sliding electronic digital calliper with 0-150 mm internal and external measuring range and 0.01 mm resolution accuracy. The index test in the current study was the CBCT linear measurements obtained from CBCT images of implant-bone models using small, midi, medium and large FOVs.

RESULTS: The results of this study showed that both medium and large FOVs showed a statistically significant difference, which could be translated into clinical relevance only in thickness measurements.

CONCLUSION: The interpretation of these results leads to the assumption that increasing the FOV size together with voxel size could adversely affect the accuracy of CBCT linear measurements, especially when small distances are to be assessed.

Introduction

Accurate and reliable linear measurements are considered very important issue in the field of oral and maxillofacial medicine, as almost all the dentists depend on such measurements in diagnosing, treatment planning and treatment outcome monitoring for multitude of cases in different dental specialities, of which, dental implantology, endodontics, forensic dentistry, orthodontics and orthognathic. CBCT was found to provide high resolution, distortion-free and accurate images for craniofacial structures without the magnification or superimposition problems of 2D images. Regarding the accuracy of linear measurements, CBCT was reported by several studies to be beneficial as it provides accurate and reliable measurements. However, a question mark is still posed regarding the radiation dose CBCT delivers to the patient as, despite its considerable merits, CBCT creates a great problem because of the higher patient's radiation dose compared to 2D radiography [1], [2], [3].

Materials

The study was performed on thirteen implant bone models obtained from Nissin Dental Products, Procedures of the study including implant bone models preparations, marking of the measurement's sites and measurements of the gold standard were performed in the Oral and Maxillofacial Radiology department, Faculty of Dentistry, Cairo University while Procedures of the study including imaging process, software manipulation of the resultant images and CBCT measurements were performed at a private radio-diagnostic centre.

The implant-bone models were randomly numbered from 1 to 13 by the principal researcher, and then on each model at the incisors region, three positions were selected and marked on the model with a permanent blue marker.

Then at each marked position three radio-opaque 'RO' markers "gutta-percha pieces" were glued on the model surfaces as following; two pieces on the facial surface one occlusally (at the alveolar crest) and one apically (at the inferior border of the model) both were on the same vertical line and perpendicular to the horizontal plane, while the third one was placed on the lingual surface opposing the occlusally placed buccal piece. The RO markers were obtained by cutting gutta-percha cones size 60 using sharp scissors into small pieces of nearly 1-1.5 mm length, and the cut pieces were glued to the selected landmarks using a cyanoacrylate gel. CBCT examinations of each bone model were performed using Cranex3Dx CBCT (Helsinki, Finland) machine. Each bone model was properly positioned in the machine with the help of the laser beam indicators of the machine such that the vertical laser beam coincided with the mid-sagittal plane (perpendicular to the floor) and the horizontal laser beam coincided with the occlusal plane (parallel to the floor).

Each model was scanned four times with standardised tube current and voltage of 12.5 mA and 90 kVp respectively at four different FOVs.

The FOVs used were as following;

Small FOV: 50 x 50 mm with voxel size 200 µm

Midi FOV: 61 x 78 mm with voxel size 300 µm.

Medium FOV: 78 x 78 mm with voxel size 300 µm.

Large FOV: 78 x 150 mm with voxel size 350 µm.

The reference standard in this study was the real linear measurements that were obtained directly on the implant-bone models using high precision sliding electronic digital calliper with 0-150 mm internal and external measuring range and 0.01 mm resolution accuracy.

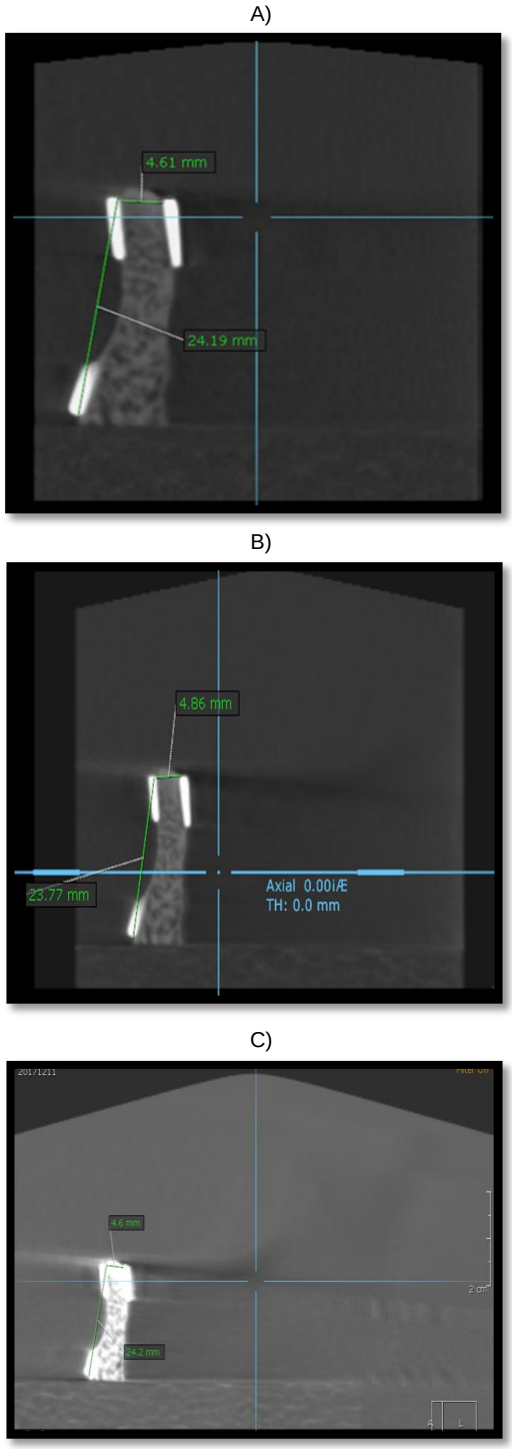

Figure 1: A) CBCT linear measurement of bone height & thickness on Small FOV CBCT images; B) CBCT linear measurement of bone height & thickness on Medium FOV CBCT images; C) CBCT linear measurement of bone height & thickness on Large FOV CBCT images

On each model at the three predetermined and marked positions the following linear measurements were taken:

Bone Height: this was measured on the facial surface of the model as the distance between the

superior end of the occlusal placed gutta-percha piece, and inferior end of the apically placed one.

Bucco-lingual "BL" Bone Thickness: this was measured as the distance from the superior end of the occlusal placed facial gutta-percha piece to the superior end of the gutta-percha piece placed on the lingual surface.

Mesio-Distal "MD" Bone Width: This was measured as the distance between the superior ends of two adjacent gutta-percha pieces on the facial surface.

CBCT DICOM files were exported to third-party software OnDemand3d* for CBCT linear measurements to be taken on a personal computer (13.3-inch LED-backlit display; 2560 x 1600 native resolution at 227 pixels/inch), where the CBCT scans were displayed on MPR screen [displaying the volumetric data set in axial, coronal, and sagittal image slices]. The CBCT linear measurements were taken in each of the marked areas as the image slices with the radio-opaque markers best visible were used for linear measurements using distance icon on the tool bar, in each area bone height, BL thickness, and MD width measurements were made exactly like those made on the bone model with the digital calliper. Both height and thickness measurements were taken on the corrected sagittal images (Figure 1A, 1B and 1C), while width measurements were taken on the corrected axial images (Figure 2A, 2B, 2C, and 2D).

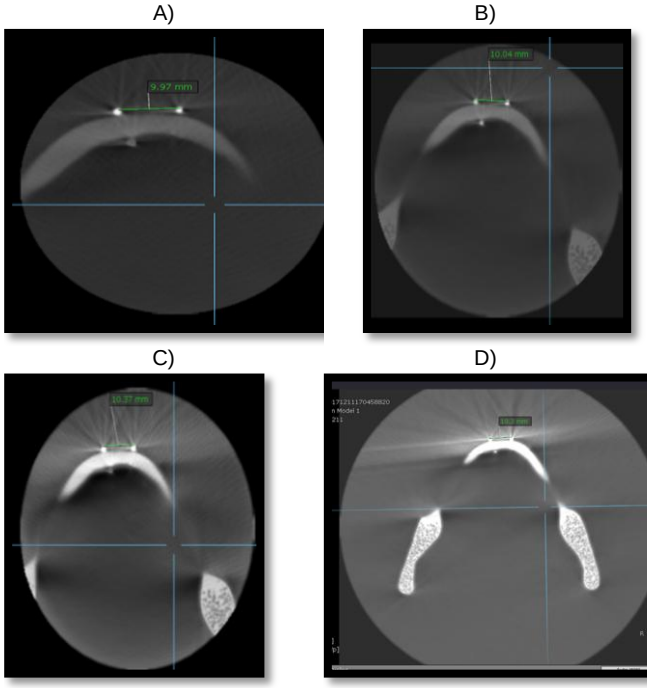

Figure 2: A) CBCT linear measurement of bone width on Small FOV CBCT images; B) CBCT linear measurement of bone width on Midi FOV CBCT images; C): CBCT linear measurement of bone width on Medium FOV CBCT images; D) CBCT linear measurement of bone width on Large FOV CBCT images

Results

Numerical data, including all the measurements taken from the gold standard (GS) and CBCT measurements in the four FOVs, were explored for normality by checking the data distribution using Kolmogorov-Smirnov and Shapiro-Wilk tests, all measurements showed normal (parametric) distribution. Data were presented as Mean ± Standard Deviation (SD), Minimum and Maximum.

Table 1: AME, and APE

	Small FOV		Midi FOV		Medium FOV		Large FOV		*P*-value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
AME (mm)	0.18 ^{B}	0.09	0.11 ^{B}	0.07	0.20 ^{A}	0.18	0.42 ^{A}	0.28	<0.001*
APE (%)	0.73 ^{B}	0.36	0.44 ^{B}	0.40	0.82 ^{A}	0.79	1.67 ^{A}	1.16	<0.001*

*: Significant at P ≤ 0.05, Different superscripts in the same row are statistically significantly different.

Checking data distribution for error measurements and percentage of error measurements showed non-normal (non-parametric) distribution. Data were presented as mean, median, standard deviation (SD), minimum, maximum and 95% Confidence Interval (95% CI) for the mean values.

Table 2: Measurement errors

	Small FOV		Midi FOV		Medium FOV		Large FOV		*P*-value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Absolute Measurement error (mm)	0.11 ^{B}	0.04	0.14 ^{B}	0.04	0.3 ^{A}	0.15	0.40 ^{A}	0.21	< 0.001*
Measurement error (%)	2.39 ^{B}	1.01	3.03 ^{B}	1.13	6.42 ^{A}	3.49	8.37 ^{A}	4.89	< 0.001*

*: Significant at P ≤ 0.05, Different superscripts in the same row are statistically significantly different.

For parametric data; Paired t-test was used to compare between FOV measurements and the standard reference. For non-parametric data; Friedman's test was used to compare between measurement errors as well as the percentage of error measurements of the four FOV. Dunn's test was used for pair-wise comparisons when Friedman's test is significant.

The significance level was set at P ≤ 0.05. Statistical analysis was performed with IBM, SPSS Statistics Version 20 for Windows.

A. Mean, standard deviation (SD) values and results of Friedman's test for the comparison between errors of height measurements by the four CBCT FOVs.

B. Mean, standard deviation (SD) values and results of Friedman's test for the comparison between errors of BL depth measurements by the four FOV.

Discussion

The first one was reported in 2010 [5]. Unlike the results of our study, they concluded that changes in FOV did not affect measurement accuracy, although they assessed small linear distances in their study (diameter & depth of chemically created periapical lesions). They utilised only two different FOVs and voxel sizes (6 inches & 9 inches FOVs using voxel sizes 0.11 & 0.19 mm^3 respectively). They reported that the difference between measurement errors in the two used FOVs was non-significant statistically, however on revising their measurements error values, high percentage error that exceeded the clinically acceptable level were found in both FOVs, as they reported that error values for 6 inches FOV ranged from -0.68 to 0.80 mm, (-11.46% to 17.03%) for diameter measurements, and from -0.73 to 0.53 mm (-13.51% to 10.82) for depth measurements, while for the larger FOV (9 inches), the error values ranged from -0.64 to 0.81 mm, (-12.41% to 16.95%) for diameter measurements, and from -0.72 to 0.52 mm (-13.50% to 10.63%) for depth measurements.

Comparing the results of the previous study [5] with ours, showed that the level of CBCT linear measurements accuracy reported in their study is much lower than ours, although both voxel sizes utilised in their study was smaller than the smallest voxel size we used. However, their FOVs used were larger than the largest FOV we used. Moreover they assessed much smaller distances than we did.

The second study found was conducted in 2014 [6]; it aimed to assess the effect of FOV on both identification and measurements (linear & volumetric) of peri-implant bone defects with different sizes. Again, they concluded that the three utilised CBCT FOVs (40 × 40, 60 × 60 and 100 × 100 mm, with voxel sizes 0.08, 0.125, 0.25 mm^3 respectively) yielded measurements that were strongly correlated to the actual real measurements; however, they didn't report the error values in their study! On the other hand, similar to the results of this study, they reported that both the detection ability and measurements accuracy were higher in larger defects, and smaller FOVs.

Again, in 2016 Ganguly et al., [7] study concluded that the reduction of FOV and Voxel size is not associated with greater accuracy of CBCT linear measurements. This study was differing from ours and other similar studies in utilizing two different CBCT machines, as each of the four cadavers used in the study was imaged twice by iCAT machine with FOV 13 × 16 cm (once with voxel sizes 0.2 mm^3 & once with 0.3 mm^3) then was imaged again by Planmeca Promax 3D machine with FOV 5 × 8 cm and 0.16 mm^3 voxel size. These scans were named as protocols 1, 2 and 3 respectively. Their mean CBCT AME values were 1.10 ± 1.3 mm, 1.2 ± 1.5 mm, and 1.1 ± 1.4 mm for protocols 1, 2, and 3 respectively. Although their error values were larger than those reported in our study, but this could be attributed to the fact that they did their study on cadaver heads with presence of soft tissues that add more radiation scatter, while ours was made on bone models with increased contrast between the external surface of the model and the surrounding air making it easier to identify landmarks and thereby explaining the higher accuracy of measurements. Contradicting our findings, Ganguly et al., [7] reported that as the measurements became larger, larger discrepancies were found between the measurements.

The last study found was published in 2016 by Anter et al., [8], who also concluded that changing the FOV doesn't affect CBCT linear measurements accuracy, this study utilized three FOVs (small 80 × 80 mm, medium 100 × 100 mm and large 200 × 100 mm) and unlike the similar studies they standardized the voxel size to be 0.2 mm in the three FOVs. Their reported mean CBCT measurement errors for the small, medium and large FOVs were 0.23 ± 0.09 mm, 0.24 ± 0.10 mm and 0.21 ± 0.09 mm respectively, which are very close to those reported in our study. However, they recommended the usage of smaller FOVs whenever possible to reduce the patient's radiation dose. The previous study was the only one that assessed the effect of FOV solely which is ideally relating the resultant effectiveness to the examined variable, however in clinical situations, most of the available CBCT machines don't allow for usage of small voxel sizes with large FOVs, and even when it is possible, it results in very high patient radiation dose.

Finally, the contradiction found between our results and those of other researchers could be attributed to any of multiple factors like difference in purposes with a resultant difference in the technical parameters used, type of the CBCT machine and type of CBCT images used for measurements. There were also differences in the qualifications and the numbers of observers who interpreted the radiographic data in the different studies.

From the results of this study, we can conclude that CBCT scans made with smaller FOVs and voxel sizes are associated with higher linear measurements accuracy than those made with larger FOVs and voxel sizes. For the same voxel size, smaller FOVs are associated with higher CBCT linear measurements accuracy than those made with larger FOVs. The shorter the distances measured, the greater is the effect of FOV and voxel size on the reported CBCT measurement accuracy.

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Prevalence of Molar Incisor Hypomineralization among School Children Aged 9 to 12 Years in Virajpet, Karnataka, India

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Abstract

BACKGROUND: The molar incisor hypomineralization (MIH) is defined as a qualitative defect of the enamel characterised by the progressive and simultaneous hypomineralization of the enamel structure of the first permanent molars which is of systemic origin, which may be associated frequently with incisors. Although the reported prevalence of MIH ranges from 2.4% to 40.2% worldwide, very little data is available from India.

AIM: To determine the prevalence of molar incisor hypomineralization among school children aged 9 to 12 years in virajpet, Karnataka.

METHODS: This cross-sectional descriptive study consisted of 1600 school children aged 9-12 years selected by stratified cluster sampling procedure. The European Academy of Pediatric Dentistry criteria were followed for MIH diagnosis. Chi-square test was used to analyse the categorical data. P ≤ 0.05 was considered for statistical significance.

RESULTS: The prevalence of MIH is 13.12% with no gender predilection. Ten-year-old children showed the highest prevalence (15%) among all the age group. Majority of children with MIH (70.2%) have lesions in both molars and incisors with demarcated opacities and atypical restorations being the most frequent defect type.

CONCLUSION: Prevalence of MIH was 13.12% in the 9-12-year child population in Virajpet. There is a need for a proper planned preventive and restorative program about the increasing prevalence of MIH.

Introduction

Dental enamel has some properties making it a unique tissue. It is the hardest tissue in the body and has a very high proportion of inorganic matter, mainly hydroxyapatite. The ameloblast has a limited reparative capacity; therefore, disturbances occurring during the mineralisation of enamel will remain as permanent marks. Defects in enamel quality or other dental hard tissues are important implications for the understanding of evolution, function, origin and relation to etiological factors behind developmental disturbances but also how environmental factors may influence on the mineralisation of the dental hard tissues. These unique properties of teeth have been widely used in research within biology, anthropology, archaeology and several other areas [1], [2].

The molar incisor hypomineralization (MIH) is defined as a qualitative defect of the enamel characterised by the progressive and simultaneous hypomineralization of the enamel structure of the first permanent molars which is of systemic origin, which may be associated frequently with incisors [3].

Since the early 1970's dentists have reported a developmental defect primarily located in the first

molars and incisors in permanent dentition, areas of demarcated hypomineralized enamel varying from opacity to more severe conditions with enamel surface breakdown were typical for the defect. This enamel disturbance was found predominately in first permanent molars and incisors. One of the first more extensive studies on the prevalence of demarcated opacities in first permanent molars and incisors was reported by Koch et al., in 1987. It was first defined by Weerheijm et al., in 2001. It is also called as "hypomineralized permanent first molars (PFMs)'', "idiopathic enamel hypomineralization", "nonfluoride hypomineralization" "demineralised PFMs'' and "cheese molars''. Knowledge about the magnitude of MIH seems desirable as it is vulnerable for consequences like rapid caries development, early enamel loss, soft structure and sensitivity [4].

The molar incisor hypomineralization is clinically presented as demarcated enamel opacities of different colours, occasionally undergoing post-eruptive breakdown (PEB) which results in atypical cavities or complete coronal distortion, requiring extensive restorative treatment. Despite the higher treatment demands, restorative treatment for these teeth is challenging for both the patient and dentist.

MIH is recognised as a global dental problem, and epidemiologic reports from all over the world are continuously published [5]. The global prevalence of MIH ranged from 2.4% to40.2%. Majority of the studies that reported MIH were from European countries with a prevalence range of 3.6% to 37.5%. Prevalence in middle and South East Asian countries ranged from 9.25% to 20.2%. Prevalence data from India were scant and reported a prevalence of 6.31% to 9.46% [1]. The prevalence is almost 40% per cent in Denmark and Brazil [6], [7]. As many as 5% of the children in a Swedish population have a severe form of MIH and will experience extensive and difficult treatment. It has been reported that children with MIH have ten times more dental treatment compared with a group of children with clinically healthy first molars [8].

The prevalence of MIH was not well documented due to several diagnostic classifications in the literature. The various indices used are Alaluusua et al., criteria (1996), developmental defects of enamel index (DDE), Kemoli criteria (2008), Koch et al., [9]. Criteria (1987), and the European Academy of Pediatric Dentistry (EAPD) 2003 criteria [10]. Knowledge about the magnitude of MIH seems desirable as it is vulnerable for consequences like rapid caries development, early enamel loss, soft structure and sensitivity [4].

Severe clinical manifestations and their consequences associated with MIH indicates the need for research to increase knowledge about its prevalence and risk factors in developing countries [11]. Thus, the aim of this study was to evaluate the prevalence of MIH in a group of children aged from 9 to 12 years in Virajpet, Karnataka, India.

Material and Methods

The present, descriptive cross-sectional study conducted during January 2018 – March 2018, the study population comprised of 9 to 12-year-old School children belonging to Virajpet taluk. Total numbers of school students were 9792. The sample size was estimated and obtained as 400 per each age group and among 4 age groups (9, 10, 11 and 12 years) the total of 1600 samples. Stratified Cluster Sampling Method was followed. Total of 171 schools in Virajpet taluk was divided into 10 clusters based on location. Each cluster contains 17 schools. Considering 10% of schools from each cluster 2 schools were chosen randomly. Total schools considered were 20. Considering the number of schools included and the sample size (400 per age group), 20 students from each age group from each school was taken. Subjects who were willing to participate and whose parents/guardians have given written informed consent and children having fully erupted all permanent first molars and incisors were included in the study.

The children with amelogenesis imperfecta, dentinogenesis imperfecta, white spot lesions, tetracycline stains, erosion, fluorosis and Turner's tooth, with appliances, undergoing orthodontic treatment, Restorations and Crowns on any of the first permanent molars and incisors were excluded. Ethical clearance was obtained from the Institutional Review Board of Coorg Institute of Dental Sciences, Virajpet. Diagnosis of MIH was done using EAPD Criteria 2003 [14].

An examination for MIH should be performed on wet teeth after cleaning. Teeth to be examined are the 4 first permanent molars (occlusal, buccal, lingual/ palatal surfaces) and 8 permanent incisors (incisal, labial, lingual/ palatal surfaces) following Type III Clinical examination with adequate natural light.

The oral examination of all the study subjects was carried out by a single investigator. Each participant was meticulously examined and the findings were compared to know the diagnostic variability agreement. The agreement was found to be 80%.

Statistical Analysis

The data was collected and transferred from pre-coded proforma to computer. The data will be analyzed using SPSS (IBM) version 23. Descriptive statistics included mean, standard Deviation, Frequency and Percentage. Inferential statistics included Chi square test. The level of significance was set at 0.05 at 95% confidence intervals.

Results

Distribution of study subjects comprised a total of 1600 (100%) participants. Among them, 786 (49.1%) were males, and 814 (50.9%) were females.

Table 1: Tooth wise Prevalence of MIH

Tooth Number	MIH	Prevalence
11	Absent	1445 (90.3%)
	Present	155 (9.7%)
12	Absent	1463 (91.4%)
	Present	137 (8.6%)
21	Absent	1450 (90.6%)
	Present	150 (9.4%)
22	Absent	1509 (94.3%)
	Present	91 (5.7%)
16	Absent	1396 (87.3%)
	Present	204 (12.7%)
26	Absent	1394 (87.1%)
	Present	206 (1.3%)
31	Absent	1534 (95.9%)
	Present	66 (4.1%)
32	Absent	1535 (95.9%)
	Present	65 (4.1%)
41	Absent	1532 (95.8%)
	Present	68 (4.3%)
42	Absent	1550 (96.9%)
	Present	50 (3.1%)
36	Absent	1398 (87.4%)
	Present	202 (12.6%)
46	Absent	1395 (87.2%)
	Present	205 (12.8%)

MIH was found to be present in 210 (13.12%) subjects, and the rest of 1390 (86.87%) subjects were unaffected.

Table 2: Prevalence of MIH according to EAPD diagnostic criteria

MIH type	Frequency	Percentage
White / creamy demarcated opacities, no PEB	15	(7.9%)
White / creamy demarcated opacities, with PEB	7	(2.6%)
Yellow / brown demarcated opacities, no PEB	70	(32%)
Yellow / brown demarcated opacities, with PEB	26	(13.7%)
Atypical restoration	72	(34.3%)
Missing because of MIH	20	(9.5%)
Total	210	(100%)

Among 210 subjects affected with MIH, 58 (14%) subjects were 9 years, 60 (15%) subjects were 10 years, 49 (12.25%) subjects were 11 years, and 43 (10.75%) subjects were 12 years of age.

Table 3: Comparison of prevalence of MIH based on Gender and Age

	Age	Prevalence	Chi Square and significance
	9 years	33 (31.1%)	
Male	10 years	26 (24.5%)	1.745
	11 years	23 (21.7%)	p=0.627
	12 years	24 (22.6%)	(NS)
	9 years	25 (24%)	
Female	10 years	33 (31.7%)	7.068
	11 years	26 (25%)	p=0.132
	12 years	19(18.3%)	(NS)

Discussion

The study recruited children aged 9 to 12 years for the assessment of MIH. Garg N et al., [12] stated that at this age, most children would have had all four first permanent molars and the majority of incisors, but these teeth would not have been exposed to the oral environment long enough to develop dental caries. Also, the permanent first molar teeth will be in a relatively good condition without excessive post-eruptive breakdown.

Table 4: Prevalence of MIH based on Arch and Segments

Prevalence		MIH		Chi-Square and significance
		Absent	Present	
Maxilla	Incisors	1407 (87.9%)	193 (12.1%)	0.728
	Molars	1391 (86.9%)	209 (13.1%)	P = 0.424 (NS)
Mandible	Incisors	1451 (90.7%)	149 (9.3%)	10.632
	Molars	1393 (87.1%)	207 (12.9%)	P = 0.001 (S)
Total	Incisors	1406 (87.9%)	194 (12.1%)	0.728
	Molars	1390 (86.9%)	210 (13.1%)	P = 0.424 (NS)

In the present study, the prevalence of MIH was 13.12% among 9-12-year-old children. This is by the study conducted by P.C. Calderara et al., [13] among school children aged 7.3 – 8.3 years living in Lissone, Northern Italy, wherein the prevalence of MIH was 13.7%. In a study conducted by Sulaiman Mohammed Allazzam et al., [14] in Jeddah, Saudi Arabia, the prevalence of MIH was 8.6% among a group of 8-12-year-old children. In a study conducted by Rahil Ahmadi et al., [15], the prevalence of MIH in a group of Iranian children aged 7-9 years was 12.7%. A study conducted by H.T Ajay Rao et al., [2] in Mangalore, Karnataka among 6-12-year school children, the prevalence of MIH was found to be 17.2%. In a study conducted by Shubha Arehalli Bhaskar [16] done among school children aged 8-13 years from Udaipur, Rajasthan, MIH prevalence was 8.9%. The prevalence in another study conducted by M Kirthiga et al., [4] among children aged 11-16 years of a city in Karnataka, Davangere was 8.9%. According to a study conducted by Savitha Deepthi Yannam et al., [11] in the child population aged between 8-12 years residing in Chennai, the prevalence of MIH was 9.7%. The study conducted by Cervantes Mendez MJ et al., [17], in South Texas, among 6-14 years subjects showed a prevalence rate of 29.5% which is on the higher side. According to Cho Sy et al., [18], the difference in MIH prevalence seen in various parts of the world may be due to the heterogeneity in ethnic and age groups being studied and the retrospective nature of the studies conducted.

In the present study, 10-year-old subjects showed comparatively higher prevalence (15%) than the other age groups and the least prevalence of MIH (10.75%) was seen in 12-year-old children. These results are by the study conducted by Savitha Deepthi Yannam [11] among the age group of 8-12 years, wherein the prevalence of MIH was highest among 10-year-old children (12.9%), and least prevalence of MIH (7.4%) was seen in 12-year old children. In a study conducted by Cristiane Maria Da Costa-Silva et al., [19], there was a higher prevalence among children with 10 years old or older (16.6%).

In the present study, it was observed that maxillary molars were more affected as compared to maxillary incisors, but the difference was not statistically significant (p = 0.728). This could be explained by the contribution of Lunt and Law [20] that modified the chronology of the deciduous human dentition and concluded that maxillary teeth are generally ahead of the mandibular teeth in development.

In the present study, according to diagnostic criteria, most of the teeth were affected by atypical restorations (34.3%) and yellow-brown demarcated opacities without PEB (32%). These results are in agreement with the study conducted by Sulaiman Mohammed Allazzam et al., [14], wherein it was found that demarcated opacities were the most frequent type of MIH and the prevalence of yellow-brown demarcations and atypical restorations can be due to the inclusion of older children, as some of the demarcated opacities may break down over time. The results are also by the study conducted by Cristiane Maria Da Costa-Silva et al., [19], in which atypical restorations were more frequently seen (20.8%). Mahoney EK et al., [21] in their study explained that Hypomineralized areas in enamel showed a reduction in mechanical properties and in mineral density [22], [23], [24], which facilitates PEB. Tooth wise prevalence based on diagnostic criteria showed that incisors were most affected with creamy white opacities without PEB, but molars were mostly affected by yellow/brown discolouration with PEB and atypical restorations. In the study conducted by Lygidakis NA et al., [25] among incisors, atypical restorations on the buccal surface were found to be more prevalent, which is in contrast to the present study results. The high incidence of atypical restorations on incisors could be related to an aesthetic concern rather than PEB, because there are generally no masticatory forces on the opacities in incisors.

In conclusion, recent research supports the assumption that MIH is a widespread problem all over the world. Findings from the present study show the following:

- The prevalence of MIH in 9-12-year-old children is 13.12 % with no gender predilection.

- Ten-year-old children showed the highest prevalence (15%) among all the age group.

- Majority of children with MIH (70.2%) have lesions in both molars and incisors with demarcated opacities and atypical restorations being the most frequent defect type.

Hence, it appears that this condition is more prevalent than was recognised until recently. Assuming the low awareness of this condition among the dentists and the general population of India, the demanding nature and the costs involved, the urgent need for further investigations into this problem becomes evident. A diligent follow-up and recall program for children who are affected is essential for developing preventive and therapeutic measures. There is also a need for formulating public awareness and prevention programs. A nationwide survey to find the prevalence of MIH is recommended.

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The Importance and Extent of Providing Compassionate Nursing Care from The Viewpoint of Patients Hospitalized in Educational Hospitals in Kermanshah - Iran 2017

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Abstract

AIM: This study is an attempt to determine the importance and extent of providing compassionate nursing care from the hospitalised patients' viewpoint in educational hospitals in Kermanshah-Iran 2017

METHODS: The study was carried out as a descriptive, analytical work in the hospitals affiliated to Kermanshah University of Medical Sciences on 300 patients in 2017. The patients were selected through convenient sampling, and Burnell Compassionate Care Scale was filled by the participants. The collected data was analysed in SPSS (v.20) using descriptive and inferential statistics.

RESULTS: The results showed that the mean and standard deviation score of importance and extent of compassionate care were 3.27 ± 0.526 and 2.80 ± 0.647 respectively. There was a significant difference between these two scores ($p < 0.001$). About all the factors in compassionate nursing care, there was a significant difference between the importance and extent of compassionate nursing care. The mean score of the importance of compassionate nursing care from female patients' viewpoint was higher than that of men ($p = 0.032$). The observers with college educations perceived the extent of compassionate nursing care less than the other groups of participants ($p = 0.008$).

CONCLUSIONS: There was a significant difference between the importance and extent of compassionate nursing care from the patients' point of view. This highlights negligence by the nurses of this critical aspect of care. It is recommended, therefore, to add compassionate nursing care to nursing programs and commission more research works on other groups of health care personnel.

Introduction

Compassion is a virtue and a necessary trait of nursing and being a nurse [1]. It is a feeling evoked by witnessing others pain that leads to taking measures to help them [2]. Compassion is the human and moral part of care, and according to many nursing literatures, compassion is the philosophical foundation and centrepiece of the nursing profession. Being compassionately responsive to the care needs of patients is one of the professional standards of nursing [3], [4], [5].

Compassion may have a direct effect on the quality of cares provided to patients so that they normally evaluate the quality of services based on the compassion demonstrated by the nurse [6], [7]. Providing compassionate nursing care can lead to higher satisfaction in patients, safer cares, saving time and cost, a sense of satisfaction and effectiveness in the personnel, higher confidence, and coping skills in them [8], [9]. However, modern nursing is mostly based on quantitative evidence and technical skills, and there is a little attention toward morality and compassion [10]. Thereby, attributes and behaviours that might be construed by patients as compassion in nurses and medical staff are not recognised, so that there are several different definitions of compassionate nursing care [11]. Papadopoulos

defined recognising the patient's need and demonstration of attention an understanding of patients' needs as the signs of compassion [12]. Van der Cingel argued that compassionate nursing care is featured with paying attention, listening, dealing with patient's problems, cooperating and accompanying the patient, helping, being available, and understanding [13]. Compassionate nursing care from the patient's point of view is featured with being considerate and accurate in dealing with patient's problems, being committed to realise and work to sooth the patient's pain while keeping a respectful relationship with the patient [14].

Compassionate nursing care is the main element in providing quality health services to patients [15], and it maybe is the best and most valuable gift that a nurse can give the patient in health systems [4], [16]. That is why nurses have always tried and been interested in meeting the patient's need through demonstrating altruism and despite all limitations and hurdles. Recent reports and studies have demonstrated, however, that the patients do not have a pleasant experience with nurses' behaviours at clinical settings. McCabe showed that the hospitalised patients complained about the lack of a proper connection and experiencing an inconsiderate, uncompassionate, and unfriendly relationship with nurses; so that according to the patients, these have led to a degradation of the quality of nursing cares [17]. All these are reported while compassion and mercy are the foundations of nursing and along with professional knowledge and clinical skills, nurses must be committed to providing humane care with compassion to the care receivers [18].

According to Dewar et al., the key point in providing compassionate nursing care is to recognise the needs and expectations of patients with cares [10]. Since compassionate nursing care is a subjective, complicated, multidimensional, and cultural based concept affected by values and social-political structure of the society [19], [20], the agreement between expectations of patients and nurse's interpretation of these expectations is a central element in providing quality and proper cares [21].

Therefore, surveying the patients' expectations and viewpoints about health cares – as receivers of cares – to determine the specifications and behaviours that resemble compassion of the nurse in providing cares is an opportunity to improve quality of nursing cares and profession.

The present paper is an attempt to determine the importance and extent of compassionate nursing care from hospitalised patients' point of view of patients hospitalised in educational hospitals in Kermanshah-Iran in 2017.

Methods

This study was done as a descriptive-analytical method in 2017. The study population consisted of the patients hospitalised in educational hospitals affiliated with Kermanshah University of Medical Sciences. Sample group included 300 patients in internal and surgery wards who were selected through convenient sampling method from three hospitals. Inclusion criteria were being at least three days in the hospital, the age of 18-65 years old, expression of consent to participate, ability to communicate orally, and stable physical status (no pain). The patients who failed to fill out the questionnaire or not interested were excluded.

For data gathering, after taking the permission from research deputy of University of Social Welfare and Rehabilitation Tehran and Kermanshah University of medical sciences, a demographics information checklist including questions about gender, marital status, education level, ward, hospitalization term, and occasions of hospitalizations in one year and Burnell Compassionate Care Scale was completed by interview and self-administering in a paper and open manner. The latter tool was first introduced by Burnell (2011) to assess compassionate nursing care in patients with cancer. Afterwards, the reliability and validity of the tool were confirmed for patients at internal, and surgery wards and the statements were decreased to 20 statements. Four factors are covered in the tool including meaningful connection (eight statements with Cronbach's Alpha 0.867), patients' expectation (five statements with Cronbach's Alpha 0.801), caring attributes (four statements with Cronbach's Alpha 0.774), and capable practitioner (three statements with Cronbach's Alpha 0.781) and also to test interdependence among the subscales of the CCAT©, the average rating of the importance for each component was computed, and then a correlation matrix was calculated for the subscale scores. All scales were significantly correlated with each other ($p < .001$), indicating consistency in the movement of one subscale in comparison to other subscales [16].

Each statement is designed based on Likert's four-point scale for important of compassionate care ("not important" to "very important). The statements about the extent of services are scored from 1 = never to 4 = most of the time. To obtain the score for each factor, the total score of the related statements is divided by the number of statements, and the higher the score, the more compassionate are the cares. The patients expressed their opinions in two fields of importance and extent or providing compassionate cares.

Before initiating data gathering process, required permissions were secured, and the researcher visited the patients and briefed them about the title and objectives of the study and secured an

informed consent was signed by candidate participants. Then the questionnaires were administered, and the participants were asked to score the 20 statement of the scale from two points of view; i) importance of each statement in provision of compassionate cares (1 = not important,…, 4 = very important); ii) the extent of providing such services by nurses (1 = never,…, 4= most of the time). For the disabled or illiterate patients, the researcher read the statements and fills out the scale for them.

Data analyses were done in SPSS (v.20) using descriptive statistics (mean, SD, frequency, and frequency percentage) and inferential statistics (Mann Whitney U, Kruskal Wallis, and Spearman correlation analysis) (P = 0.05).

Results

Of the 300 individuals participated in the study, 191 (63.7%) were male and 226 (75.3%) married. About 56% had an educational level less than a diploma, and 51% hospitalised in the internal units (Table 1). Mean, and standard deviation (SD) of age, duration of hospitalisation, and a number of hospitalisations were 43.94 ± 13.76 years, 10.23 ± 12.49 days, and 3.67 ± 3.82, respectively.

Table 1: Demographic characteristics of the samples

Variables		Frequency	Frequency per cent
Gender	Male	191	63.7
	Female	109	36.3
Marital status	Married	226	75.3
	Single	74	24.7
Unit of hospitalisation	Internal	153	51.0
	Surgical	147	49.0
Educational level	Under diploma	169	56.3
	Diploma	86	28.7
	Academic	45	15.0

The mean of Importance of compassionate and providing it was 3.27 ± 0.526 and 2.80 ± 0.647, respectively, in which Wilcoxon test showed a significant difference between them (Z = -10.22, P < 0.001). This difference was also different about all compassionate care factors (Table 2).

Table 2: Relationship between the importance of compassionate care and its providing in the viewpoint of patients

Variables		Mean	Mean rank	Statistical test
Whole score of the questioner	Importance	3.28	163.52	Z = 10.25
	Providing	2.80	76.03	*P < 0.001
Meaningful connection	Importance	3.21	144.92	Z = 9.27
	Providing	2.78	76.65	*P < 0.001
Patient expectation	Importance	3.37	142.73	Z = 9.55
	Providing	2.85	73.74	*P < 0.001
Caring attributes	Importance	3.24	149.71	Z = 8.98
	Providing	2.71	87.47	*P < 0.001
Capable practitioner	Importance	3.39	132.83	Z = 9.28
	Providing	2.89	70.31	*P < 0.001

In viewpoints of female compassionate care was more important than male (Z = 2.149, P = 0.032), but they have a no different idea about providing compassionate care by nurses (Z = 0.171, P = 0.864). Also the compassionate care was not related to a marital status, unit of hospitalisation. The people academic education level took more compassionate care than diploma and lower level (K2 = 9,727, P = 0.008) (Table 3).

Table 3: Importance of compassionate care based on the demographic characteristics

Variables		Mean	Mean rank	Statistical test
Gender	Male	3.23	142.37	Z=2.149
	Female	3.38	164.74	*p=0.032
Marital status	Married	3.31	154.40	Z=1.361
	Single	3.20	138.59	P=0.173
Unit of hospitalization	Internal	3.26	147.35	Z=0.643
	Surgical	3.31	153.78	P=0.520
Educational level	Under diploma	3.26	145.25	F=2.66
	Diploma	3.35	163.40	P=0.263
	Academic	3.24	145.59	

However, there was no correlation between age, duration of hospitalisation and number of hospitalisation with important and providing compassionate care (Table 4).

Table 4: Providing compassionate care based on the demographic characteristics

Variables		Mean	Mean rank	Statistical test
Gender	Male	2.80	151.15	Z = 0.171
	Female	2.79	149.37	P = 0.864
	Married	2.81	152.40	Z = 0.663
Marital status	Single	2.76	144.70	P = 0.508
Unit of hospitalization	Internal	2.79	148.28	Z = 0.453
	Surgical	2.81	152.81	P = 0.651
Educational level	Under diploma	2.88	159.91	F = 9.72
	Diploma	2.78	150.83	P = 0.008
	Academic	2.53	114.54	

There was no correlation between providing and importance of compassionate care with quantitative demographic variables (Table 5 and Table 6).

Table 5: correlation between the importance of compassionate care and its factors with the variables of age, duration of hospitalisation and number of hospitalisations

Variables	Age	Duration of hospitalisation	Number of hospitalisations
Importance of compassionate care	R = 0.055	R = 0.022	R = 0.029
	P = 0.344	P = 0.706	P = 0.720
Meaningful connection	R = 0.074	R = 0.056	R = -0.022
	P = 0.201	P = 0.332	P = 0.779
Patient expectation	R = 0.020	R = 0.030	R = -0.086
	P = 0.726	P = 0.602	P = 0.267
Caring attributes	R = 0.012	R = 0.026	R = 0.054
	P = 0.841	P = 0.651	P = 0.485
Capable practitioner	R = 0.062	R = 0.066	R = -0.010
	P = 0.288	P = 0.254	P = 0.899

Table 6: Correlation between providing compassionate care and its factors with the variables of age, duration of hospitalisation and number of hospitalisations

Variables	Age	Duration of hospitalisation	Number of hospitalisations
Providing of compassionate care	R = -0.019	R = 0.080	R = 0.042
	P = 0.746	P = 0.169	P = 0.590
Meaningful connection	R = -0.018	R = 0.073	R = 0.045
	P = 0.760	P = 0.205	P = 0.566
Patient expectation	R = 0.022	R = 0.102	R = 0.052
	P = 0.706	P = 0.079	P = 0.506
Caring attributes	R = -0.064	R = 0.020	R = 0.057
	P = 0.267	P = 0.726	P = 0.461
Capable practitioner	R = 0.002	R = 0.039	R = -0.022
	P = 0.971	P = 0.501	P = 0.773

Discussion

The result showed compassionate nursing care from the viewpoint of patients hospitalised in educational hospitals in Kermanshah-Iran in 2017 was important (3.27 ± 0.526), and also the extent of providing compassionate nursing care was 2.80 ± 0.647.

The results showed that there was a significant difference between what was important for the patients' in terms of compassionate nursing care and what was provided to them. There was a significant difference between the mean score of importance and extent of providing compassionate nursing care in general and from the four subscales point of view – i.e. meaningful connection, patients' expectation, caring attributes, and capable practitioner. These findings indicate that the extent of providing compassionate nursing care was less than what was expected and desired by the patients. In other words, there was a difference between the expectations of the patients and actual compassionate nursing care provided to them. This is consistent with Modic (2016), Sinclair et al., (2016), Lown (2017) and Joolaee (2014) [22], [23], [24], [25].

The literature review showed that the nurses do not have comprehensive knowledge about their patients and their wants. In most of the cases, the patients' needs are not surveyed and fulfilled thoroughly [24], [26], [27]. Hajime had et al. reported a significant difference between nurses and patients in terms of their attitudes about nursing personnel's caring behaviours [28]. It is essential therefore for the nurses to pay attention to those aspects of care that are more important for patients. Lown (2010) reported that 50% of hospitalised patients in the USA stated that compassion is a missing part in care services [8]. An agreement between patients' expectations and nurses' interpretation of these expectations is the key point in providing proper cares.

In light of these, nurses need to see things (e.g. issues, concerns, disabilities) from the patient's eyes to make a nursing care program based on care priorities identified by patients and deal with concerns and disabilities of patients [20], [24], [29].

From the participants' point of view capable practitioner was the most important aspect and obtained the highest score of provision. Meaningful connection and caring attributes were the least and second least important and provided aspects respectively in terms of providing compassionate nursing care. These findings are consistent with Khademian, Hajinejad, and Wolf who reported that the patients found technical caring behaviours more important than emotional caring behaviours [28], [30], [31]. Palese et al. studied the importance of caring behaviours of nurses from the viewpoint of patients in internal and surgery wards in six European countries and concluded that "knowledge and skill" were of the highest importance (most important caring behaviour) and "positive connection" was of the least importance [31]. Meanwhile many studies such as Meyer and Thing et al. showed that the attention to psychosocial aspects of care, especially the proper communication with patients, is more than the technical aspects of care, which leads to the satisfaction of patients [19], [32].

In the present study, the high importance and extent of provision of technical and physical aspects of cares by nurses from the patients' point of view might be explained by the fact that these aspects are more tangible and observable. In other words, these behaviours are more objective and easier to perceive by the patients. Additionally, the larger extent of providing these aspects might be due to stricter supervision of providing them comparing with the other humanistic aspects. Moreover, these aspects might be more important from the nurses and nurses' skills point of view [25], [33], [34].

Studies have shown that although nurses find compassionate nursing care a key part of their professional tasks, failure of the managers to emphasise on that part and omitting it in nursing performance evaluation have had a negative effect on this aspect of nurses' performance [25], [35]. As a result, compassion has become such an unimportant and trivial matter in the nursing profession that whether or not to observe it is a personal choice of nurses and a moral aspect [18]. The ability to combine tangible science (quantitative) with morality and spirituality in nursing care has become a serious challenge in the nursing profession [5].

The findings also showed that women put more emphasis on compassionate nursing care than men, while there was no difference between the patients based on a ward of hospitalisation and marital status. This finding is consistent with Brunel and Eagen (2013) who argued the importance of compassionate nursing care was higher for women compared with men [16]. This difference between men and women may be explained by the fact that women have the different emotional background and life experience from men so that women have different perception and attitude about compassionate nursing care and find it more important.

The extent of providing compassionate nursing care was significantly different based on education level so that the participants with a college degree experienced a lower level of compassionate nursing care. That is, the higher the education level, the higher the patient's expectation for quality cares, so that educated patients expect a larger opportunity to participate in the treatment process rather than being a mere patient. On the other hand, people with higher education level tend to have better social communication skills and access to information so that they have keener eyes for shortages and

imperfections of the system. As a result, this group of patients are harder to satisfy. This finding is consistent with Péfoyo (2013), Joulali (2014) and Kazemeini (2011) who mentioned that patients with higher education degree Perceived nursing care as lower quality [24], [36], [37].

In this study, a significant difference was found between the importance of compassionate caring from the patients' point of view and the extent of providing such services to patients. This hints the failure of nurses to pay adequate attention to this aspect of cares. Nurses need to see the patient's caring needs and expectations from the patient's point of view and pay more attention to the aspects that are more important for the patients. Through this, they can better help the patients with their concerns and disabilities. Paying more attention to compassionate nursing care in nursing textbooks is recommended, and the nurses should receive in-service educations in this regard. There is a need for further qualitative and quantitative research works on compassionate care in the personnel of different fields of health cares, patients in intensive wards, and family members of patients.

Ethics Approval and Consent to Participate

The study is confirmed by the research ethics committee of the University of Social Welfare and Rehabilitation

Consent for Publication

All the authors and the University of Social Welfare and Rehabilitation consented to publish the study in your journal

Availability of Data and Material

Data available by contacting the corresponding author

Authors' Contributions

AD, AS, AVR, KN, AE and MR made the conceptual work and the designing of study, AVR and participated in data collection, and data analysed by AVR, AD and MR. The final report and article were written, read and approved by all the authors

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