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***Basic Science***

# Study of Azole - Resistant and *Cyp51a* Gene in *Aspergillus Fumigatus*

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**Keywords:** Azole-resistant; *Cyp51 A gene; Aspergillus fumigatus*

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**Abstract**

**AIM:** The main goal of the present study was to find azole-resistant and molecular analysis of *cyp51A* gene in *Aspergillus fumigatus*.

**MATERIALS AND METHODS:** Fifty-eight *A. fumigatus* strains including environmental, clinical and reference isolates were assessed in this investigation. Azole susceptibility testing for itraconazole and voriconazole was carried out for *A. fumigatus* isolates. PCR was performed based on *cyp51A* gene sequence for all isolates.

**RESULTS:** Susceptibility testing verified the minimum inhibitory concentrations (MICs) for itraconazole (0.125 to 2 µg/ml) and voriconazole (0.125 to 4 µg/ml). Nine (15.5%) *A. fumigatus* isolates were resistant to voriconazole with MIC 4 µg/ml. A 1500 bp DNA fragment was amplified using *cyp51A* gene for all tested Aspergillus isolates. The sequences of the fragments showed 99% identity with *A. fumigatus cyp51A* gene in the GenBank. No point mutation was found at *cyp51A* gene codons.

**CONCLUSION:** In the current study, we detected the voriconazile resistant in *A. fumigatus* isolates. Susceptibility tests should be considered in patients who infected by *A. fumigatus*.

## Introduction

*Aspergillus fumigatus* is one of the most common airborne fungal pathogen which causes invasive aspergillosis. The spores are capable of spreading to air and inhaled and eventually cause infection in a susceptible host. *A. fumigatus* infections occur in excessive morbidity and mortality in immunocompromised hosts [1] [2]. The azoles are antifungal drugs that inhibit the ergosterol biosynthesis pathway by the inhibition of 14α-demethylase. Azoles, for example, itraconazole, voriconazole, and posaconazole are among the advised first-line agents in the management and prophylaxis of aspergillosis [3].

The appearance of azoles resistance in yeast species has encouraged researchers of the mechanisms associated with this resistance. Several genes encoding 14α-demethylase (*ERG11*/*cyp51*) have been identified for fluconazole-resistant of the clinical isolates of *Candida albicans* [4] [5].

The mechanisms for azole drug resistance in *A. fumigatus* appear to be extremely dissimilar from that in *Candida* spp. There are two various but related *Cyp51* proteins which are encoded by *cyp51A* and *cyp51B* genes [6] [7].

Two models of resistance to azoles have discovered in *A. fumigatus*. First, the *A. fumigatus* could turn into resistant during exposure to azoles in the patient. This model was detected in chronic pulmonary aspergillosis and aspergilloma cases in the United Kingdom [8]. In this pattern, eighteen dissimilar

amino acid substitutions were distinguished in the *cyp51A* gene [8]. Second, the *A. fumigatus* can turn into resistance in the environment during the exposure to azole fungicides which are applied in agriculture and material conservation.

This model was suggested in the Netherlands [9]. In this pattern, a replacement at codon 98 of the *cyp51A* gene combined with a tandem repeat of 34 bp in the promoter (TR/L98H), was detected in 94% of the resistant strains [10].

Treatment of invasive aspergillosis is mainly limited to therapy by the polyene agent amphotericin B, and triazoles such as itraconazole, voriconazole and echinocandin caspofungin. Amphotericin B is very toxic and can consequence in nephrotoxicity [11], while triazoles are fungistatic and subject to development of resistance [12].

Here, we explain the analysis of *cyp51A* gene *A. fumigatus* which is responsible for the phenotype of *A. fumigatus* azole-resistance.

## Material and Methods

A total of 58 *A. fumigatus* strains were used in the study including 45 environmental, 9 clinical and 4 reference isolates. The following strains were used as a reference: PTCC 5009, IBRC-M 30033, IBRC-M 30040, IBRC-M 30048. Environmental strains were recovered from soil or air.

One hundred ml of yeast extract peptone dextrose (YEPD) medium in Erlenmeyer flasks was inoculated with 1 ml of thick spore suspension and incubated at 37°C for 72 h 200 rpm under agitation to obtain mycelium growth. The mycelia were harvested, washed with 0.5 M EDTA and sterile dH2O and using liquid nitrogen and a pestle and mortar ground into a fine powder. The DNA was extracted with vivantis is GF-1 plant DNA extraction kit, Malaysia.

The primer sets, P450-A1 (5'-ATGGTGCCGATGCTATGG-3') and P450-A2 (5'-CTGTC-TCACTTGGATGTG-3') was used to amplify an ~ 1500 bp DNA fragment of the full coding sequences of *cyp51A* gene. PCR reactions were carried out with a volume of 30 µl, comprised of 3 µl 10X reaction buffer, 200 µM each dNTP, 2.2 mM $MgCl_2$, 2.5 units of *Taq* DNA polymerase (CinnaGen, Tehran, Iran), 30 ng template DNA and 50 pmol of each primer.

Initial denaturation for 5 min at 94°C was followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 58˚C for 1 min and extension at 68˚C for 2 min. The PCR product (5 µl) was electrophoresed on 1% agarose gel in TAE buffer and stained with ethidium bromide.

Spore suspension for each isolate was set up in sterile normal saline and adjusted at a concentration of $10^6$ spores/ml, consequent to 68 to 82% transmittance at 530 nm [13]. Broth microdilution susceptibility test was carried out as explained in clinical and laboratory standards institute (CLSI) method with modifications. The antifungal drugs used were itraconazole (Sigma-Aldrich, Germany) and voriconazole (Sigma-Aldrich, Germany).

Stock solutions were prepared in 100% dimethyl sulfoxide (CinnaGene, Karaj, Iran), then diluted in RPMI 1640 medium and dispensed into 96-well microdilution trays. The final concentration of voriconazole and itraconazole in the wells ranged from 0.015 to 8.0 µg/ml. The stock spore suspension ($10^6$ spores/ml) was diluted to a final concentration of $5 \times 10^4$ CFU/ml and dispensed into the microdilution wells. The inoculated microdilution trays were kept at 35°C and read after 48 h. The minimum inhibitory concentration (MIC) for voriconazole and itraconazole was described as the lowest concentration that created prominent growth inhibition.

Some *cyp51A* gene amplicons were submitted for direct sequencing (Bioneer Corporation, Daejeon, South Korea). The obtained sequences were searched for in the National Center for *Biotechnology Information* (NCBI) database (http://www.ncbi.nih.gov/).

The sequences showed 99% similarity with *A. fumigatus cyp51A* gene sequences deposited in NCBI database. The computer software package MEGA5 (http://www.megasoftware.net) was employed for sequences alignment.

## Results

A total of 58 samples were tested including 45 environmental, 9 clinical and 4 reference isolates, according to the European committee for antibiotic susceptibility testing (Eucast) methodology suggestion breakpoints for *A. fumigatus,* itraconazole and voriconazole [14]. For itraconazole and voriconazole, <2 µg/ml (susceptible), 2 µg/ml (intermediate) and >2 µg/ml (resistant). Susceptibility testing verified the MICs for itraconazole (0.125 to 2 µg/ml) and voriconazole (0.125 to 4 µg/ml) (Table 1).

Nine (15.5%) *A. fumigatus* isolates were resistant to voriconazole with MIC 4 µg/ml. Twelve (20.7%) isolates exhibited intermediate susceptibility to itraconazole with MIC 2 µg/ml. The PCR amplification of *cyp51A* gene with set primers P450-A1 and P450-A2 produced a 1500 bp fragment for all tested *Aspergillus* isolates (Fig. 1).

**Table 1: Results of susceptibility testing voriconazole and itraconazole for *A. fumigatus* isolates**

| Isolate number | Source | MIC (µg/ml) for voriconazole | MIC (µg/ml) for itraconazole |
|---|---|---|---|
| 3/M | Environmental | 2 | 1 |
| 4/M | Environmental | 2 | 0.5 |
| 5/M | Environmental | 2 | 1 |
| 6/M | Environmental | 0.5 | 0.5 |
| 8/M | Environmental | 2 | 1 |
| 11/M | Environmental | 1 | 1 |
| 12/M | Environmental | 1 | 2 |
| 13/M | Environmental | 4 | 0.5 |
| 14/M | Environmental | 0.5 | 1 |
| 15/M | Environmental | 0.5 | 2 |
| 16/M | Environmental | 2 | 2 |
| 17/M | Environmental | 4 | 0.5 |
| 19/M | Environmental | 2 | 0.5 |
| 20/M | Environmental | 1 | 1 |
| 21/M | Environmental | 2 | 1 |
| 22/M | Environmental | 2 | 1 |
| 23/M | Environmental | 0.125 | 0.5 |
| 24/M | Environmental | 4 | 0.5 |
| 26/M | Environmental | 2 | 1 |
| 27/M | Environmental | 0.5 | 2 |
| 28/M | Environmental | 2 | 1 |
| 29/M | Environmental | 1 | 1 |
| 30/M | Environmental | 1 | 2 |
| 31/M | Environmental | 2 | 1 |
| 32/M | Environmental | 1 | 1 |
| 34/M | Environmental | 2 | 2 |
| 36/M | Environmental | 2 | 1 |
| 37/M | Environmental | 4 | 0.5 |
| 38/M | Environmental | 2 | 1 |
| 44/M | Environmental | 2 | 0.5 |
| 45/M | Environmental | 4 | 0.5 |
| 46/M | Environmental | 2 | 1 |
| 6/1 | Environmental | 2 | 1 |
| 6/2 | Environmental | 2 | 2 |
| 6/3 | Environmental | 4 | 2 |
| 7/1 | Environmental | 2 | 1 |
| 7/2 | Environmental | 2 | 0.5 |
| 7/3 | Environmental | 2 | 0.5 |
| 7/4 | Environmental | 2 | 0.5 |
| 7/5 | Environmental | 4 | 0.5 |
| 7/6 | Environmental | 2 | 1 |
| 9/1 | Environmental | 2 | 0.5 |
| 10/1 | Environmental | 2 | 2 |
| 11/1 | Environmental | 2 | 1 |
| 18/1 | Environmental | 2 | 0.5 |
| 1/B | Clinical | 2 | 1 |
| 2/B | Clinical | 2 | 0.5 |
| 3/B | Clinical | 2 | 1 |
| 4/B | Clinical | 2 | 0.5 |
| 70/B | Clinical | 1 | 2 |
| 72/B | Clinical | 1 | 1 |
| 98j/B | Clinical | 1 | 1 |
| 1010/B | Clinical | 0.25 | 2 |
| MEH | Clinical | 0.5 | 2 |
| PTCC 5009 | Reference | 1 | 0.125 |
| IBRC-M 30033 | Reference | 2 | 0.5 |
| IBRC-M 30040 | Reference | 4 | 0.5 |
| IBRC-M 30048 | Reference | 4 | 0.5 |

Several *cyp51A* gene amplicons including nine voriconazole resistant isolates were sent for direct sequencing. The sequences were searched in the NCBI database.

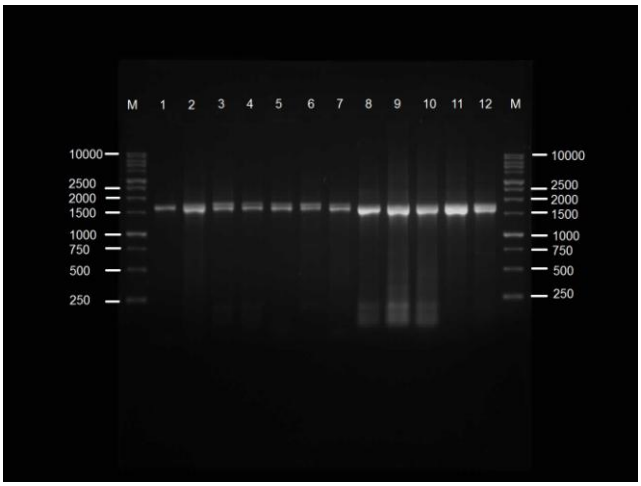


*Figure 1: Agarose gel electrophoresis of cyp51A gene products (1500 bp) of Aspergillus fumigatus isolates (lanes 1, 2, reference strains; lanes 3-7, clinical isolates; lanes 8-12, environmental isolates). Lane M, 1 kb ladder; lane 1, 30040; lane 2, 30048; lane 3, 2/B; lane 4, 4/B; lane 5, 72/B; lane 6, 1010/B; lane 7, Meh; lane 8, 7/4, lane 9, 10/1; lane 10, 18/1; lane 11, 19/M; lane 12, 22/M*

The sequences showed 99% identity with *A. fumigatus* sequences deposited in the NCBI database. The computer software MEGA5 was applied for sequences alignment. Although nine isolates were found to be resistant to voriconazole, we did not find any isolate with a point mutation in their *cyp51A* gene codons.

## Discussion

Rapid recognition of *Aspergillus* infections with a precise assessment of possible drug resistance is vital for successful management of patients with invasive infection. Clinically, triazole resistance rates are different between 2 and 6.6% among samples [15] [16]. In 1997 the first case of *A. fumigatus* itraconazole resistant was reported [17]. Research from the Netherlands reported that 3 of 114 *A. fumigatus* isolates were resistant to itraconazole although all had MICs for all of them were low for voriconazole [18].

Some researchers reported that a point mutation that substituted the glycine at codon 54 of CYP51A was created itraconazole resistant in *A. fumigatus* [19][20]. Mechanisms of resistance to azole have been explained in other fungi, particularly *C. albicans*. Triazoles are the foundation of therapy with voriconazole the first-choice treatment for invasive aspergillosis [21]. Nevertheless, azole resistance reports have appeared, not only after long time azole usage [8] but also after a short time using and in azole-naive cases [10].

Resistance to azole in *A. fumigatus* has been connected with mutations in *cyp*51*A* gene that is a target for antifungal azoles. The occurrence of *cyp*51*A* mutations has been related to a failure in treatment.

Management of invasive aspergillosis is complicated because of negative cultures is frequent, and many laboratories do not carry out susceptibility assessments on isolates of *Aspergillus*. Therefore, the frequency of azole-resistant to *Aspergillus* likely underdiagnosed, with a possible risk of unsuitable treatment.

In our study MICs for itraconazole between 0.125 to 2 mg/ litre and voriconazole between 0.125 to 4 mg/ litre were obtained. Nine of the isolates including 7 environmental isolates and 2 standard isolates were resistant to voriconazole with MIC 4mg/ litre. None of the resistance strains showed point mutation in *cyp51A* gene.

We consider that the results of our investigation and the rising reports on azole-resistance propose that susceptibility examinations of *A. fumigatus* isolates must be regularly carried out.

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# Fetal Biometric Charts and Reference Equations for Pregnant Women Living in Port Said and Ismailia Governorates in Egypt

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**Abstract**

**AIM:** To construct new fetal biometric charts and equations for some fetal biometric parameters for women between 12$^{th}$ and 41$^{st}$ weeks living in Ismailia and Port Said Governorates in Egypt.

**MATERIAL AND METHODS:** This cross-sectional study was carried out on 656 Egyptian women (from Ismailia and Port Said governorates) with an uncomplicated pregnancy, and all were sure of their dates. The selected group was between the 12$^{th}$ and 41$^{st}$ weeks of gestation, recruited from the district general hospital in Ismailia and Port Said to measure ultrasonographically biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC) and femur length (FL), then for each measurement separate regression models were fitted to estimate both the mean and the Standard deviation at each gestational age.

**RESULTS:** New Egyptian charts were reported for BPD, HC, AC, and FL. Reference equations for the dating of pregnancy were presented. The mean of the previous measurements at 12$^{th}$ and 41$^{st}$ weeks were as follows: (23.37, 98.72), (83.05, 336.12), (67.85, 332.57) and (12.50, 74.92) respectively.

**CONCLUSION:** New fetal biometric charts and regression equations for pregnant women living in Port Said & Ismailia governorates in Egypt.

## Introduction

Appropriate intrauterine fetal growth and development are fundamental for newborn health and lifelong welfare. Both intrauterine growth restriction (IUGR) where the fetus failed to reach the recommended growth potential [1] usually as a result of placental insufficiency and macrosomia (exaggerated intrauterine growth, frequently associated with maternal obesity and/or diabetes), are associated with in utero fetal death, neonatal morbidity and mortality, and remote future risks to health [2]. IUGR is a common condition affecting about 10-15% of the general maternity population [3], while in developing countries along with Egypt it reaches up to 30% and constitutes 50-60% of low birth weight neonates ( birth weight below 2500 g) [4].

Fetal growth abnormalities such as large-for-gestational-age, small-for-gestational-age, low birth weight and macrosomia are determined based on the standard growth charts taken from the growth of what we termed "normal fetuses". This issue is of specific consequences because many fetal growth references did not consider many factors that can affect the construction of such references. Furthermore, some charts are based on fetuses from normal and abnormal pregnancies, without sufficient acknowledgement of the implications for normative interpretation using percentiles [5].

Many changes affect fetal growth along with physiological and pathological changes, such as weight and height of pregnant women, drug or tobacco hazards, fetal sex [6], genetic syndromes, placental failure and congenital anomalies.

Many of published charts or curves showing the normal values of measurement in fetal biometry are established mainly depending on studies from western or American populations [7]. Such standards may be unsuitable for other populations; indeed, ethnic variations in fetal size and growth have been demonstrated in several studies [8] [9] [10]. The ethnic factor is essential in the fetal growth pattern, making it impossible for reference ranges of fetal biometric parameters from the homogeneous population to be applied in other populations, mainly heterogeneous populations [11]. In an American study with singleton pregnancies between 17 and 22.9 weeks, Afro-American fetuses have a smaller abdominal circumference (AC) than Caucasian fetuses. As AC contributes heavily to the estimated fetal weight, the Afro-American fetuses could be mistakenly underestimated [12].

Several other authors have stressed the value of using customised fetal biometry charts that consider variables such as maternal weight, parity, and race [13]. Cross-sectional and longitudinal ultrasound studies have demonstrated racial variations in fetal growth [10] [14] [15]. The fetuses of Turkish and Moroccan women had been reported having a shorter femur, smaller head and abdominal circumferences than Belgian women, and in Africa, Nigerian AC and BPD were found to be smaller than those of the British population [10] [16].

If we excluded all pathological conditions still ethnicity [10] contributes significantly to the fetal growth, and accordingly, each specific population or ethnic group should have their reference charts for the different fetal anthropometrical variables to maintain the most precise fetal assessment. Moreover, fetal nomograms need to be revised at regular intervals as fetal size has changed in the last decades [6].

Biparietal diameter provides the closest correlation with gestational age in the second trimester. Head circumference is an adequate alternative in case of presence of differences in skull shape. Abdominal circumference is the most useful dimension to evaluate fetal growth, while femur length is the best framework for evaluating skeletal dysplasia. Using multiple predictors improves the accuracy of such estimates [17].

The objective of this study is to construct new fetal biometric charts and equations for some fetal biometric parameters for women living in Ismailia and Port Said Governorates in Egypt.

## Material and Methods

This cross-sectional study was carried out on 656 Egyptian women (from Ismailia and Port Said governorates) with an uncomplicated pregnancy. All those included were sure of their dates and were attending for routine antenatal care. The selected group was between the $12^{th}$ and $41^{st}$ weeks of gestation, recruited from the district general hospital in Ismailia and Port Said. We chose a lower gestational age limit of $12^{th}$ weeks as sometimes there is difficulty in getting the ideal fetal position for measuring crown-rump length. Accordingly, BPD and FL are appropriate at such early gestational age. For each measurement, separate regression models were fitted to estimate both the mean and the standard deviation at each gestational age.

Menstrual history was recorded including last menstrual period (LMP) and regularity of the cycle. Women who came in the first trimester had their dates being confirmed by measuring crown-rump length (CRL)**.** While those attending in the second and third trimesters had their dates confirmed by the documented early first-trimester scan.

The range of each week is from week$^{+0}$ days to week$^{+6}$ days. The inclusion criteria for women with regular cycle (26-30 days), sure of their LMP and carrying singleton pregnancy, age between 18-40 years, without congenital fetal anomalies or maternal diseases that could affect fetal growth and not taking drugs that could affect the growth of her baby were included in the study.

Whereas, those with irregular cycles or without early ultrasound dating or a difference of more than 10 days in the GA (between their LMP and early ultrasound scan) or suffering from diseases that disturb normal fetal growth as diabetes mellitus, hypertension, autoimmune disorders or those on anticoagulant and antiplatelets were excluded from the study.

The BPD, HC, AC and, FL were measured by 3.5MHz convex abdominal probe as the standard fetal biometric profile, according to the guidelines proposed by the International Society of Ultrasound in Obstetrics and Gynecology [18] using (General Electric, LOGIQ 3, Milwaukee, Wisconsin, USA) and(Mindray DP-5, Nanshan, Shenzhen, China) ultrasound machines. BPD was measured from the outer proximal edge to the inner proximal edge of the fetal skull border in an axial plane showing the third ventricle, cavum septum pellucidum, and the thalami,

HC was measured directly by placing the ellipse of ultrasound device around the outside of the skull bone echoes. The AC measurement was taken at the widest part of the fetal abdomen, across the liver where, the transverse section should include the fetal stomach, spine and deep portion of the umbilical vein. The femur length was obtained with a linear array transducer along the long axis of diaphysis using a straight line from the tip of the greater trochanter to the lateral epicondyle.

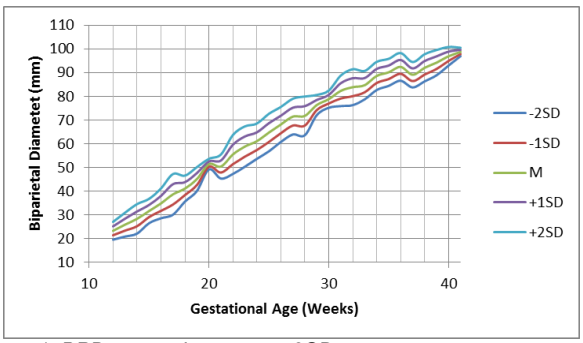


*Figure 1: BPD regression curve ± 2SD*

Statistical analysis was performed using SPSS program (version 14). The BPD, HC, AC, and FL measurements were expressed as mean ± SD and maximum and minimum values. A Polynomial regression model was used to obtain biometric charts for the GA from the above biometric measurements. Charts were figured out by plotting the predicted means and two SD at each week of the GA as shown in Figures 1-4.

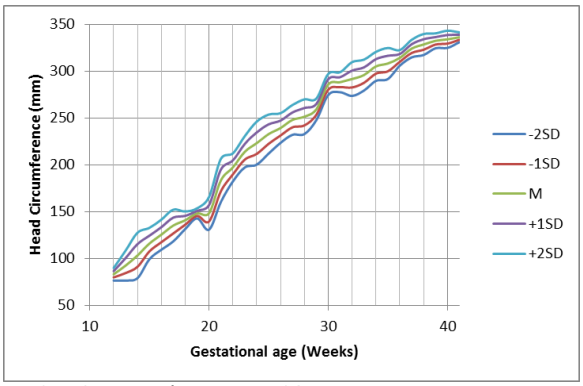


*Figure 2: HC regression curve ± 2SD*

Regression analysis has been used to produce an analytic description and to obtain the best-fitted model polynomial equation for the fetal biometric parameters. Quadratic functions were used to find the best interrelation between the measured fetal parameter and GA according to the least squares criteria. The goodness of fit was evaluated by measurement of the coefficient of determination $r^2$ (the nearer to one the better the correlation). Predicted parameter values for GA were calculated using the most appropriate models.

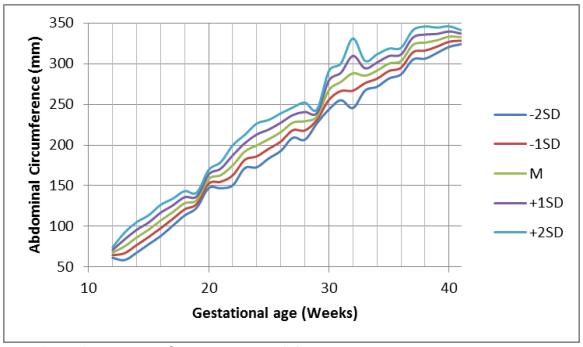


*Figure 3: AC regression curve ± 2SD*

We compared the results of fetal biometric measurements from our population with those from different countries as United Kingdom [19], Korea [7] and North America [20].

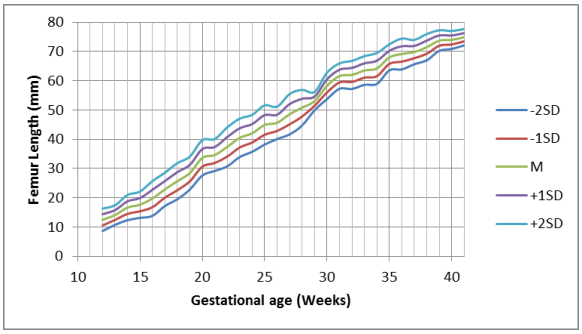


*Figure 4: FL regression curve ± 2SD*

## Results

The mean, standard deviation (SD), minimum and maximum of BPD(mm), HC(mm), AC(mm) and FL(mm) of the study group at each gestational age were tabulated (Tables 1-4). The mean of the previous measures at 12$^{th}$ and 41$^{st}$ weeks were as follows: (23.37, 98.72), (83.05, 336.12), (67.85, 332.57) and (12.50, 74.92) respectively.

**Table 1: Descriptive Statistics for BPD**

| GA (wks) | Ismailia & Port Said cases | | | | | GA (wks) | Ismailia & Port Said cases | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | Mean | SD | Min | Max | | N | Mean | SD | Min | Max |
| 12 | 4 | 23.37 | 1.87 | 21.3 | 25.2 | 27 | 16 | 71.47 | 3.76 | 64.7 | 77.3 |
| 13 | 16 | 25.97 | 2.51 | 22.5 | 30.4 | 28 | 17 | 71.78 | 4.09 | 61.2 | 80.0 |
| 14 | 21 | 28.40 | 3.10 | 22.1 | 34.4 | 29 | 14 | 76.36 | 2.13 | 72.6 | 79.3 |
| 15 | 30 | 31.69 | 2.56 | 27.8 | 36.5 | 30 | 13 | 78.81 | 1.81 | 76.7 | 83.6 |
| 16 | 24 | 34.93 | 3.14 | 30.4 | 41.8 | 31 | 14 | 82.32 | 3.22 | 78.3 | 87.6 |
| 17 | 25 | 38.73 | 4.28 | 32.5 | 47.2 | 32 | 28 | 83.87 | 3.78 | 77.8 | 89.4 |
| 18 | 30 | 41.16 | 2.73 | 37.6 | 48.0 | 33 | 36 | 84.74 | 3.0 | 79.2 | 90.9 |
| 19 | 11 | 45.18 | 2.51 | 41.9 | 50.1 | 34 | 28 | 88.62 | 2.96 | 81.8 | 93.5 |
| 20 | 5 | 51.46 | 1.11 | 50.2 | 53.2 | 35 | 31 | 90.13 | 2.85 | 85.0 | 96.4 |
| 21 | 18 | 50.44 | 2.53 | 46.7 | 54.4 | 36 | 18 | 92.42 | 2.92 | 88.1 | 99.1 |
| 22 | 13 | 55.53 | 4.10 | 50.0 | 63.2 | 37 | 35 | 89.12 | 2.67 | 85.3 | 96.2 |
| 23 | 16 | 58.86 | 4.25 | 53.0 | 65.3 | 38 | 53 | 92.04 | 2.85 | 86.7 | 98.3 |
| 24 | 21 | 61.12 | 3.72 | 55.3 | 67.1 | 39 | 44 | 94.23 | 2.65 | 88.2 | 98.4 |
| 25 | 19 | 64.75 | 3.95 | 58.9 | 71.6 | 40 | 27 | 96.91 | 1.95 | 93.1 | 99.6 |
| 26 | 25 | 68.16 | 3.69 | 62.0 | 74.8 | 41 | 4 | 98.72 | 0.88 | 98.0 | 99.8 |

The polynomial regression equations that best described the interrelation between BPD, HC, AC, FL and gestational age were as follows:

**Table 2: Descriptive Statistics for HC**

| GA (wks) | Ismailia & Port Said cases | | | | | GA (wks) | Ismailia & Port Said cases | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | Mean | SD | Min | Max | | N | Mean | SD | Min | Max |
| 12 | 4 | 83.05 | 3.37 | 79.4 | 87.1 | 27 | 16 | 247.89 | 7.96 | 234.9 | 263.3 |
| 13 | 16 | 92.27 | 7.98 | 80.0 | 105.7 | 28 | 17 | 251.29 | 9.26 | 236.7 | 271.3 |
| 14 | 21 | 103.14 | 12.11 | 83.2 | 117.4 | 29 | 14 | 259.18 | 5.76 | 245.6 | 271.3 |
| 15 | 30 | 115.85 | 8.39 | 93.1 | 127.2 | 30 | 13 | 285.76 | 5.63 | 276.0 | 269.2 |
| 16 | 24 | 125.28 | 8.03 | 106.4 | 139.2 | 31 | 14 | 288.13 | 5.36 | 279.2 | 296.9 |
| 17 | 25 | 135.06 | 8.45 | 120.8 | 154.7 | 32 | 28 | 291.53 | 9.01 | 275.5 | 310.3 |
| 18 | 30 | 140.66 | 4.71 | 130.4 | 149.2 | 33 | 36 | 295.76 | 8.25 | 297.2 | 311.1 |
| 19 | 11 | 148.04 | 2.69 | 142.9 | 152.3 | 34 | 28 | 305.02 | 7.74 | 290.4 | 321.8 |
| 20 | 5 | 184.18 | 8.73 | 171.8 | 192.3 | 35 | 31 | 308.10 | 8.27 | 287.9 | 320.4 |
| 21 | 18 | 183.42 | 11.53 | 167.2 | 209.0 | 36 | 18 | 314.11 | 4.16 | 305.0 | 320.8 |
| 22 | 13 | 197.15 | 7.49 | 183.5 | 209.1 | 37 | 35 | 324.02 | 4.69 | 315.3 | 335.6 |
| 23 | 16 | 213.85 | 8.37 | 200.6 | 228.5 | 38 | 53 | 328.41 | 5.60 | 314.7 | 337.4 |
| 24 | 21 | 223.04 | 11.46 | 201.4 | 241.4 | 39 | 44 | 332.33 | 3.97 | 324.7 | 338.4 |
| 25 | 19 | 232.74 | 10.40 | 210.8 | 249.7 | 40 | 27 | 333.94 | 4.59 | 322.7 | 338.9 |
| 26 | 25 | 239.21 | 7.93 | 226.5 | 259.6 | 41 | 4 | 336.12 | 2.60 | 333.2 | 338.7 |

BPD (mm) = - 0.051(GA) ² + 5.403(GA) – 37.934

HC (mm) = - 0.174(GA) ² + 18.555(GA) – 126.302

AC (mm) = - 0.107(GA) ² + 15.6475(GA) – 115.157

FL (mm) = - 0.026(GA) ² + 3.739(GA) – 32.088

GA (days) = 0.235(BPD) + 0.061(HC) + 0.312(AC) + 1.132(FL) + 36.706

$R^2$ was 0.98; the mode was highly significant as $P<0.05$.

**Table 3: Descriptive Statistics for AC**

| GA (wks) | Ismailia & Port Said cases | | | | | GA (wks) | Ismailia & Port Said cases | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | Mean | SD | Min | Max | | N | Mean | SD | Min | Max |
| 12 | 4 | 67.85 | 3.24 | 63.5 | 71.3 | 27 | 16 | 227.56 | 9.32 | 217.3 | 251.0 |
| 13 | 16 | 75.49 | 8.44 | 60.2 | 87.3 | 28 | 17 | 229.33 | 11.38 | 207.9 | 245.6 |
| 14 | 21 | 86.39 | 9.37 | 62.8 | 100.6 | 29 | 14 | 235.14 | 4.24 | 229.1 | 241.6 |
| 15 | 30 | 95.85 | 8.91 | 80.9 | 113.2 | 30 | 13 | 267.39 | 11.83 | 251.7 | 284.2 |
| 16 | 24 | 107.28 | 9.58 | 88.4 | 120.2 | 31 | 14 | 277.66 | 11.28 | 256.4 | 291.4 |
| 17 | 25 | 117.40 | 8.30 | 100.6 | 130.0 | 32 | 28 | 288.21 | 21.36 | 236.5 | 386.4 |
| 18 | 30 | 128.43 | 7.49 | 113.8 | 139.6 | 33 | 36 | 285.21 | 9.15 | 263.2 | 304.5 |
| 19 | 11 | 132.24 | 4.66 | 123.6 | 140.3 | 34 | 28 | 291.51 | 9.95 | 237.6 | 307.8 |
| 20 | 5 | 158.24 | 5.59 | 150.2 | 165.7 | 35 | 31 | 300.19 | 9.17 | 277.2 | 315.6 |
| 21 | 18 | 162.97 | 8.04 | 150.1 | 178.1 | 36 | 18 | 303.23 | 8.15 | 289.3 | 314.9 |
| 22 | 13 | 175.11 | 12.28 | 159.3 | 193.5 | 37 | 35 | 323.19 | 9.04 | 302.9 | 338.7 |
| 23 | 16 | 192.05 | 10.23 | 171.9 | 207.7 | 38 | 53 | 325.91 | 9.86 | 302.8 | 350.7 |
| 24 | 21 | 199.57 | 13.44 | 177.4 | 221.8 | 39 | 44 | 328.82 | 7.79 | 306.4 | 338.4 |
| 25 | 19 | 207.27 | 11.83 | 190.7 | 223.9 | 40 | 27 | 333.24 | 6.30 | 319.7 | 343.8 |
| 26 | 25 | 215.92 | 11.53 | 196.0 | 238.3 | 41 | 4 | 332.57 | 4.32 | 328.3 | 336.4 |

On comparing the mean of fetal biometric measures (BPD, HC, AC, FL) of our study population with that of other published ones from different countries as United Kingdom, Korea, and North America we found that the mean of BPD measurement appeared to be quietly larger in UK women than Egyptian ones till reaching maximum difference at 37$^{th}$ week with 6mm difference, as shown in (Figure 5A). While the mean of BPD appeared to be quietly bigger in Egyptian women than in Korean and North American women till reaching maximum difference at 20$^{th}$ week (5 mm and 4 mm respectively).

**Table 4: Descriptive Statistics for FL**

| GA (wks) | Ismailia & Port Said cases | | | | | GA (wks) | Ismailia & Port Said cases | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | Mean | SD | Min | Max | | N | Mean | SD | Min | Max |
| 12 | 4 | 12.50 | 1.92 | 10.3 | 14.3 | 27 | 16 | 48.60 | 3.45 | 43.8 | 54.1 |
| 13 | 16 | 14.14 | 1.67 | 10.2 | 16.7 | 28 | 17 | 50.84 | 3.04 | 45.9 | 56.4 |
| 14 | 21 | 16.69 | 2.15 | 12.9 | 19.8 | 29 | 14 | 53.07 | 1.57 | 51.3 | 56.7 |
| 15 | 30 | 17.67 | 2.25 | 13.2 | 21.6 | 30 | 13 | 58.28 | 2.25 | 54.4 | 61.3 |
| 16 | 24 | 19.81 | 2.97 | 14.9 | 25.9 | 31 | 14 | 61.63 | 2.17 | 58.0 | 64.6 |
| 17 | 25 | 22.92 | 2.84 | 18.4 | 29.1 | 32 | 28 | 62.03 | 2.40 | 55.9 | 65.3 |
| 18 | 30 | 25.72 | 3.09 | 20.3 | 31.5 | 33 | 36 | 63.53 | 2.45 | 58.4 | 68.3 |
| 19 | 11 | 28.49 | 2.82 | 24.7 | 32.4 | 34 | 28 | 64.24 | 2.64 | 59.5 | 69.6 |
| 20 | 5 | 33.68 | 3.01 | 30.9 | 38.5 | 35 | 31 | 68.04 | 2.22 | 64.3 | 71.3 |
| 21 | 18 | 34.67 | 2.73 | 30.5 | 41.1 | 36 | 18 | 69.18 | 2.63 | 65.1 | 72.5 |
| 22 | 13 | 37.45 | 3.33 | 32.7 | 43.2 | 37 | 35 | 69.85 | 2.08 | 67.1 | 74.6 |
| 23 | 16 | 40.53 | 3.28 | 34.1 | 45.3 | 38 | 53 | 71.52 | 2.24 | 66.5 | 75.4 |
| 24 | 21 | 42.08 | 3.15 | 38.0 | 48.1 | 39 | 44 | 73.76 | 1.75 | 69.0 | 76.8 |
| 25 | 19 | 44.93 | 3.35 | 39.9 | 52.4 | 40 | 27 | 73.97 | 1.54 | 70.4 | 76.3 |
| 26 | 25 | 45.65 | 2.76 | 41.8 | 51.2 | 41 | 4 | 74.92 | 1.40 | 72.9 | 76.1 |

Also, it appeared that the mean of HC is gently higher in the UK and North American women than Egyptian ones till 25$^{th}$ week (Figure 5B). This difference increases after that until reaching its maximum (18 mm) at 29$^{th}$ week. While there was unstable variability between Korean and Egyptian women, the maximum difference was at the 19$^{th}$ and 29$^{th}$ weeks (10 mm and 11 mm, respectively).

There was unremarkable inconstancy between the mean of AC in the UK, North American and Egyptian women till 25$^{th}$ weak were the mean of AC was mildly higher in the UK and North American women than Egyptian ones reaching maximum difference of 21 mm at 36$^{th}$ week (Figure 5C), while there was unstable flippancy between those of Korean and Egyptian women with the maximum difference (15 mm) was at 29$^{th}$ week. Finally, in (Figure 5D), there was no remarkable variability between the mean of FL of UK, North American and Egyptian women, while regarding Korean women the mean of FL was lower than that of Egyptian women reaching maximum difference at 31$^{st}$ and 39$^{th}$ week (the difference was 5 mm).

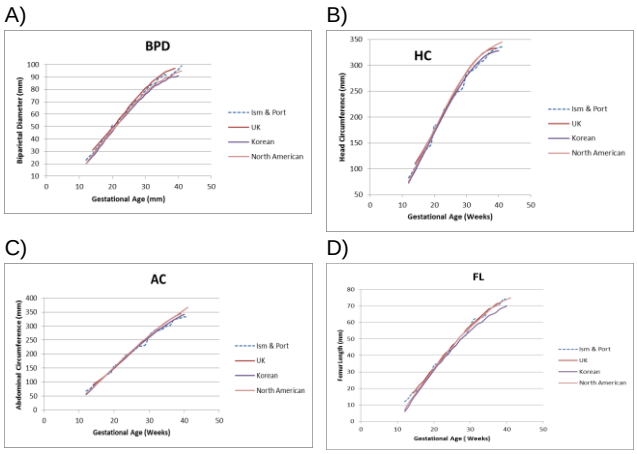


*Figure 5: A) Comparison of our BPD with that of UK, Korean & North America populations; B) Comparison of our HC with that of UK, Korean & North America populations; C) Comparison of AC among the study group and that of UK, Korean & North America populations; D) Comparison of FL measurements within study group and that of UK, Korean and North America populations*

## Discussion

Nowadays examination and measurement of fetal biometry using ultrasound devices have become a basic part of recent obstetric care. These measurements helped in measuring the GA and assessment of fetal development. Choosing the appropriate reference charts is of great importance to guarantee an accurate diagnosis [21]. Several studies have demonstrated the influence of ethnicity on fetal biometry [10] [22].

A Pilot study was done by Zaki *et al*. (2012) in Egypt to compare the fetal biometric measurements of Egyptian women with those of other western ones. They found that Egyptian data are different from other western data and they recommended the development of a national fetal ultrasound biometric reference charts that can be used in clinical practice and the assessment of fetal growth. Unfortunately, this study was a limited pilot study applied to only 71 pregnant women between 14$^{th}$ & 24$^{th}$ weeks of gestation, not through the whole pregnancy. This

study also did not include a wide and diverse range of Egyptian population from different governorates [23].

Accordingly, this study was designed to provide fetal biometric charts and regression equations for biometric measurements of pregnant women between 12$^{th}$ and 41$^{st}$ weeks of gestation living in Ismailia and Port Said Governorates in Egypt as a part of a larger project to create an Egyptian growth curve based on all governorates.

The noted difference of the BPD and HC among the UK women and the Korean and North American women than the Egyptian women may mainly be attributed to the method or the way BPD and HC measures were taken, ethnic, racial factors and the shape of the head.

AC was higher in the UK and North American women than Egyptian ones especially in the third trimester, while there was an unstable variability between Korean and Egyptian women. This may be related to women height and size as well as other epigenetic factors as the nutritional status, level of pollution and socioeconomic standards of our women.

Egyptian fetuses have almost comparable femur length as those from the UK and North American fetuses. While fetuses of Korean women had shorter femur than that of Egyptian counterparts. Fetal FL measurement can be underestimated by obtaining oblique images of the femur or overestimated by including the non-ossified portions of the femur [24]. There was no systemic bias in our study as we included only the ossified portion of the femur shaft, and all the measurements were done in the same way on all fetuses. It might be important to pay more attention to the effect of ethnic variations on fetal FL measurements as short femur has been reported as an important soft marker for Down syndrome [23].

In conclusion, the fetal growth is not uniform and varies between different groups of citizens. These differences in the various fetal biometric measurements among the dissimilar inhabitants emphasise the importance of selecting suitable charts for every population separately. Otherwise, over or underestimation of fetal growth abnormalities will include normally growing babies according to their normal population potential. This has a tremendous impact on the national health and economic resources. We endorse on the need to establish national Egyptian fetal biometric growth references.

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# *In Vivo* Anti-Inflammatory, Anti-Bacterial and Anti-Diarrhoeal Activity of *Ziziphus Jujuba* Fruit Extract

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**Abstract**

**BACKGROUND:** *Ziziphus jujuba* belongs to family Rhamnaceae widely distributed in subtropical and tropical countries. It is used traditionally for several pharmacological purposes including anti-inflammation, antidiarrhoeal and antibacterial, as well as tonic and sometimes as hypnotic (sedative).

**AIM:** To determine the in vivo antidiarrhoeal, antibacterial and anti-inflammatory activities of *Z. jujuba* fruit ethanolic extract.

**METHOD:** The fruit was macerated and extracted by 95% (v/v) ethanol. The antidiarrhoeal activity was evaluated using castor oil and *Escherichia coli* induced diarrhoea mouse model. The antidiarrhoeal and antibacterial activity was investigated at graded doses (400-1200 mg/kg). The anti-inflammatory effects were tested using the carrageenan-induced paw oedema in female Wistar rats. Rat's treatment groups received tragacanth, 100 mg/kg diclofenac sodium, 800 mg/kg, 1200 mg/kg or 1600 mg/kg of an ethanolic extract of *Z. jujuba* (EEZJ). All treatment groups were fed with the compounds one hour before carrageenan injection at of rat's paw. Also, the EEZJ was further analysed by HPLC-PDA system for identification of the presence of betulinic acid and quercetin.

**RESULTS:** EEZJ different doses did not show inhibitory activity against castor oil induced diarrhoea except for the higher (1200 mg/kg) dose. However, the frequency of defecation of stools and watery stool were reduced significantly when compared to control group ($P \leq 0.05$ and $P \leq 0.01$ respectively), resulted in overall 67% inhibition of diarrhoea. Our anti-inflammatory results demonstrated that EEZJ was able to inhibit the carrageenan-induced paw oedema in rats to a significant degree ($p \leq 0.05$) and the paw volume and thickness of both left and right paw were affected compared to the negative control group.

**CONCLUSION:** EEZJ possesses antidiarrhoeal and antibacterial activity in a dose depending manner and may provide a pharmacological basis for its clinical use in diarrheal diseases. The activity may partially be due to the presence of betulinic acid and quercetin.

## Introduction

*Ziziphus jujuba* Mill. (family: Rhamnaceae), is a thorny tree of medium height, whose fruit resembles buckthorn or olive fruit and had been widely consumed as both fruit and remedy for a long time all around the world. It has been listed among the first most valuable fruits in ancient Chinese medicine books. Moreover, in the Chinese herbal medicine, it is considered as one of the superior herbal medicines and thought to possess the effect of prolonging lifespan by purifying and nourishing blood, treating insomnia and help in digestion. Nowadays, *Z. jujuba* fruit is believed to be one of the nutritious foods as it contains plenty of nutrients which consists of amino acids, carbohydrates, minerals and vitamins [1] [2].

Regarding the geographical distribution, *Z. jujuba* is widely found in the tropical and subtropical regions of Asia and America as well as in the Mediterranean regions [3]. It is well known in the Arabian Peninsula since ancient time. However, it is believed to be originated in the Algerian town of Annaba, due to which it has been named Annab. Furthermore, the mature fruit of *Z. jujuba* is red to purplish black, resembling small dates. Therefore in China are known as Chinese red date or Chinese

jujuba. The dried pulp of *Z. jujuba* is a source of essential unsaturated fatty acids. The main fatty acids in the jujube are oleic, linoleic (omega-6), palmitic, and palmitoleic acids. Jujube fruits contain various types of amino acids and proteins. The protein and free asparagines content are influenced and accumulated potentially during the ripening and harvesting stage [4].

Dietary fibre and fructose contents of the jujube fruit play a role in the regulation of blood sugar levels by slowing digestion [5]. The major sugars found in the jujube fruit are glucose, fructose, sucrose, rhamnose and sorbitol. The fruit is also abundant in vitamin C, which is one of the water-soluble antioxidants [6]. The postharvest sorting process is important for increasing the economic benefits and dietary values of the jujube fruit, especially vitamin C content protection during storage and marketing [7]. Moreover, the jujube is enriched, nevertheless to a lesser extent, with other vitamins including thiamin, riboflavin, niacin, vitamin $B_6$, and vitamin A. Jujube fruit is also considered a good source of minerals such as magnesium, phosphorus, potassium, sodium, and zinc [6]. Various studies have shown that the jujube fruit contains many bioactive compounds, including triterpenic acids, flavonoids, cerebrosides, phenolic acids, α-tocopherol, β-carotene, and polysaccharides. Each constituent of the jujube presents some health benefits, thus making it a healthy food choice [8]. The total phenolic compounds in jujube fruit which is accounted for the antioxidant activities are higher compared to other common fruits, such as cherry, apple, persimmon, or red grape [9]. Flavonoids, phenolic acids, tannins, stilbenes, and lignans are derivatives of phenolic compounds [10] [11] [12].

The Indian jujuba, *Ziziphus mauritiana* Lam. and *Z. jujuba* Mill. Are the two main domesticated jujubes. The pantropical genus *Ziziphus* Mill. Includes approximately 170 species with a few species occurring in temperate regions. Recent studies on the biological activities of this fruit have supported the health benefits of jujube as both food and medicinal herb. Different parts of *Z. jujuba* are used traditionally for curing many kinds of illness including diabetes, diarrhoea, liver complaints, urinary disorders, obesity, skin infections, respiratory infections, anaemia, insomnia, cancer, and also for blood purification and modification of the gastrointestinal tract [ [6] 13] [14]. *Z. jujuba* is used traditionally as a tonic and sometimes as hypnotic-sedative. Additionally, there are studies that had been carried out to test its anxiolytic, anticancer, antimicrobial, anti-inflammatory, anti-allergy, cognitive, anti-nephritic, antioxidant and wound healing properties [8] [15].

The anxiolytic effect *in vivo* had been reported in a polyherbal substance which consists of seed extract of *Z. jujuba* [16]. The possibility of improving cytotoxic activity was suggested to be due to the presence of coumaroyl moiety at the carbon-3 position of the lupanetype triterpene, extracted from *Z. jujuba* [17]. Selective toxicity against cultured human melanoma cells is also performed by the triterpenes acid and betulinic acid extracted from *Z. jujuba* and *Ziziphus mauritiana* [18]. Notable inhibitory activity performed by ethanolic extract of *Z. jujuba* root on fungi *Candida albicans, C. tropicalis, Aspergillus flavus* and *A. niger* showed convincing antifungal activity [19]. Additionally, extract of root bark of *Z. jujuba* exhibited antibacterial activity against 20 bacteria [20].

A traditional Chinese prescription, Huangqin Tang with fruit content from *Z. jujuba* had shown remarkable anti-inflammatory and antispasmodic effect [21]. Additionally, *Ziziphus mauritiana* leaf extracts were also found to possess significant anti-inflammatory activity against carrageenan induced rat paw oedema [22]. The anti-allergic activity of the aqueous extracts of leaves of *Z. jujuba* was studied by measuring its inhibitory effect on hyaluronidase or bovine testes activation in vitro. *Z. jujuba* was shown to have strong antiallergic and anti-anaphylactic activity [23] [24]. A component of *Z. jujuba* extract, oleamide poses possible potential against Alzheimer's disease as it could be a useful chemopreventative agent [25] [26]. Methanolic extract of *Z. jujuba* was found to show activation effect on choline acetyltransferase in vitro as high as 34.1%.

Moreover, *Z. jujuba* also exhibits possibly anti-nephritic effect by increasing renal blood flow and thus reducing inflammation of kidney [27]. Two reports studied 70 antioxidant Korean medicinal plants listed the in vitro antioxidant effect of *Z. jujuba* [28] [29]. The wounding healing effect had been reported on the extract of *Z. jujuba* root [30]. Furthermore, a rat model which uses an ointment formula at a dose 0.5% and 1% on topical application proved the wound healing activity of the extract of *Z. jujuba* root [31]. The main aim of the current studies was to further explore the anti-inflammatory and anti-diarrhoeal activities of ethanolic extract of Ziziphus jujube fruit.

## Materials and Methods

The 1 ml and 10 ml disposable syringes, amber bottles, oral gavage and 27G needle, were purchased from Terumo Tokyo–Japan. Digital Plethysmometer (model 7140, Ugo Basile, Italy), weighing balance (A & D, Tokyo, Japan), freezer (Action International, Kuala Lumpur, Malaysia), refrigerator (Sharp Malaysia), cell culture incubator (CL-170B-8, ESCO, Singapore), rotary evaporator (Butchi Rotavapour model R-114, Büchi Labortechnik AG, Flawil Switzerland). The plastic cages, water bottles, white cloth, tissue paper, masks, gloves,

vacuum pump and vortex, were from Autovortex SA6, Stuart Scientific, UK. HPLC machine (Waters 2695; WATERS CORPORATION, Milford, MA 01757 USA), Ultra-purified water machine (ELGA Labwater Purification System, High Wycombe HP14 3BY, UK).

The dried granules of fruits (2744.58 g) were extracted by cold maceration for 72 hours with 95% of ethanol. The solutions were filtered using vacuum filtration. The filtrates were then concentrated in a rotary evaporator to eliminate ethanol and yielded semi-solid extract. Immersion, filtration and rotary evaporation process were repeated for three times. The extract of *Z. jujuba* was preserved at 10$^{o}$C until further use [24].

Different concentration of EEZJ, loperamide, and diclofenac sodium salt were freshly prepared in tragacanth compound powder suspension prior administration. Carrageenan powder was added into normal saline to create 1% w/v carrageenan solution at 4$^{o}$C and stirred immediately. Any remaining lumps were dispersed by using vortex. The solution was warmed to 50$^{o}$C with stirring. After that, the solution was incubated at 40$^{o}$C for less than 24 hours before use [32].

Sample (EEZJ) and standards (betulinic acid and quercetin) were weighed and transferred into three 2 ml vials, respectively. After dissolved in 100% methanol, solutions were filtered through 0.45 µm membrane filters before HPLC analysis, Guo et al. [33].

The bacterial inoculum suspension of *E. coli* (ATCC 25927) was prepared by direct transfer of bacteria from stock culture into nutrient broth contained in the universal bottle. Few loops of stock culture were transferred into the sterile nutrient broth and incubated for 24 hours at 37$^{o}$C prior administration. *E. coli* suspension was then compared with McFarland standards to obtain the desired turbidity of bacterial suspension. The minimum effective dose obtained from this test will determine the doses of choice in the *in vivo* antidiarrhoeal experiment and *in vivo* antibacterial experiment [33].

Following Sahoo et al. work [34] the preliminary *in vivo* assay using mice, six mice were separated randomly into two groups of three animals each. The turbidity of *E. coli* inoculum suspensions was adjusted to McFarland Standard No. 1 (3.0x10$^{8}$ CFU /ml), No. 2 (6.0x10$^{8}$ CFU/ml) prior administration to mice. Group 1 received 1 ml of *E. coli* inoculum suspension of McFarland Standard No.1 while group 2 received 1 ml of *E. coli* inoculum suspension of McFarland Standard No.2. Following treatment of *E. coli* suspension, mice were placed and observed for subsequence eight hours in the separated beaker which consists of white filter paper. The filter paper was changed hourly. Several parameters such as the weight of stools, the frequency of total stools and watery stools were taken for measuring purpose. After eight hours of observation, mice were sacrificed, and the intestines were removed. Any abnormalities such as ulceration, perforation and redness were observed and recorded. The minimum concentration of *E. coli* that induced abnormalities in intestines of the mice was used in the antibacterial activity experiment.

Base on experiment used by Ayalate and his colleagues [36], thirty mice with a weight of 20-35 g were separated into six groups of five animals each. All five groups of animals were fed orally with 1 ml of *E. coli* suspension using gavage needle with turbidity similar to McFarland Standard No. 1 three hours prior administration of suspension. Group 1 received 0.2 ml of tragacanth 2% orally and served as the control group while group 2 received antibiotic (Amoxicillin 260 mg/kg) and served as positive control. Group 3, 4 and 5 received 400 mg/kg, 800 mg/kg and 1200 mg/kg of EEZJ, respectively. Following treatment with drug and extraction suspension, mice were separated and placed individually into a different beaker containing a white filter paper and observed for the subsequent four hours. The white filter papers were changed hourly.

RA was calculated according to the following formula:

$$\text{Relative area} = \frac{\text{Total mucosal area}}{\text{Total ulcerated area}}$$

Meanwhile, approximately 2000 mg of stools of each mouse were collected and suspended into 5 ml of sterile phosphate-buffered saline (PBS) to produce suspension **A**, which underwent vigorous shaking by a vortex mixer for at least 15 minutes. Then, 100 µl of the suspension A was diluted into 10 ml of sterile PBS to form suspension B. Afterwards, 100 µL of the suspension B was spread on to the MacConkey agar plates and incubated at 37$^{o}$C for 24 hours. Later the growth of bacterial colony was observed and counted by using colony counter.

A preliminary study was carried out to determine the dose of carrageenan to induce paw oedema in rats. Moreover, the effective doses of plant extract that exert an observable anti-diarrhoeal effect on castor oil induced diarrhoea, as well as anti-inflammatory effects on carrageenan-induced paw oedema were determined in the current study.

Following Wang et al. method [33], 30 mice were selected and divided randomly into six groups of five animals each. The weight of all mice was in the range of 20-35 g. Group 1 and group 2 were administered with 0.2 ml of 2% tragacanth and 4.2 mg/kg loperamide HCl respectively. Group 3, 4 and 5 received 400 mg/kg, 800 mg/kg and 1200 mg/kg of EEZJ, respectively. Mice acute diarrhoea was induced with 0.2 ml of castor oil one hour after administration of oral suspension. After the supply of castor oil, animals were placed in the separated beakers which consist of white filter paper. The filter papers were

changed every hour for subsequence four hours. Mice were under direct observation for the onset of diarrhoea, weight of stools, and frequency of total stools and watery stools during the four hours. The proportion of the weight of watery stools was calculated based the formula that developed by Wang et al., [33].

$$\text{Proportion of weight of watery stool} = \frac{\text{Weight of watery stool}}{\text{Total weight of stool}} \times 100$$

The percentage of inhibition of defaecation and diarrhoea were calculated according to the formula that developed by Lumpu et al., [37].

$$\text{Percentage inhibition of defaecation} = \frac{\text{Pc - Ps}}{\text{Pc}} \times 100$$

Where Pc is the average number of defecation of control group while Ps is the average number of defaecation of the test group.

$$\text{Percentage inhibition of diarrhoea} = \frac{\text{Dc - Ds}}{\text{Dc}} \times 100$$

Where Dc is the average number of diarrhoea of control group, while Ds is the average number of diarrhoea of test group.

Acute inflammation was induced by subplantar injection of 0.1 ml carrageenan solution (1% w/v in normal saline) in the hind paw of each rat. Before injection, the rat paw was held still and steady to ensure the injection site was cleared and observable. Different groups of rats were administered either solution of *Z. jujuba* ethanolic extract (800 mg/kg, 1200 mg/kg or 1600 mg/kg), diclofenac sodium (100 mg/kg) or negative control solution (2% tragacanth) orally, one hour before carrageenan injection. The paw volume and thickness were measured by plethysmometer and digital calliper respectively immediately before carrageenan injection (0 h) and the consecutive six hours with the one-hour interval between two readings [38].

Paw volume was measured by dipping the rat paw into the tube until marked level, and the pedal switch was pressed to get the data value after the figure stabilised. The rat feet were kept still and steady during measurement process to improve accuracy and consistency. Every time after data value was taken, the meter was zeroed before next measurement. The thickness of the paw was taken at the marked line to ensure the consistency and accuracy of the data value by using a digital calliper.

The extent of inflammation was expressed as percent (%) oedema and calculated as below [39]:

$$\% \text{ oedema} = \frac{\alpha - \beta}{\beta} \times 100$$

Where α and β are the paw volume/ thickness after carrageenan injection and paw volume/ thickness before carrageenan injection respectively. The anti-inflammatory effects were deduced from the extent of inflammation. The greater the extent of inflammation, the less anti-inflammatory effects exerted by that treatment group.

HPLC analysis was performed on a Waters 2695 Alliance HPLC system (Waters Corporation, United States of America), equipped with a quaternary pump solvent management system, an auto-sampler, and an on-line degasser. The separation was carried out with an XBridge™ C18 column (4.6 mm x 250 mm) while raw data were detected by Waters 2998 PDA and processed with EmpowerTM Software. The column temperature was 25◦C.

The mobile phase for betulinic acid HPLC qualification was composed of A (acetonitrile) and B (0.1 % acetic acid) at the flow rate of 1.0 ml/min. The elution concentration of mobile phase were: 0-5 min (A: 20%; B: 80%), 6-25 min (A: 70%; B: 30%), 26-45 min (A: 90%; B: 10%) and detected at the wavelength of 205nm. Re-equilibration duration was 15 minutes between individual runs [40].

HPLC analysis for qualification of quercetin was carried out by gradient elution beginning with a mobile phase A (acetonitrile) and B (0.2% of acetic acid) at the flow rate of 0.5 ml/min. The elution concentration of mobile phase were: 0-12 min (A: 30%; B: 70%), 12-13 min (A: 33%; B: 67%), 13-31 min (A: 48%; B: 52%), 31-35 min (A: 63%; B: 37%), 35-80 min (A: 100%; B: 0%) and detected at the wavelength of 205nm [41]. Re-equilibration duration was 15 minutes between individual runs.

The chromatographic peaks from EEZJ were identified by comparing the retention time to the reference standard compounds which underwent the same condition of elution. Each sample and reference standard were analysed for three times for the precision of the analysis.

Data obtained were analysed statistically using GraphPad PRISM. All data are presented as means a ± standard error of the mean (S.E.M). Data were evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. The level of statistical significance was set at $P \leq 0.001$ (***) or $P \leq 0.01$ (**) or $P \leq 0.05$ (*) when compared to control group.

## Result

The yield of a crude ethanolic extract of *Z. jujuba* was 1620.68 g, from 2744.58 g. The yield percentage was 59.05%. 90% of the extract was subjected to *in vivo* test while another 10% was subjected to qualification analysis.

Two parameters were employed in this test, which is the number of colonies growing in the petri dish and the ulcer index. Positive control greatly reduces the number of colonies growing on petri dish; nevertheless, the results were not sufficient to be statistically significant. Test groups postulate a decreasing trend in reducing the number of colonies in a dose depending manner. However, the results are not statistically significant.

However, Table 1 shows that the EEZJ inhibited the growth of bacteria *in vivo* in a dose depending manner, although there were no ulcers observed in our experiment. Orally administered E. coli demonstrated some redness like inflammation and ulcerative presentation at the intestinal tissue during post-mortem session.

**Table 1: Antibacterial activity**

| Compound | Dosage (mg/kg) | Number of Colonies | Ulcer Index |
|---|---|---|---|
| Control | - | 162.83 ± 49.57 | 0.82 ± 0.02 |
| Amoxicillin | 260 | 60.67 ± 18.11 $^{ns}$ | 0.80 ± 0.00 $^{ns}$ |
| EEZJ | 400 | 140.83 ± 48.14 $^{ns}$ | 0.82 ± 0.04 $^{ns}$ |
| EEZJ | 800 | 122.83 ± 52.64 $^{ns}$ | 0.77 ± 0.05 $^{ns}$ |
| EEZJ | 1200 | 117.00 ± 38.26 $^{ns}$ | 0.88 ± 0.02 $^{ns}$ |

(means ± S.E.M; n = 6); ns P ≥ 0.05 compared to control (One-way ANOVA followed by Dunnett's Test); *P ≤ 0.05 compared to control (One-way ANOVA followed by Dunnett's Test)' **P ≤ 0.01 compared to control (One-way ANOVA followed by Dunnett's Test).

Based on Table 2, the extractions produced no significant results in parameters such as the proportion of watery stools, and the onset of diarrhoea when compared statistically to the control group. However, there is a trend of reducing the proportion of the weight of watery stool in mice treated with different concentration of EEZJ. Not only that, but mice treated with EEZJ also delayed the onset of diarrhoea compared to control group. However, the results obtained were not statistically significant. Among three concentrations of EEZJ, only EEZJ 1200 mg/kg produced a significant result (P ≤ 0.01) in reducing a number of watery stools. This significant level was comparable with positive control as it also produced a significant result (P ≤ 0.01). Furthermore, EEZJ 1200 mg/kg was the only test group that produced a significant result (P ≤ 0.05) when compared to control group in reducing a total number of the stool. From Table 3, it shows increasing inhibition of both defaecation and diarrhoea frequency with increasing concentration of EEZJ. Positive control produced the similar significance level when compared to negative control (P ≤ 0.05) regarding reducing a total number of stool. Loperamide was the only group that showed the significant result in all parameters when compared to control group.

**Table 2: Preliminary antidiarrhoeal test**

| Compound | Dosage (mg/kg) | The proportion of Weight of Watery Stool (%) | The onset of Diarrhoea (hour) | Total Number of Defecation | Number of Watery Stool |
|---|---|---|---|---|---|
| Control | - | 92.73 ± 6.70 | 1.00 ± 0.00 | 12.67 ± 1.20 | 11.67 ± 1.76 |
| EEZJ | 100 | 96.19 ± 3.62 $^{ns}$ | 1.33 ± 0.33 $^{ns}$ | 12.67 ± 1.76 $^{ns}$ | 11.67 ± 2.33 $^{ns}$ |
| EEZJ | 400 | 75.47 ± 8.81 $^{ns}$ | 2.67 ± 0.33** | 13.67 ± 1.76 $^{ns}$ | 9.00 ± 1.00 $^{ns}$ |

(means ± S.E.M; n = 3); ns P ≥ 0.05 compared to control (One-way ANOVA followed by Dunnett's Test); *P ≤ 0.05 compared to control (One-way ANOVA followed by Dunnett's Test); **P ≤ 0.01 compared to control (One-way ANOVA followed by Dunnett's Test).

As shown in Table 4 and Table 5, anti-inflammatory activity of *Z. jujuba* is significant when compared to negative control group (2% tragacanth) all the time. The experimental findings from the carrageenan-induced rat paw oedema model showed that the *Z. jujuba* ethanolic extract reduced the paw volume significantly (P≤0.001) from 1 h to 6 h. Inhibition of paw oedema formation was shown in three doses of *Z. jujuba* ethanolic extract. However, the anti-inflammatory effect exerted by *Z. jujuba* ethanolic extract was less potent than that found with positive control drugs. Furthermore, the onset of anti-inflammatory action of *Z. jujuba* ethanolic extract was found to be comparable with that of control positive drug.

**Table 3: In vivo antidiarrhoeal activity**

| Compound | Dosage (mg/kg) | The proportion of Weight of Watery Stool (%) | The onset of Diarrhoea (hour) | Total Number of Stools | Number of Watery Stool | Inhibition of Defecation (%) | Inhibition of Diarrhoea (%) |
|---|---|---|---|---|---|---|---|
| Control | - | 83.07 ± 5.98 | 1.83 ± 0.31 | 16.33 ± 1.50 | 13.17 ± 2.06 | 0.0 | 0.0 |
| Loperamide | 4.2 | 42.49 ± 14.57* | 3.67 ± 0.56* | 6.83 ± 2.75* | 3.83 ± 1.72** | 58.16 | 70.89 |
| EEZJ | 400 | 77.34 ± 3.70 $^{ns}$ | 2.33 ± 0.21 $^{ns}$ | 11.17 ± 1.90 $^{ns}$ | 9.00 ± 0.97 $^{ns}$ | 31.63 | 31.66 |
| EEZJ | 800 | 73.00 ± 3.90 $^{ns}$ | 2.33 ± 0.33 $^{ns}$ | 10.50 ± 1.38 $^{ns}$ | 8.67 ± 1.01 $^{ns}$ | 35.70 | 34.17 |
| EEZJ | 1200 | 66.23 ± 12.06 $^{ns}$ | 2.83 ± 0.48 $^{ns}$ | 8.50 ± 1.82* | 4.33 ± 1.45** | 47.95 | 67.09 |

(means ± S.E.M; n = 6); ns P ≥ 0.05 compared to control (One-way ANOVA followed by Dunnett's Test); *P ≤ 0.05 compared to control (One-way ANOVA followed by Dunnett's Test); **P ≤ 0.01 compared to control (One-way ANOVA followed by Dunnett's Test.

According to Table 4 and 5, both three doses of *Z. jujuba* ethanolic extract successfully controlled the percentage of paw oedema under 40%. 1200 mg/kg *Z. jujuba* ethanolic extract treatment group has a higher percentage of paw oedema inhibition when compared to that of 800 mg/kg and 1600 mg/kg *Z. jujuba* ethanolic extract treatment groups. 800 mg/kg *Z. jujuba* ethanolic extract treatment group has weakest anti-inflammatory effect at the corresponding time point.

**Table 4: Percentage of right paw oedema (%) calculated from the change in paw volume after treatment**

| Group | Treatment | Dose (mg/kg) | Percentage of right paw oedema (%) after treatment (Mean ± SEM) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 1 h | 2 h | 3 h | 4 h | 5 h | 6 h |
| 1 | *EEZJ* | 800 | 7.60 ± 1.12*** | 14.00 ± 1.58*** | 20.80 ± 1.72*** | 25.40 ± 1.69*** | 30.20 ± 2.08*** | 36.00 ± 2.68*** |
| 2 | *EEZJ* | 1200 | 8.00 ± 1.27*** | 13.40 ± 1.20*** | 18.40 ± 1.50*** | 24.00 ± 1.79*** | 30.00 ± 2.43*** | 32.80 ± 3.41*** |
| 3 | *EEZJ* | 1600 | 6.00 ± 0.45*** | 16.00 ± 1.76*** | 21.40 ± 1.33*** | 26.80 ± 1.78*** | 30.40 ± 1.86*** | 35.00 ± 2.24*** |
| 4 | Diclofenac Sodium | 100 | 3.20 ± 1.07*** | 8.00 ± 1.30*** | 13.60 ± 2.34*** | 18.00 ± 2.67*** | 21.20 ± 2.56*** | 23.20 ± 2.35*** |
| 5. | Control | 100 | 28.00 ± 4.72 | 45.80 ± 5.00 | 68.00 ± 5.06 | 89.20 ± 6.59 | 105.20 ± 10.10 | 114.00 ± 10.07 |

(means ± S.E.M; n = 5); ns P ≥ 0.05 compared to control (One-way ANOVA followed by Dunnett's Test); * P ≤ 0.05 compared to control (One-way ANOVA followed by Dunnett's Test); ** P ≤ 0.01 compared to control (One-way ANOVA followed by Dunnett's Test); *** P ≤ 0.001 compared to control (One-way ANOVA followed by Dunnett's Test).

Apart from that, all three doses of *Z. jujuba* ethanolic extract showed a similar trend of oedema development. Additionally, the *Z. jujuba* ethanolic extract produced the dose-dependent significant anti-inflammatory activity as the lowest dose, 800 mg/kg

showed least anti-inflammatory activity when compared to other two with the higher dose.

According to Table 6 and 7, the three doses of *Z. jujuba* ethanolic extract successfully controlled the thickness of paw that due to oedema when compared with that of the negative control group. The 1200 mg/kg *Z. jujuba* ethanolic extract treatment group has a higher percentage of paw oedema inhibition when compared to the 800 mg/kg and 1600 mg/kg *Z. jujuba* ethanolic extract treatment groups. However, all the three doses of *Z. jujuba* ethanolic extract exhibited lower anti-inflammatory activity than the positive control group.

**Table 5: Percentage of left paw oedema (%) calculated from the change in paw volume after treatment**

| Group | Treatment | Dose (mg/kg) | Percentage of left paw oedema (%) after treatment (Mean ± SEM) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 1 h | 2 h | 3 h | 4 h | 5 h | 6 h |
| 1 | *EEZJ* | 800 | 6.80 ± 1.16*** | 13.40 ± 2.54*** | 19.80 ± 3.09*** | 25.80 ± 3.80*** | 31.00 ± 4.38*** | 37.00 ± 4.45*** |
| 2 | *EEZJ* | 1200 | 6.00 ± 0.63*** | 12.60 ± 1.75*** | 17.40 ± 2.11*** | 24.40 ± 4.13*** | 29.40 ± 4.68*** | 32.20 ± 4.12*** |
| 3 | *EEZJ* | 1600 | 5.80 ± 0.37*** | 12.80 ± 1.36*** | 17.40 ± 1.17*** | 25.00 ± 1.82*** | 29.00 ± 1.92*** | 34.20 ± 2.48*** |
| 4 | Diclofenac Sodium | 100 | 2.20 ± 0.37*** | 6.40 ± 0.81*** | 10.20 ± 2.60*** | 14.00 ± 2.59*** | 17.60 ± 2.46*** | 19.80 ± 2.29*** |
| 5. | Control | 100 | 23.60 ± 4.78 | 41.80 ± 4.60 | 60.00 ± 4.37 | 78.00 ± 6.72 | 93.20 ± 6.58 | 102.60 ± 6.81 |

Means ± S.E.M; n = 5); ns P ≥ 0.05 compared to control (One-way ANOVA followed by Dunnett's Test); *P ≤ 0.05 compared to control (One-way ANOVA followed by Dunnett's Test); **P ≤ 0.01 compared to control (One-way ANOVA followed by Dunnett's Test); *** P ≤ 0.001 compared to control (One-way ANOVA followed by Dunnett's Test).

The HPLC chromatograms of the reference standards and EEZJ are shown in the Fig. 1A, 1B, 1C and 1D, respectively. UV absorption, retention time and identification results of peaks are presented in Table 8.

**Table 6: Percentage of right paw oedema (%) calculated from the change in paw thickness after treatment**

| Group | Treatment | Dose (mg/kg) | Percentage of right paw oedema (%) after treatment (Mean ± SEM) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 1 h | 2 h | 3 h | 4 h | 5 h | 6 h |
| 1 | *EEZJ* | 800 | 14.00 ± 4.93$^{ns}$ | 19.33 ± 4.41$^{ns}$ | 21.67 ± 3.38** | 25.33 ± 2.91* | 20.33 ± 9.33** | 26.67 ± 7.13** |
| 2 | *EEZJ* | 1200 | 6.33 ± 1.45* | 14.33 ± 2.33* | 20.67 ± 1.76** | 22.67 ± 3.48** | 23.00 ± 5.20* | 21.00 ± 6.81*** |
| 3 | *EEZJ* | 1600 | 10.33 ± 0.88$^{ns}$ | 20.00 ± 1.53$^{ns}$ | 25.33 ± 0.88* | 24.67 ± 6.57* | 26.33 ± 5.78* | 24.67 ± 1.20** |
| 4 | Diclofenac Sodium | 100 | 3.00 ± 0.58** | 8.00 ± 1.00** | 10.00 ± 1.53*** | 8.66 ± 3.28*** | 10.00 ± 4.50** | 13.33 ± 3.18*** |
| 5. | Control | 100 | 19.33 ± 3.53 | 31.00 ± 4.73 | 39.00 ± 4.36 | 46.00 ± 2.52 | 56.00 ± 4.51 | 62.33 ± 3.71 |

Means ± S.E.M; n = 5); ns P ≥ 0.05 compared to control (One-way ANOVA followed by Dunnett's Test); *P ≤ 0.05 compared to control (One-way ANOVA followed by Dunnett's Test); **P ≤ 0.01 compared to control (One-way ANOVA followed by Dunnett's Test); *** P ≤ 0.001 compared to control (One-way ANOVA followed by Dunnett's Test).

Peak 1 represented the present of betulinic acid at the retention time of 42.169 minutes from reference compound. EEZJ showed a peak at the same retention time. Hence, EEZJ consists of betulinic acid as the peak was comparable to reference compound (Fig. 1).

Peak 2 appeared at 16.821 minutes in the reference compound and represented as quercetin. EEZJ showed a peak at the same retention time which indicates the presence of quercetin in the extract tested (Fig. 1).

## Discussion

People in different communities commonly use plant or plant derivatives to cure illnesses without scientific evidence. The current studies aimed to evaluate anti-diarrhoeal as well as anti-inflammatory activity potential of *Z. jujuba* fruit. Castor oil was given to mice to imitate the diarrhoea symptom while carrageenan was given to rats to induce inflammation at the paw of the rats.

**Table 7: Percentage of left paw oedema (%) calculated from the change in paw thickness after treatment**

| Group | Treatment | Dose (mg/kg) | Percentage of left paw oedema (%) after treatment (Mean±SEM) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 1 h | 2 h | 3 h | 4 h | 5 h | 6 h |
| 1 | *EEZJ* | 800 | 12.67 ± 3.18** | 22.00 ± 7.55$^{ns}$ | 28.67 ± 6.33$^{ns}$ | 27.33 ± 5.55** | 30.33 ± 6.94** | 30.67 ± 4.41*** |
| 2 | *EEZJ* | 1200 | 7.00 ± 0.58*** | 15.00 ± 1.53* | 19.33 ± 0.88** | 19.00 ± 1.16*** | 22.67 ± 1.67*** | 19.67 ± 1.76*** |
| 3 | *EEZJ* | 1600 | 10.33 ± 0.33** | 19.67 ± 2.73$^{ns}$ | 25.00 ± 3.00* | 31.33 ± 1.20* | 28.00 ± 4.36** | 27.33 ± 3.53*** |
| 4 | Diclofenac Sodium | 100 | 3.00 ± 0.58*** | 6.00 ± 1.00** | 8.33 ± 0.67*** | 8.33 ± 1.33*** | 6.33 ± 1.33*** | 12.00 ± 7.69*** |
| 5. | Control | 100 | 22.33 ± 2.03 | 31.33 ± 1.67 | 40.33 ± 2.33 | 46.67 ± 2.67 | 53.33 ± 1.20 | 56.00 ± 2.08 |

Means ± S.E.M; n = 5); ns P ≥ 0.05 compared to control (One-way ANOVA followed by Dunnett's Test); * P ≤ 0.05 compared to control (One-way ANOVA followed by Dunnett's Test); ** P ≤ 0.01 compared to control (One-way ANOVA followed by Dunnett's Test); *** P ≤ 0.001 compared to control (One-way ANOVA followed by Dunnett's Test)

The minimum effective dose of EEZJ that determined in the preliminary test was brought into the experimental test. Moreover, two higher concentration of EEZJ (800 mg/kg and 1200 mg/kg) were added into the test to evaluate the effectiveness in gradual increasing dose.

**Table 8: Method employed, retention time, and ultraviolet absorption of betulinic acid, quercetin and ethanolic extract of *Ziziphus jujuba***

| Compound | Method | Constituent | Retention Time (min) | UV Absorption (nm) |
|---|---|---|---|---|
| Standard 1 | Zhang et al. 2008 | Betulinic Acid | 42.169 | 205 |
| EEZJ | Zhang et al. 2008 | Betulinic Acid | 42.169 | 205 |
| Standard 2 | Guo et al. 2011 | Quercetin | 16.821 | 205 |
| EEZJ | Guo et al. 2011 | Quercetin | 16.821 | 205 |

Loperamide (loperamide hydrochloride) served as a positive control group in the experiment as it acts directly at the opioid receptors on the circular and longitudinal intestinal mucosa. Similar to other μ-receptor agonists, loperamide inhibits peristalsis of the intestine and prolongs the transit time of the digested content. Loperamide also stimulates the reabsorption process and alters the transport of water and electrolytes. Thus, it reduces the faecal volume and decreases the fluid and electrolyte loss. Loperamide not only used in acute diarrhoea patient but also successfully applied in chronic diarrhoea patient for several years without evidence of tolerance [42].

Based on the results obtained and presented in Table 3, mice treated with different concentrations of EEZJ produced a reduced number of watery dropping as well as a total number of faeces.

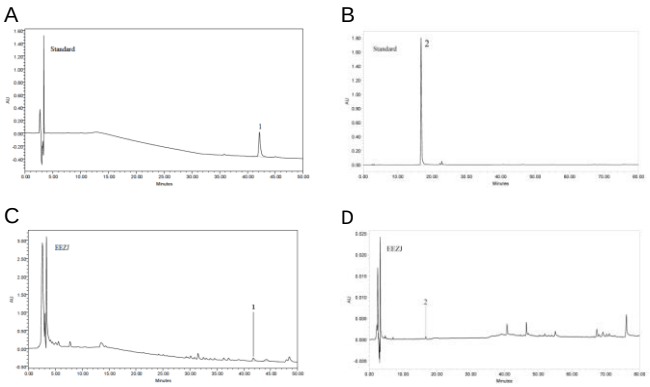


*Figure 1: A) Chromatographic profile of standard; 1 = Betulinic acid; B) Chromatographic profile of standard; 2 = Quercetin; C) Chromatographic profile of ethanolic extract of Ziziphus jujube; 1 = Betulinic acid; D) Chromatographic profile of ethanolic extract of Ziziphus jujube; 2 = Quercetin*

This result is similar to the studies done by Oh and Ryu [43], where they proposed that Korean oriental medicine which containing *Z. jujuba* fruit, can reduce the number of faeces of mice in five hours of observation. A similar observation was reported by Hu et al. [44] where a Chinese traditional medicine (Wei-Chang-An-Wan extract) containing *Z. jujuba* fruit was able to reduce the total number of faeces significantly and the number of watery dropping.

Stool weight depends on many factors such as water composition, number of bacteria and fibre content in the stool. In addition to this, total stool weight is highly correlated with the frequency of defaecation [45]. In our experiment, EEZJ did not only reduce the number of stool but also induced mice to produce a lesser weight of watery stool. Muller et al. [46] in their work concluded that the gastrointestinal transit time would affect the weight of stool where longer gastrointestinal transit time will produce lighter stool weight. Which was also tested by Rao and Lakshimi [44], where they also displayed the aqueous extract of *Z. jujuba* leaves to increase the gastrointestinal transit time. Hence, it may indicate that *Z. jujuba* fruit in our experiment may reduce stool weight by increasing the gastrointestinal transit time.

EEZJ did not only reduce stool weight and some faeces, but it also delayed the onset of first watery dropping compared to control group. *Z. jujuba* can reduce the intestinal motility, and this has been described by Rao and Lakshimi [47]. Hu et al. [44] demonstrated that *Z. jujuba* containing traditional medicine able to attenuate the gastrointestinal tract motility in a dose depending manner. Based on the same studies, *Z. jujuba* containing traditional medicine also decreased the spontaneous contraction of isolated jejunum in animals. In our study, the result showed that EEZJ could reduce the number of faeces as well as the weight of faeces in a dose depending manner. Moreover, it significantly attenuated the onset of first watery dropping from the mice. With these characteristics, EEZJ can be considered as possessing antidiarrhoeal activity against castor oil-induced diarrhoea.

Inflammation process causes an alteration in human body's physiological responses and ultimately resulting in pain, heat, redness, swelling and loss of function. Carrageenan-induced paw oedema is an example of acute inflammation which results in swelling. It had been widely used as an experimental animal model to discover and evaluation of anti-inflammatory potential [48].

Carrageenan is a complex group of polysaccharides made up of repeating galactose-related monomers. The inflammatory response is usually quantified by an increase in paw size which is observed for around five hours after carrageenan injection [32]. Inhibition of carrageenan-induced inflammation has been shown to be highly predictive of anti-inflammatory activity in human inflammatory disease and doses of nonsteroidal anti-inflammatory drugs (NSAIDs) in this model correlate well with effective dose in patients [49]. Using antagonists of various mediators of inflammation showed that the inflammatory response to carrageenan consisted of three phases [50]. The primary phase is mediated by both histamine and 5-hydroxytryptamine. The second phase starts after one hour and persists for six hours after carrageenan injection. This phase is kinin-mediated.

The final phase is attributed to the local production of prostaglandins, which is derived from arachidonic acid by the action of cyclooxygenase (COX) enzymes. Inhibition of these enzymes is the basis of action of the NSAIDs of major clinical importance in the treatment of pain and inflammation [51]. Cyclooxygenase 2 (COX-2) is a pro-inflammatory enzyme which is in charge of making prostaglandins (PG), and it is also the site of action targeted by non-steroidal anti-inflammatory drugs such as diclofenac and COX-2 inhibitors. However, COX-2 is normally produced within the second hour after carrageenan is induced to cause inflammation. This means PG is not involved in oedema formation in the very first hour but instead contribute to oedema within second and the subsequent hours.

According to Di Lorenzo et al. [52], carrageenan injection evokes neutrophil infiltration immediately after injection and persists for six hours. $O_2^-$, OH and $H_2O_2$ which are derived from neutrophils, are suggested to cause a sustained increase in permeability through damaging the endothelial cells [53]. Thus, carrageenan-induced paw oedema has been an important tool in the development of NSAIDs.

A role for neutrophil-derived reactive oxygen species, nitric oxide, and peroxynitrite in carrageenan-induced inflammation has also been identified, and some specific inhibitors have been identified which have potential clinical use.

Diclofenac sodium is superior to the other clinically established non-steroidal anti-inflammatory agents in carrageenan-induced paw oedema [54]. It exerts their effect by blocking prostaglandin synthetase system. In the present study, diclofenac sodium significantly expressed its anti-inflammatory activity in Table 4-7. The result of the present study showed and solidified that diclofenac sodium has significant anti-inflammatory activity.

According to the anti-inflammatory experiments that were carried out, *Z. jujuba* ethanolic extract successfully controlled the inflammation of the paw. At 1200 mg/kg dose of *Z. jujuba* ethanolic extract displayed the highest paw oedema inhibition. The mechanisms of action of *Z. jujuba* ethanolic extract may be similar to that diclofenac sodium whereby the anti-inflammatory effect observed might be due to the inhibition of expression and activity of COX-2.

Based on the chemical analysis done, it was found that *Z. jujuba* fruit contains both betulinic acid and quercetin in it. Earlier studies showed that most of the medical plants could counter dysentery and diarrhoeal incident. Most of the medical plants contain alkaloids, saponins, flavonoids, sterols and triterpenoid [55] and they are responsible for the mechanism of antidiarrhoeal activity. Betulinic acid belongs to the pentacyclic triterpenoid group [56], whereas quercetin belongs to flavonoids group [13]. From the earlier statement, we understand that both triterpenoid and flavonoids capable of possessing antidiarrhoeal activity. This is further support by Ezekwesili et al. [57] studies, where he described that quercetin relaxes the intestinal smooth muscle and inhibits bowel contraction which probably due to inhibition of intracellular release of calcium ions from the sarcoplasmic reticulum. Other than that, Begum et al. [58] claimed that triterpenoid also had been shown to possess antispasmodic activity in a dose depending manner.

From previous studies, betulinic acid is considered to have a potent anti-inflammatory activity where betulinic acid can reduce the production of TNF-α as well as nitric oxide in mice. Additionally, betulinic acid also found to promote the concentration of IL-10 upon LPS stimulation [59][60], and quercetin was able to regulate down the inflammatory response of *in vitro* bone marrow-derived macrophages. They further proof that quercetin to inhibit the release of cytokine and induce the nitric oxide synthase via inhibition of NF-κB pathway without modification of c-Jun N-terminal kinase activity.

Besides that, different kinds of methods were tried during the chemical analysis for better resolution and separation of target peak. All of it was run at the maximum wavelength of 205 nm as the study compound from *Z. jujuba* has wavelength maximum at 205 nm. According to Guo et al. [33], suggested acetonitrile to be used as a mobile phase because acetonitrile has low wavelength maximum compared to methanol where acetonitrile avoids blank interference during the analysis. Therefore, low peaks of EEZJ can be observed due to less blank interference. Guo et al. group reported the mobile phase with a buffered acid to produce a better separation of peaks. This is particularly in case of betulinic acid analysis as buffer acid improved the peak shape and eliminated the tailing of the target peak [61].

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# A New Horizontal Plane of the Head

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**Keywords:** Cephalometrics; Frankfurt Horizontal; True vertical; Natural head position

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**Abstract**

**AIM:** This study aimed to attempt to introduce a new extracranial horizontal plane of the head (K plane that extends from SN to SAE bilaterally) that could act as a substitute to the Frankfurt horizontal intracranial reference plane; both clinically and radiographically.

**MATERIAL AND METHODS:** The new K plane depended on three points of the head. The first was the soft tissue nasion (NS) on the interpupillary line when the subject looked forward at a distant point at eye level. The other two points were the superior attachments of the ears (SAE).

**RESULTS:** The student "t" test comparing mean values of K/V and FH/V was not significant; -0.21. The coefficient of correlation between different variables was highly positively significant (r = 0.98 with probability = 0.001).

**CONCLUSION:** Within the limitations of this prospective study, the new K plane was found to be both reliable and reproducible. It can be used as a reliable reference plane instead of Frankfort horizontal plane both clinically and radiographically; as it is an accurate tool for head orientation in the natural head position.

## Introduction

Before the development of the roentgenographic cephalometric technique, anthropologists had studied and recorded craniofacial structures using direct craniometric techniques for many years. Brodbent, [1] in 1931 introduced a "new X-ray technique" which provided a standardised method of recording craniofacial structures. The Frankfort horizontal plane; a plane extending from left Orbitale to both Porion points, was officially defined in 1884 in the anthropologic conference in Frankfort, Germany. This plane had been accepted as the plane most reflective of the true horizontal when the patient's eyes were cast at the horizon [2] [3] [4] [5] [6] [7]. Initially, it was established as a craniometric reference plane for classification purposes. By the development of precise X-ray techniques, radiographic cephalometry became the natural extension of craniometry, and Frankfort horizontal was adopted as a reliable reference plane for cephalometric studies.

However, Krogman and Sussouni [8] claimed that defining Porion by cephalometric instrumentation might be brought by through possible error since the ear rod positioning and the "ear-hole size and inclination" were considerably variable. Downs [4] [5] [6] used Frankfort horizontal, in his cephalometric analysis, with the angle of the facial plane (N-Pog) to classify facial forms. He found that it was tipped upward anteriorly about +1.3°. Sassouni [10] also reported that the optic plane was tipped up anteriorly. Also, when Moorees and Kean [7] used the true vertical as an extracranial reference plane (with the patients in the natural head position); they found Frankfort Horizontal to be 92° to the true vertical opening postro-inferiorly. Some authors hence, believed that the patient positioning technique could be standardised with minimal error; [4] while others, because of reported low measurement reliability, favoured dropping Porion and thus Frankfort horizontal [2] [8].

The vestibular method of orientation, specifically by the lateral semicircular canals, has

perceived the attention of many researchers [3] [11] [12] [13].

De Beer [3] showed that most mammals, except man, maintained a head posture in the alert state with the lateral canals being parallel to the true horizontal. The man, however, does not exhibit an identical situation to animals. In fact, it has been shown that the lateral semicircular canals were canted down and back approximately 30° about Frankfort horizontal when a person was in the natural head posture with his eyes cast at the horizon [14] [15].

Lundstrom et al., [16] studied the accuracy and validity of the natural head orientation. They concluded that the extra-cranial reference line "sella-horizontal" should substitute, or at least supplement the use of intracranial reference lines for the cephalometric analysis of patients with malocclusion. They also recommended the use of estimated natural head orientation (NHO) performed by experienced clinicians, in addition to the natural head position concept.

Consequently, it was important to attempt to introduce a new extracranial horizontal plane of the head (K plane that extends from SN to SAE bilaterally) that combined anatomical points and the natural head position, at the same time. This plane was evaluated for its reliability and reproducibility as a clinical and radiographic substitute to the Frankfurt horizontal intracranial reference plane.

## Material and Methods

The new plane (K plane) extended through three points on the head. The first was the soft tissue nasion (SN) on the interpupillary line; when the subject looked forward at a distant point at the eye level. The other two points were the superior attachments of the ears (SAE). The three points were confirmed by a metal frame "Orienter" which is a preformed three armed stainless steel wire with a cross-section of 1.5 mm; Figure 1. The frontal arm rested on the SN point (marked by a black wax pencil) and was secured in place by a scorching tape. The two lateral arms were perpendicular to the front arm and in the same horizontal plane. They could be adjusted according to the width of the face and rested on the SAE bilaterally. The metal Orienter is radio-opaque; this facilitated its identification in X-ray (Figure 2). The design of the front arm included two semicircles facing the eyes to clear the vision of the subject.

The sample of this prospective study consisted of 40 adult subjects (20 males and 20 females) with an age range of 18-24 years. They were orthodontic patients selected from the outpatient clinic of Orthodontic Department, Faculty of Oral and Dental Medicine, Cairo University.

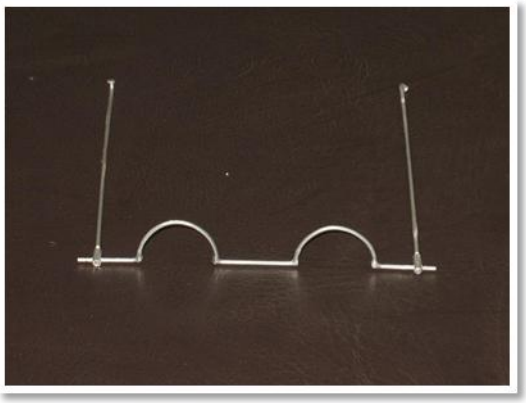

*Figure 1: The Orienter*

All subjects did not receive any previous orthodontic treatment and were free from any history of significant medical diseases. All the subjects signed informed consent. Lateral cephalometric radiographs were taken for each subject in the natural head position according to Solow and Tallagren, [17] except that the Orienter was fixed in place.

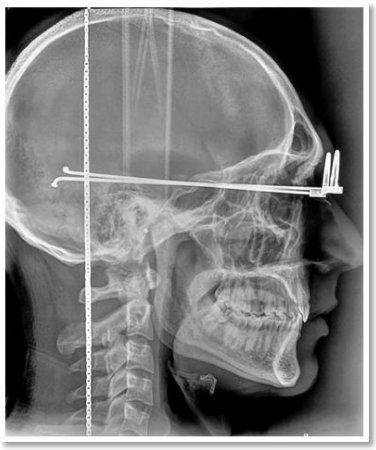

*Figure 2: A Lateral cephalometric radiograph with the Orienter in place*

### *Clinical Procedure*

1- The subjects were instructed to stand upright with their arms at their sides, looking straight forward at a distant point at the same level of their eyes. The SN point was identified by a black wax pencil between the forehead and the nasal bridge on the interpupillary line.

2- The Orienter was scotched in place over the mark; the two lateral arms rested on the SAE after their adjustment to fit the subject's facial width.

3- After the subjects oriented their heads in the natural head position (NHP), the posterior inferior angle between the lateral arm of the Orienter and the true vertical was measured by the plump line

method; according to Vig and associates [18] (weight hanging from a lead line or a metal chain on the center of a protractor, Figure 3).

4- The readings were recorded three times at 5-minute intervals, where the subjects were asked to tilt their heads during rest and then return to the NHP using the same procedure. The mean values of these angles were taken to establish the clinical orientation angle of the subjects.

5- The orientation angle of each subject was used to orient the subject's head during the cephalometric X-ray (Figure 3).

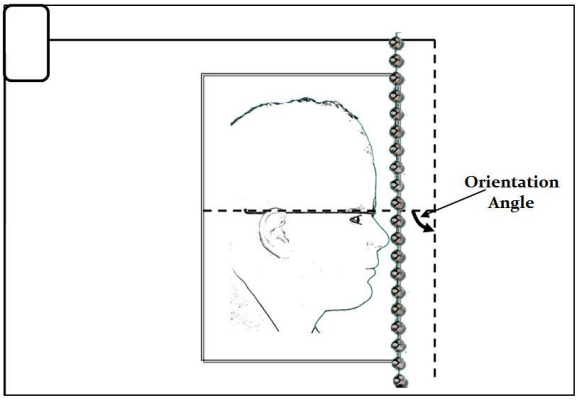


*Figure 3: Orientation angle*

*The cephalometric reference lines used:*

(FH): Frankfort horizontal plane: passing through orbitale and porion (the midpoint of the upper contour of the external auditory meatus).

(SN): a line passing through nasion and sella.

(KP): the image of the metal orienter on the film either by one line or two parallel lines.

(TV): true vertical; the vertical line on the film represented by the suspended metal chain.

*The cephalometric angular measurements used:*

(KP/FH): the inferior posterior angle between KP and Frankfort Horizontal.

(KP/SN): the inferior posterior angle between KP and SN line.

(FH/SN): the inferior posterior angle between Frankfort Horizontal and SN line.

(FH/TV): the inferior posterior angle between Frankfort Horizontal and the true vertical.

(SN/TV): the inferior posterior angle between SN line and the true vertical.

(KP/TV): the inferior posterior angle between KP and the true vertical.

*Error detection:*

After tracing the cephalometric radiographs the orientation angle, which had been made by the image of the Orienter and the true vertical (posterior-inferior angle), was checked by comparing it to the original clinical orientation angle of the subject. Any deviation or error during exposure was adjusted without repeating the exposure by modifying the orientation angle of the tracing to fit the original clinical orientation angle. This was done by adjusting the true vertical (the image of the metal chain).

The reliability and reproducibility of placement of the Orienter in the natural head position were examined through taking five measurements of the natural head position for 5 consecutive days. Similarly, the recorded plumb-line measurements with and without the Orienter were compared. Measurements were found to be nearly the same; which showed that the Orienter did not influence the reliability and reproducibility of the natural head position.

The lateral cephalometric radiographs were repeated for 10 subjects using the same method and technique after one month to assess the reproducibility of the method. Dahlberg's formula (method error) was used to assess the method error where (d) was the difference between the first and second records and (n) was the number of double determination. The mean difference was found to be 0.29° for both FH/TV and KP/TV.

$$\sqrt{\frac{\Sigma d^2}{2n}}$$

*Statistical analysis was performed using SAS computer software to calculate:*

A) Descriptive statistics (mean values, standard deviation, standard error and range).

B) The coefficient of correlation between different variables.

C) Paired "t" test to examine the significance of differences between variables.

## Results

Descriptive statistics of the different measurements were shown in Table 1.

**Table 1: Descriptive statistics of the different measurements (in degrees)**

| Variables | No. | Range | Mean | SD | SE |
|---|---|---|---|---|---|
| KP/FH | 40 | 0-1 | 0.06 | 0.22 | 0.04 |
| KP/SN | 40 | 2.5-12 | 7.60 | 2.64 | 0.54 |
| FH/SN | 40 | 2.5-12 | 7.58 | 2.55 | 0.52 |
| FH/TV | 40 | 88.5-96 | 89.28 | 4.65 | 0.74 |
| SN/TV | 40 | 78.2-87.3 | 82.28 | 4.24 | 0.67 |
| KP/TV | 40 | 88.5-96 | 89.05 | 4.94 | 0.78 |

The coefficient of correlation between different variables showed a high positive significance (r = 0.98 with probability = 0.001) shown in Table 2.

**Table 2: Coefficient of correlation between the different variables**

| V1 | V2 | Correlation | Probability |
|---|---|---|---|
| FH/SN | KP/SN | r = 0.98 | 0.001** |
| FH/TV | KP/TV | r = 0.98 | 0.001** |

The results of the paired "t" test between the original clinical angle and the cephalometric orientation angle were not significant; 89.98 ± 4.94 and 89.05 ± 4.94, respectively (Table 3).

**Table 3: Paired "t" test between the original clinical angle and the cephalometric orientation angle**

| Clinical orientation angle | Cephalometric orientation angle | Mean % Difference | "t" value | Probability |
|---|---|---|---|---|
| 89.98 ± 3.4 | 89.05 ± 4.94 | 0.90% | 0.981 | 0.330 NS |

The student's "t" test comparing mean values of KP/TV and FH/TV was not significant; -0.21, as shown in Table 4.

**Table 4: Independent student "t" test comparing the mean values of KP/TV and FH/TV**

| Mean ± SD | | "t" value | Probability |
|---|---|---|---|
| *KP/TV* | *FH/TV* | | |
| 89.05 ± 4.94 | 89.28 ± 4.65 | -0.21 | 0.83 NS |

Mean values of KP/TV and FH/TV were illustrated in Figure 4.

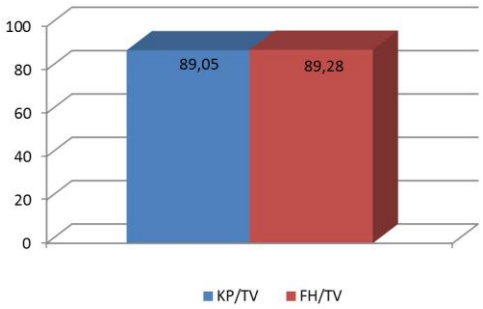


*Figure 4: A bar graph illustrating the comparison of mean values of KP/TV and FH/TV*

## Discussion

A reliable reference plane was the first demand for cephalometric interpretation. Many reference planes were used, most of them relied on anatomical points or intracranial reference points. The natural head position (NHP) was an alternative to the traditional cephalometric method. It was a trial to relate the face and cranium to extracranial reference lines; the true vertical and true horizontal lines [18] [21].

A new horizontal plane, the K plane, was introduced in this study; it was determined according to anatomical and physiological reference points. Its anatomical reference point anteriorly was soft tissue nasion (SN) while posteriorly the reference points were the superior attachments of the ears (SAE). Both SN and SAE points have been used in profile photographs, as well as the interpupillary line in facial photographs, to orient the head in the horizontal plane [22] [23]. The visual axis was physiologically determined when the subject looked at the horizon; thus the head was oriented in the NHP. Every time this was done the pupils of the eyes were fixed in the same position without any change. Hence this showed that it was both reproducible and reliable [21].

The new K plane (KP) was found to be nearly parallel to the Frankfort horizontal plane (FH) with a very high positive correlation (r = 0.98) and a range of 0-1°. Also, KP to FH was found to be 0.06° ± 0.22 with a range of 0-1°. KP to SN and FH to SN were found to be 7.60° ± 2.64 and 7.58° ± 2.55, respectively. From these results, it could be deduced that the K plane might be used instead of the FH plane.

The metal Orienter, when fixed in position, represented the new K plane clinically, photographically, and cephalometrically as it was radio-opaque in the X-ray. Accordingly, it could be used in any radiographic imaging technique; like the cone-beam computed tomography. Using the Orienter to determine the clinical orientation angle facilitated the measurement of the inferior posterior angle, before and after cephalometric radiography, to assess any errors (Figures 2 & 3). The mean percentage difference, in this study, was found to be 0.90% while the "t" value was not statistically significant; 1.39. This denoted that some errors could occur during cephalometry which could not be noticed using the ordinary method for recording the NHP without the use of the Orienter.

As a result, 2D and 3D radiographic imaging, as well as 2D and 3D photographs performed with the Orienter in place, might give us the opportunity to analyse the face and the profile in the NHP according to the new horizontal K plane of the head. Also, any error during recording could be adjusted by modifying the orientation angle on the film according to the original clinical orientation angle recorded before imaging, for every patient (Figure 3); which reduced the possible errors. This is considered as a valuable finding as it was shown by Madsen et al., [24] that the true vertical and the true horizontal planes were more valid when recorded from the NHP. Shetty et al., [25] also reported that the Frankfurt horizontal plane was recommended as a reference plane only when the radiographs were not recorded in the natural head position.

Furthermore, the K plane was found, during the analysis, to be on the same level of the insertion of the trapezius muscle (neuchal line at the back of

the head) separating the skull into 2 portions; cranial and facial. These observations need further studies.

In conclusion, within the limitations of this prospective study, the new K plane was found to be both reliable and reproducible. It can be used as a reliable reference plane instead of Frankfort horizontal plane both clinically and radiographically; as it is an accurate tool for head orientation in the natural head position.

A new appliance design was constructed based on the new K plane with the Orienter to fix the head in the natural head position. This appliance will be used as a reliable 3D non-radiographic; extra-oral and intra-oral, mean of diagnosis, to overcome the errors produced by traditional methods and prevent patient irradiation. The appliance can perform static and dynamic measurements at the same time, and it could be digitised as well.

Studies will be conducted on the new appliance.

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# Angiotensin-Converting Enzyme (*ACE*) D Allele as a Risk Factor for Increase Serum Interleukin-6 and Interleukin-8 in Psoriasis Patients

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**Keywords:** *ACE* polymorphism; Serum IL-6; serum IL-8; Psoriasis

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**Abstract**

**BACKGROUND:** Psoriasis is a chronic, recurrent inflammatory skin disease. It is characterised by autoimmune, environmental factors and complex genetic disorder.

**AIM:** To explore the role of IL-6, IL-8, and *ACE* I/D polymorphism in the pathogenesis of Psoriasis and investigation of the relationship between *ACE* polymorphism and occurrence of psoriasis.

**PATIENTS AND METHODS:** In this study, we took 73 psoriasis patients and 47 healthy patients as a control. These two groups subjected to analysis for *ACE* gene I/D polymorphism by PCR and biochemical methods.

**RESULTS:** The serum levels of ACE, IL-8 and IL-6 were statistically significantly higher in psoriasis patients compared to healthy subjects ($P < 0.001$). ID and DD polymorphism were more common in psoriasis patients than healthy subjects. Also, D allele was significantly over-represented in patients compared to controls (52.7% Vs 35.1%).

**CONCLUSION:** *ACE* gene polymorphism might grant susceptibility to develop psoriasis.

## Introduction

Psoriasis is a chronic, recurrent inflammatory skin disease that can have a great effect on a patient's self-esteem [1]. It is affected by autoimmune, environmental factors and complex genetic disorder [2]. The effect of the disease is not only limited solely to the skin but also causes permanent joint damage in nearly 30% of the patients [3].

Angiotensin-converting enzyme (ACE) is a zinc metallopeptidase, located on chromosome 17q23. It contains an insertion (I)/deletion (D) polymorphism within intron 16 that contain the most genetic variables of the variability of serum ACE activity and is associated with the development of psoriasis [4].

Several studies indicated that Angiotensin-converting enzyme is a major and effective factor in creating angiotensin II (Ang II) and inactivating bradykinin [5] [6].

Active angiotensin II increases the production of reactive oxygen species (ROS) and the synthesis of cytokines such as interleukin-6 (IL-6) and IL-8 which play an important role in the development of psoriasis [7]. Also, inactivation of bradykinin by ACE stimulates the synthesis of cytokines such as IL-6, IL-8 and nitric oxide (NO) [8] [9] [10].

Interleukin 6 (IL-6) is a major inducer of regulated expression of many cytokines [11]. IL-6 is one of the normal skin components, and it was immunologically founded in endothelial cells, keratinocytes, and fibroblasts [11]. IL-6 has been suggested to function as an autocrine mitogen in the psoriatic epidermis [12].

Interleukin 8 (IL-8) is one of the most common chemokines that is elevated in the psoriatic lesion [13]. Moreover, both mRNA and peptide IL-8 have been detected in situ in psoriatic patients [14]. Elevated IL-8 blood levels are considered as a marker for the systemic inflammatory disorders [15].

Our main goal was to explore the role of IL-6, IL-8, and *ACE* I/D polymorphism in the pathogenesis of Psoriasis. Also, our specific aim is to investigate the relationship between *ACE* polymorphism and occurrence of psoriasis.

## Subject and Methods

The present study was performed at Outpatient Dermatology Clinic, Buraidah Central Hospital, Qassim region, Saudi Arabia between October 2016 and May 2017.

A total of 73 patients (42 male and 31 females) were enrolled for this case-control study. The diagnosis was established by clinically-physical examination as the diagnosis was striated forward (All patients had characteristic erythematosquamous plaques located on the trunk and limbs). Patients had an only cutaneous form of psoriasis, with no systemic involvements were included in the study. None of the patients had received any systemic immunosuppressive medications or used any local treatment at the site of biopsies for 4 weeks before study participation.

Patients were classified according to body surface area (BSA) into severe psoriasis vulgaris greater than 10% of the body surface, moderate psoriasis vulgaris 5%-10% of the body surface and mild psoriasis vulgaris less than 5% of the body surface [16].

The other 47 subjects were healthy volunteers who were age and gender-matched with the psoriasis group (27 male and 20 females), they had no clinical evidence or family history of psoriasis or any other autoimmune disorder.

Both groups had undergone complete physical and clinical examinations, genetics studies and biochemical tests.

Before the initiation of the study, informed consent was obtained from all individuals chosen for the study. The aim and the value of the work were explained to them in a simplified manner. This study was approved by the Local Medical Ethical Committee and according to their instructions.

Serum ACE concentrations were measured, utilising the Human ACE Quantikine ELISA Kit from R&D Biotech brand system [17].

The interleukin-6 level was determined using a commercially available ELISA kit (Quantikine, human IL-6R & D Systems, Minneapolis, USA) by the manufacturer's instructions [18].

Serum IL-8 samples from all patients were tested in a sandwich ELISA using according to the manufacturers' instructions (R&D, Minneapolis, USA; Bender, Vienna; Amersham, Germany) [19].

Blood samples were collected on $Na_2EDTA$ as an anticoagulant. Genomic DNA was purified from 200 µl whole blood with the QIAamp® DNA BloodMini Kit according to manufacture instruction for Blood protocol.

To determine the *ACE* gene I/D polymorphism, a genomic DNA fragment on intron 16 of the *ACE* gene was amplified by using Polymerase Chain Reaction (PCR) method with a pair of oligonucleotide primers: The upstream of primer sequence was: 5`-CTG GAG ACC ACT CCC ATC CTT TCT -3` and the downstream was: 5`- GAT GTG GCC ATC ACA TTC GTC AGA T -3` (20). The primers were blasted to the gene bank database https://blast.ncbi.nlm.nih.gov/Blast.cgi [21].

Data was presented by means ± SD and percentages. The compiled data were computerised and analysed by SPSS PC+, version 12. The following tests of significance were used: Analysis of variance (ANOVA) test between more than two means, t-test between means we used to analyse the mean difference, t-test between percentage to analyse percent difference and chi-square. A level of significance with $p \leq 0.001$ was considered highly significant and $p > 0.05$ was considered insignificant.

## Results

In our present study, we analysed *ACE* gene polymorphism for Seventy–three (42 male and 31 females) psoriasis patients and Forty –seven (27 male and 20 females) healthy controls they had no clinical evidence or family history of psoriasis or of any other autoimmune disorder. Clinical and General Data of all the patients and controls are shown in Table 1. It was noted that serum ACE, serum IL-8 and serum IL-6 were statistically significantly higher in psoriasis patients than in healthy subjects ($P < 0.001$).

Clinical presentation of psoriasis showed that 23 patients (31.5%) have severe psoriasis vulgaris, 26

patients (33.5%) have moderate psoriasis vulgaris and 24 patients (33%) have mild psoriasis.

**Table 1: General and laboratory characteristics of psoriatic patients and healthy control**

| Parameters | Patients N ( 73) Mean ± SD | Control N (47) Mean ± SD | P-value |
|---|---|---|---|
| Age/years (Mean ± SD) | 41.2 ± 4.8 | 38.1 ± 6.8 | 0.411 |
| Male/Female (N, %) | 42(57.5%)/31(42.5%) | 27(57.4%)/20(42.6%) | 0.442 |
| Severe psoriasis vulgaris (N, %) | 23 (31.5%) | | |
| Moderate psoriasis vulgaris (N, %) | 26 (33.5%) | | |
| Mild psoriasis vulgaris (N, %) | 24 (33%) | | |
| Presence of Family history (N, %) | 46/63% | | |
| Absent of Family history (N, %) | 27/37% | | |
| Serum ACE (IU/L) | 21.7 ± 5.5 | 7.7 ± 4.1 | <0.001* |
| Serum IL-6 (pg/ml) | 17.5 ± 0.48 | 8.9 ± 0.60 | <0.001* |
| Serum IL-8 (pg/ml) | 15.9 ± 0.9 | 7.6 ± 1.5 | <0.001* |

SD, standard deviation; ACE, angiotensin-converting enzyme; IL-6, interleukin-6; IL-8, interleukin-8; N, Number; *Significance between healthy Subjects and psoriatic patients (P<0.05).

Comparison of ACE genotypes in patients and controls showed that I/D were the most common (45.2%) followed by D/D (30.1%) then I/I (24.7%). I/I was the most common in healthy subjects (46.8%) while I/D and D/D genotypes were found to be 36.2% and 17% respectively (Table 2).

**Table 2: Comparison of ACE genotype in psoriatic patients and healthy control**

| Genotypes | Patients (N,%) | Control (N,%) | P-Value |
|---|---|---|---|
| I/ I | 18 (24.7 %) | 22 (46.8%) | 0.007* |
| I/D | 33 ( 45.2 %) | 17 (36.2%) | 0.872 |
| D/D | 22 ( 30.1 % ) | 8 (17 %) | 0.001* |

N, number; *p value<0.05 is statistically significant.

The allele frequency was significantly different between patients and controls (P = 0.005). The results indicated that the D allele was significantly over-represented in patients compared to the controls (52.7% vs 35.1 %) (Table 3).

**Table 3: Comparison of allele frequency in psoriatic patients and healthy control**

| Alleles frequency | Patients (N, %) | Control (N, %) | P-Value |
|---|---|---|---|
| I | 69 (47. 3%) | 61 (64.9 %) | 0.005 |
| D | 77 (52.7 %) | 33 (35.1%) | 0.002 |

N, number; *p value<0.05 is statistically significant.

Patients with DD genotypes have statistically significantly higher levels of serum ACE (P < 0.001), higher serum IL-8 (P < 0.001) and higher serum IL-6 (P < 0.001), Table 4.

**Table 4: Comparison of biochemical parameters and *ACE* genotypes in psoriatic patients**

| Parameters | I/ I (n = 18) | I/D (n = 33) | D/D (n = 22) | P-value |
|---|---|---|---|---|
| Serum ACE (IU/L) | 16.88 ± 4.22 | 23.66 ± 1.66 | 28.07 ± 3.21 | 0.001* |
| Serum IL-6 (pg/ml) | 23.67 ± 2.9 | 39.24 ± 1.54 | 47.22 ± 2.5 | 0.001* |
| Serum IL-8 (pg/ml) | 12.66 ± 2.3 | 18.34 ± 1.6 | 28.32 ± 4.1 | 0.001* |

SD, standard deviation; ACE, angiotensin-converting enzyme; IL-6, interleukin-6; IL-8 interleukin - 8; N, Number; * Significance between healthy Subjects and psoriatic patients (P < 0.05).

Comparison of biochemical parameters and variable clinical types of psoriasis indicated that the levels of serum ACE, serum IL-6, and IL-8 higher in Severe psoriasis vulgaris patients than in those with Moderate psoriasis and Mild psoriasis vulgaris with P-values 0.012, 0.001 and 0.008 respectively (Table 5).

**Table 5: Comparison of biochemical parameters and variable clinical types of psoriasis in our patients**

| Parameters | Severe psoriasis vulgaris (N = 23) | Moderate psoriasis vulgaris (N = 26) | Mild psoriasis vulgaris (N = 24) | P-value |
|---|---|---|---|---|
| Serum ACE (IU/L) | 23.25 ± 1.21 | 18.9 ± 2.88 | 14.79 ± 2.7 | 0.012* |
| Serum IL-6 (pg/ml) | 38.5 ± 4.5 | 21.56 ± 3.9 | 17.44 ± 2.55 | 0.001* |
| Serum IL-8 (pg/ml) | 22.3 ± 3.8 | 14.99 ± 2.88 | 11.23 ± 1.3 | 0.008* |

SD, standard deviation; ACE, angiotensin-converting enzyme; IL-6, interleukin-6; IL-8 interleukin -8; N, Number; * Significance between healthy Subjects and psoriatic patients (P < 0.05).

Our results showed that D/D genotype was more common with severe psoriasis vulgaris (52.2%). On the other side, I/D was more frequent in Moderate psoriasis vulgaris and Mild psoriasis vulgaris patients (50% and 58.3% respectively) (Table 6).

**Table 6: Comparison of ACE genotypes and variable clinical types of psoriasis in our patients**

| Parameters | I/I (N, %) | I/D (N, %) | D/D (N, %) | P-value |
|---|---|---|---|---|
| Severe psoriasis vulgaris (N = 23) | 5 (21.7%) | 6 (26.1 %) | 12 (52.2 %) | 0.001* |
| Moderate psoriasis vulgaris (N = 26) | 6 (23.1%) | 13 (50%) | 7 (26.9%) | 0.001* |
| Mild psoriasis vulgaris (N = 24) | 7(29.1%) | 14 (58.3%) | 3 (12.6%) | 0.001* |

*Significance between healthy Subjects and psoriatic patients (P < 0.05).

## Discussion

The present study provides a relationship between ACE I/D polymorphism, IL-6, IL-8 and psoriasis as a risk factor was determined.

Our results indicated that psoriasis patients have serum IL-6 and serums IL-8 were significantly higher than in controls (P < 0.001). Our results are in line with several studies [22] [23] [24] they reported that elevated levels of serum IL-6 and serum IL-8 in psoriasis vulgaris patients.

The results of the current study indicated that the ACE polymorphism of the I/I genotype was more frequent in controls (46.8%) than in psoriasis patients (24.7%), while the I/D and the D/D genotypes were more abundant in psoriasis vulgaris patients (45.2% and 30.1% respectively). However, this difference was statistically not highly significant, and this can be explained by the small sample size of our study. Also, we observed that DD genotype was more common in patients with severe psoriasis vulgaris (52.2%) than those with intermediate (26.9%) and mild (12.6%) disease. These results are consistent with Song et al., 2015 who revealed that frequency of DD and ID in case more than II [25].

Our results revealed that D allele was significantly higher in the psoriasis patients (52.7%) than in controls (35.1%). These results were consistent with Min Huang et al., 2017. Who found that the D allele was frequency higher in psoriasis patients than in the controls (43.8% and 31.8% respectively) [26].

Multiple studies have been performed to detect the polymorphisms as an important factor in the development of psoriasis: *TNF α* gene -238G/A

polymorphism [27] -2518 A/G *MCP-1* and -403 G/A *RANTES* promoter gene polymorphisms [28], *CARD14 rs11652075* polymorphism [29] and support the genetic hypothesis in psoriasis.

Our findings showed that a significant relationship between the serum ACE levels in patients and healthy subjects (P < 0.001). Moreover, we showed a significant difference of the serum ACE levels with the different *ACE* genotypes; psoriasis patients with *ACE* DD genotype showed the highest mean ACE serum level (28.07 ± 3.21), while patients with *ACE* II genotype had the lowest mean serum ACE level (16.88 ± 4.22). In the present study, we observed that DD was associated with increased levels of IL-6 and IL-8 (47.22 ± 2.5 and 28.32 ± 4.1 respectively). Also, comparison of biochemical parameters and variable clinical types of psoriasis in our patients showed increased levels of all variables in patients with severe psoriasis vulgaris compared to those with Moderate psoriasis vulgaris and mild psoriasis vulgaris.

Increased levels of serum ACE, IL-6 and IL-8 in psoriasis patients were due to the important role of ACE in inflammation. Where ACE converts Ang I into Ang II and inactivates bradykinin [30]. Ang II activates cytokines like IL-6 and IL-8, thus exerting proinflammatory effects [6]. This indicates an important role of ACE in the pathogenesis of psoriasis [31].

In conclusion, *ACE* gene polymorphism might confer susceptibility to the development psoriasis.

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***Clinical Science***

# Comparison of Effect of Leukotriene Biosynthesis Blockers and Inhibitors of Phosphodiesterase Enzyme in Patients with Bronchial Hyperreactivity

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**Keywords:** Bronchial asthma; Zileuton; Diprophylline and tiotropium bromide

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**Abstract**

**AIM:** Blocking effect of leukotriene biosynthesis–zileuton and blocking the effect of phosphodiesterase enzyme–diprophylline in the treatment of patients with bronchial asthma and bronchial increased reactivity, and tiotropium bromide as an antagonist of the muscarinic receptor studied in this work.

**METHODS:** Parameters of the lung function are determined with Body plethysmography. The resistance of the airways (Raw) was registered and measured was intrathoracic gas volume (ITGV), and specific resistance (SRaw) was also calculated. For the research, administered was zileuton (tabl. 600 mg) and diprophylline (tabl. 150 mg).

**RESULTS:** Two days after in-house administration of leukotriene biosynthesis blocker–zileuton (4 x 600 mg orally), on the day 3 initial values of patients measured and afterwards administered 1 tablet of zileuton, and again measured was Raw and ITGV, after 60, 90 and 120 min. and calculated was SRaw; ($p < 0.01$). Diprophylline administered 7 days at home in a dose of (2 x 150 mg orally), on the day 8 to same patients administered 1 tablet of diprophylline, and performed measurements of Raw, ITGV, after 60, 90 and 120 min, and calculated the SRaw ($p < 0.05$). Treatment of the control group with tiotropium bromide - antagonist of the muscarinic receptor (2 inh. x 0.18 mcg), is effective in removal of the increased bronchomotor tonus, by also causing the significant decrease of the resistance (Raw), respectively of the specific resistance (SRaw), ($p < 0.05$).

**CONCLUSION:** Effect of zileuton in blocking of leukotriene biosynthesis is not immediate after oral administration, but the effect seen on the third day of cys-LTs' inhibition, and leukotriene B4 (LTB4) and A4 (LTA4) in patients with bronchial reactivity and bronchial asthma, which is expressed with a high significance, ($p < 0.01$). Blockage of phosphodiesterase enzyme–diprophylline decreases the bronchial reactivity, which is expressed with a moderate significance, ($p < 0.05$).

## Introduction

Hyperactivity of the airways is manifested with acute bronchoconstriction. Recent studies indicate that blocking the effect of leukotriene biosynthesis is efficient in the treatment of hyperactivity and bronchial asthma. Effect of this medication is about the treatment of slight and moderate forms of bronchial asthma [1]. Effects of cys-LTs' appears not only because of the activation of cys-LT1 receptor.But, also through cys-LTs' which trigger the vascular smooth muscle contraction [2] and stimulate activity of the P-selectin produced by endothelial cells through receptor LT2 [3].

Inhibitors of 5-lipoxygenase (5LO) and of the protein which activate 5LO (5-lipoxygenase activating protein–FLAP) such as GSK-2190915 (FLAP-inhibitor) acts not only in cysteinyl-leukotrienes but also inhibits the creation of LTB4, which can be useful at neutrophile asthma [4]. It also inhibits the early and late asthmatic reaction during provoking test inhalator allergen. It also decreases the number of $LTB_4$ eosinophils in sputum.

Most of the authors agree that medicines which block the biosynthesis of leukotriene are the first line of asthma therapy, as an efficient alternative for reduction of inhaling doses of corticosteroids [5].

In the latest medical literature, highest importance is attributed to the caffeine, namely its capability to block the adenosine receptor. Adenosine receptor act through G-protein and that is why the possibility of new synthesis and their introduction to the specific blocker therapy is intensively studied, more powerful to these receptors. Adenosine causes contraction of airways smooth muscles and increases the release of histamine by mastocyte [6].

In the isolated preparation of frenicus-diaphragm, caffeine and theophylline increase the contractility of the diaphragm during the direct and indirect stimulation. In the isolated preparation of frenicus-diaphragm, methylxanthines cause the fatigue to be removed. Also, caffeine and theophylline remove the tiredness of the diaphragm during chronic obstructive pulmonary diseases (COPD). Deemed that this effect causes the removal of dyspnoea at the severe airways obstruction [7].

Inhibitors of phosphodiesterase such as diprophylline block the synthesis and secretion of inflammatory mediators from many types of cells including mastocytes and basophils. This effect of diprophylline may be caused as consequence of PDE inhibition and can be imitated in largest part with medicines that selectively inhibit the isoenzyme PDE4 [8]. In therapeutic concentration, therapeutic effects of diprophylline are related more with its anti-inflammatory effect rather than with bronchodilation effect. However, this remains to be proved.

Administration of these medicines to patient leads to the improvement of symptoms, lung function parameters, reduce of medicines usage, less aggravated breathing through the night, respectively improvement in all parameters necessary in the disease control process. They are also administered concomitantly with other antiasthmatics, such: agonists beta$_2$-adrenergic receptor, corticosteroids, and antagonists of the muscarinic receptor, to which they have synergist and additive effect [9] [10].

Inhibitors of phosphodiesterase 4 (PDE4) have an immune modulatory effect. In the treatment of severe asthma, necessary are many doses which cannot be applied due to side effects to the gastrointestinal tract [11].

Inhalation forms of the medicines can improve these deficiencies and their therapeutic index [12].

Blocking effect of leukotriene biosynthesis – zileuton, orally administered and blocking the effect of phosphodiesterase enzyme-diprophylline administered in the treatment of patients with bronchial asthma and bronchial increased reactivity, and tiotropium bromide as an antagonist of the muscarinic receptor studied in this work.

## Material and Methods

Examination performed in 14 patients with bronchial asthma and increased bronchial reactivity. At least 48 hours before the research of bronchial reactivity response, patients have not administered any of the bronchodilator substances. Examined were informed regarding the method of the functional pulmonary tests. Patients had asthma, with or without associated bronchitis.

Pulmonary function, composed of measurement of vital capacity (VC), forced expiratory volume in the first second ($FEV_1$), resistance in the airways (Raw) and intrathoracic gas volume (ITGV), was defined at the rest. The overall quantity of the intrathoracic gas volume (ITGV) was measured with the plethysmography method, including non-ventilated closed gas. If the residual functional capacity is taken from the ITGV, obtained by the plethysmography method, we will gain information regarding the quantity of closed gas due to a severe obstruction, cystic lungs, or pneumothorax. In healthy subjects with a normal pulmonary function, the volume of the intrathoracic gas is equal to the residual functional capacity. From the beta and alpha angles, assisted by tables, values of the airways resistance and volume of the intrathoracic gas are calculated. From gained values, the specific resistance was calculated:

$$SRaw = Raw \times ITGV$$

Raw and the SRaw were taken for analyses. Research of the bronchial response to different substances was done with the measurement of Raw and the SRaw as very sensitive indicators.

Basic and pulmonary function features researched provided in Table 1.

**Table 1: Basic and pulmonary function features**

| n | Age (yrs) | Height (cm) | Weight (kg) | VC (%) | $FEV_1$ (%) | Raw (kPa L/s) | ITGV (L) |
|---|---|---|---|---|---|---|---|
| 14 | 45 ± 1.60 | 177.13 ± 1.5 | 71.77±0.5 | 3.41±3.5 | 3.02±1.26 | 0.19.5±0.1 | 3.05±3.05 |

Zileuton, as a blocker of leukotriene receptor (600 mg, tablet) administered per os 2 days in a row at home (4 x 600 mg) and on the 3$^{rd}$ day reported to the ambulance and measured initial values. One tablet administered orally at the ambulance, and measured Raw and ITGV after 60, 90 and 120 min. In the end, as control, administered was tiotropium bromide in the form of an aerosol and a dose of (2 inh. x 18 mcg), and Raw and ITGV values were measured again, and SRaw was calculated.

Diprophylline, as a blocker of leukotriene receptor, administered in-house for 7 days (2 x 150 mg) and on the 8$^{th}$ to the same patient administered 1 tablet of diprophylline, measured Raw and ITGV after 60, 90 and 120 min., with SRaw that was calculated afterwards. As above, the control group was treated

with tiotropium bromide-aerosol (antagonist of the muscarinic receptor), and after 5 minutes of administration, Raw and ITGV values were measured, and SRaw was calculated.

Used was the hypothesis that changes in the respiratory system are not important, not related to the development of bronchial asthma or other obstructive diseases, and not related to allergic manifestation.

Gained results grouped and analysed. Statistic data processing included determination of the average values (X), standard deviation (SD), standard mistake (SEM).

Gained results tested with a t-test to ascertain significant changes in between examined groups. To compare groups, utilised was statistic computer software GraphPadInStat III.

## Results

Results of this research, in patients with bronchial asthma, indicate that patients with increased bronchial reactivity 2 days after in-house administration of zileuton, a blocker of leukotriene biosynthesis, at a dose of (2 x 600 mg) reported to the ambulance and measured initial values.

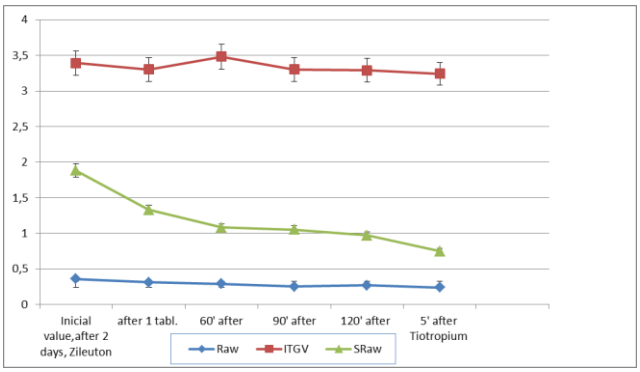


*Figure 1: Effect of zileuton (p < 0.01), (2 x 600 mg, tablet), and tiotropium bromide (p < 0.05), (2 inh. x 18 mcg); in Raw, ITGV and SRaw. 2 days after in-house administration of zileuton (4 x 600 mg); (n = 7; X ± SEM)*

One tablet administered orally and measured the Raw and ITGV again after 60, 90 and 120 min., and because of significant leukotriene biosynthesis inhibition (p < 0.01) reduced was the increased bronchomotor tonus. Diprophylline, as blocker of phosphodiesterase enzyme after in-house administration for 7 days (2 x 150 mg) and on the 8$^{th}$ administered 1 tablet of diprophylline, measured Raw and ITGV after 60, 90 and 120 min., with SRaw that was also calculated; as result of phosphodiesterase enzyme blockage (diprophylline), significantly was reduced the increased bronchomotor tonus (p < 0.05); also, as treatment of the control group with tiotropium bromide (2 inh. X 18 mcg), antagonist of the muscarinic receptor, which is effective in removal of the increased bronchomotor tonus, by causing significant decrease of the resistance (Raw), respectively of the airways specific resistance (SRaw), (p < 0.05).

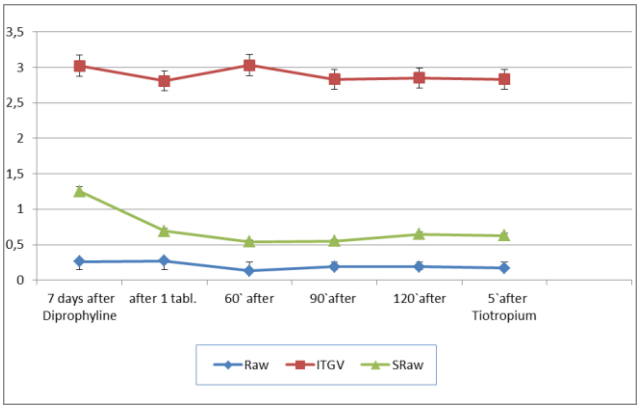


*Figure 2: Effect of diprophylline (p < 0.05), (2 x 150 mg, tablet), and tiotropium bromide (p < 0.05), (2 inh. x 18 mcg); in Raw, ITGV and SRaw. 2 days after in-house administration of diprophylline (n = 7; X ± SEM)*

## Discussion

Clinical trials with blocking medicines of leukotriene biosynthesis were quite heterogeneous in response to the therapy, with patients that can be classified in two groups, those “responding” on the treatment and those “not responding” to it. For patients responding to the treatment with blockers of leukotriene receptor, pulmonology institution has recognised these medicines as an alternative to inhaled steroids, in small doses, to maintain slight chronic asthma under control.

Zileuton, as a blocker of the leukotriene biosynthesis, is a powerful selective inhibitor of the activity of 5-lipoxygenase and as such inhibits the biosynthesis of its products. Consequently, besides inhibition of cys-LTs’**,** zileuton also inhibits the biosynthesis of leukotriene B4 (LTB4), which is a powerful chemotactic and another eicosanoid too, which depend on the synthesis of leukotriene A4 (LTA4). Theoretically**,** therapeutic effects of 5-lipoxygenase should include all those seen at the antagonist cys-LT1, but also other effects which include inhibition of the LTB4 and other products of 5-lipoxygenase. It is deemed that LTB4 acts through receptor LT1 and LT2, by causing accumulation of neutrophils, but their role remains yet unclear [13] [14].

Some clinical trials indicated that blockers of the leukotriene biosynthesis have an affinity in the reduction of the dose of inhaled steroids necessary to control asthma exacerbation [15] [16]. If so, this can

be quite important, especially in children suffering from a more severe asthma.

Blocking effect of leukotriene biosynthesis-zileuton, in the treatment of patients with bronchial asthma and increased bronchial reactivity in comparison to the control group treated with tiopentium bromide applied via inhalation (as an antagonist of the muscarinic receptor) studied in this work. Two days after in-house administration of zileuton, and because of significant blockage of leukotriene biosynthesis ($p < 0.01$) decreased was the increased bronchomotortonus in patients with emphasized reactivity; effect of tiopentium bromide is efficient in removal of increased bronchomotor tonus, causing significant decrease of the resistance (Raw), namely of the specific resistance (SRaw), ($p < 0.05$).

Blockers of the leukotriene biosynthesis at doses applied 2 days after in-house administration of zileuton to the same patient, cause lowering of systolic and diastolic blood pressure (BP), but not significantly ($p > 0.1$) [17].

Blockers of adenosine receptors and inhibitors of PDE can play a role in lung bronchodilation effect. Adenosine does not contract the isolated smooth muscle of human bronchi directly, but when inhaled it acts as powerful bronchoconstrictor at asthmatics [18]. Thus, inhibition of the adenosine function can contribute to the bronchodilation triggered by diprophylline at some asthmatics. Inhibition of PDE4 and PDE5 effectively relaxes the isolated smooth muscle of human bronchi. Thus, seems that inhibition of PDE may contribute to the bronchodilation effect of theophylline. Studies conducted with methylxanthine enprofylline (3-propylxanthine), which is studied a lot for asthma treatment in Europe, supports the role of PDE inhibition in bronchodilation effects of theophylline. Regarding bronchodilation, Enprofylline is more powerful than theophylline but is less powerful at inhibition of largest part of adenosine receptor types. The latter is to be carefully interpreted. Activation of subtype $A_{2B}$ of adenosine receptor causes some pro-inflammatory effects, and both theophylline and enprofylline are powerful competitive antagonists of the $A_{2B}$ adenosine receptor [19] [20].

Selective inhibitors of PDE4 are assessed in various clinical trials in asthma treatment and chronic obstructive pulmonary disease (COPD). In a study, cilomilast (Ariflo 15 mg two times a day for 10 weeks) significantly reduced infiltration of inflammatory cells, which is seen in the bronchial biopsies of patients with COPD. Further studies are necessary to determine the role of PDE4 inhibitors in asthma and COPD, but these medicines are promissory regarding new approaches to asthma therapy [21].

Our research indicates that due to result of inhibition of phosphodiesterase (doxofylline) reduced was the significantly increased bronchomotor tonus ($p<0.05$)**;** also, as treatment of the control group with tiotropium bromide (antagonist of the muscarinic receptor), which is effective in removal of the increased bronchomotor tonus, by causing significant decrease of the resistance (Raw), respectively of the airways specific resistance (SRaw), ($p < 0.05$).

Further studies are necessary to determine the role of these medicines in moderated and severe asthma. Some clinical trials have shown that blockers of the leukotriene biosynthesis possess an affinity to reduce doses of inhaled steroids necessary to control asthma exacerbations [22]. Even though leukotriene inhibitors are efficient in the prophylactic treatment of slight asthma; their role in the asthma therapy is not defined. Most of the clinical trials with these medicines studied at the patients with slight asthma, who do not administer glucocorticoids. In general, studies show a modest, but important improvement to the pulmonary function and a decrease of symptoms and asthma exacerbation.

Based on results gained, it can be concluded as follows:

In patients with bronchial asthma and emphasised bronchial reactivity, an orally administered blocker of leukotriene biosynthesis-zileuton, 2 times a day (4 x 600 mg), causes emphasised and a significant decrease of the specific resistance (SRaw) of airways, ($p < 0.01$).

Blocker of phosphodiesterase enzyme-diprophylline, orally administered for 7 days in a row at doses of 2 x 150 mg, also causes a significant decrease of the specific resistance (SRaw) of airways, ($p < 0.05$).

Treatment of the control group with tiotropium bromide-antagonist of the muscarinic receptor (2 inh. x 0.18 mcg), is effective in removal of the increased bronchomotor tonus, by causing the significant decrease of the resistance (Raw), respectively of the specific resistance (SRaw), ($p < 0.05$).

Effect of a blocker of leukotriene biosynthesis – zileuton, applied to patients with reversible bronchial reactivity, is powerful only after two days of the intake, causing cys-LTs' inhibition, and blocking of leukotriene B4 (LTB4) and A4 (LTA4) biosynthesis, ($p < 0.01$).

Results, also indicate that blocker of phosphodiesterase enzyme significantly blocks connection to the adenosine receptor ($p < 0.05$), but their effect is weaker in the respiratory system rather that of the leukotriene biosynthesis blockers.

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***Clinical Science***

# Socio-Demographic Characteristics of the Patients with a Post Stroke Depression from the Municipality of Tetovo, Republic of Macedonia

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**Keywords:** PSD; Age; Education; Gender; Nationality; Occupational status

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**Abstract**

**BACKGROUND:** Although post-stroke depression (PSD) is the most common neuro-psychiatric consequence after a stroke there is still some obscurity regarding its aetiology and risk factors, which complicates its management. A better knowledge of the predictors will enable better prevention and treatment.

**AIM:** The aim of this work was the identification of the risk factors for PSD, typical for the Macedonian population, which will help in early prediction, timely diagnosis and treatment of the disease.

**MATERIALS AND METHODS:** We carried out a prospective study at the Clinical Hospital in Tetovo, the Republic of Macedonia to determine the prevalence of PSD and to analyse the socio-demographic characteristics as possible risk factors in 100 patients on discharge and after 5 months. The depression symptoms were quantified using the Hamilton Depression Ranking Scale (HAM-d) and the Geriatric Depression Scale (GDS).

**RESULTS:** The average age of the patients with PSD on the first examination is 65.0 ± 8.3, whereas on the second examination is 64.5 ± 9.2. According to the Mann-Whitney U test, the difference between the average ages on both examinations is statistically insignificant for $p > 0.05$. On both examinations, the statistically significant dependence of $p > 0.05$ between PSD and the occupational status and PSD and education is not recorded. On both examinations, the PSD in male patients was 78.0% and 62.7%, while in female patients it was 85.4% and 68.3% not recording the statistically significant dependence of $p < 0.05$ between PSD and the gender.

**CONCLUSION:** The socio-demographic characteristics of the patients with PSD cannot be considered as predictors of the disease.

## Introduction

Post-stroke depression (PSD) is the most common complication after stroke with a negative effect on the patient's rehabilitation [1]. The epidemiological studies report a widely variable prevalence of PSD which ranges from 10-64% of patients that suffered a stroke [2] [3] [4] [5] [6] [7]. Some studies indicate a highest PSD prevalence in the first 3-6 months after the stroke with a gradual decline after the first year following the stroke. This is an early reactive stage of PSD. The depression that occurs later, after the 6$^{th}$ month, is considered a late stage of PSD [8].

In clinical practice, the disease is frequently underdiagnosed and untimely treated.

So far in scientific findings, the etiopathogenesis of the disease has been described as multifactorial. The epidemiological characteristics point out that PSD is a disease of the older population and occurs more frequently in and after the sixth decade of life, more often in the male sex [2] [9]. Most commonly mentioned risk factors are: functional disability and a severe stroke, often localised in the left orbitofrontal cortex, disturbing the neurotransmitting circles between the cortex and the basal ganglia [3] [10] [11].

As a result, to all of this, there was a need to research identification of the risk factors for PSD, typical for the Macedonian population, which will help in early prediction, timely diagnosis and treatment of the disease.

## Material and Methods

We carried out a prospective, longitudinal, epidemiological study to identify the prevalence and the risk factors for PSD on the discharge form the hospital and after 5 months following the stroke. The study was carried out at the Department of Neurology at the Clinical Hospital in Tetovo, Republic of Macedonia. The study included all the patients, which fulfilled the inclusion criteria, clinically treated at the department due to an acute stroke, clinically verified and confirmed by computed tomography of the brain in the period from 1st September 2016 to 28th February 2017. Inclusion criteria: normal Mini-Mental Score according to the patient's education, maintained verbal communication ability, maintained sensorium, age ≤75. The study did not include patients with another comorbidity that seriously disturbed the general somatic condition and patients that were previously diagnosed with a psychiatric disorder. All the patients gave informed consent previously approved by the Ethical committee of the Clinical Hospital.

Quantification of all the depression symptoms was done on all the patients using the Hamilton Depression Rating Scale (HAM-d). The Geriatric Depression Scale (GDS) was additionally used for self-evaluation of the patients at age ≥65. According to the results, the patients were divided into two groups, with and without PSD on discharge and after 5 months. The socio-demographic characteristics were collected from the hospital's documentation and interviews with the patients and their relatives. The study included 100 patients, 97 of those were monitored 5 months and 3 deaths were recorded.

**The Hamilton Depression Rating Scale (HAM-D**) for quantification of depression symptoms, a form that is consisted of 21 questions. They provide answers for a potential existence of typical or atypical depression symptoms, according to the ICD-10, with a multiple choice possibility out of 4. For the study, we used an official Macedonian translation from the Psychiatric clinic in Skopje. The scale score provides a ranking of the subject in one of the following groups:

- 0-7 normal;
- 7-13 mild depression;
- 14-18 moderate depression;
- 19-22 severe depression;
- >23 very severe depression.

**The Geriatric Depression Scale (GDS)** is a scale for self-evaluation of the depression among the geriatric population, ≥65 years of age. It consists of 30 questions, which refer to the daily mood, attitudes and feelings, answered by the patient or with the help of the examiner by choosing "yes" or "no". An official translation of the scale by the Ministry of health was used in the study. The scale score provides ranking in one of the following groups:

- 0-10 normal;
- 11-20 mild depressives;
- 21-30 severe depressives.

The statistical analysis was done with statistical software: STATISTICA 7.1; SPSS 17.0, using the following statistical methods: difference test, average and standard deviation, Mann-Whitney U test, Person correlation coefficient (r) and $\chi 2$ test. A statistical significance level of 0.05 (p) is defined as a confidence interval (95% CI).

## Results

On the first examination, PSD was diagnosed in 81.0% of the patients, while on the second examination 65.0% of the patients had PSD, the percentage difference is statistically significant for $p < 0.05$ ($p = 0.0108$ Difference test) (Table 1). According to the Index of dynamics, there is a decreasing rate of PSD by 19.8%.

**Table 1: Patients with PSD**

| PSD/control | First | | Second | |
|---|---|---|---|---|
| | N° | % | N° | % |
| Without | 19 | 19.1 | 32 | 32.0 |
| With | 81 | 81.0 | 65 | 65.0 |
| Lethal outcome | 0 | 0.0 | 3 | 3.0 |

Using HAM-D, a normal score was recorded in 19.0% of the patients, while in 81.0% a post-stroke depression was recorded. In the majority of the patients, 55%, mild depression is recorded, followed by 14% with moderate depression, 11.0% with severe depression and 1.0% with very severe depression (Table 2). The percentage difference between the mild depression versus the rest of the depression modalities is statistically significant for $p < 0.05$ ($p = 0.0000$, Difference test).

Using the Geriatric Depression Scale-GDS, on the first examination, a normal score was recorded in 34.6% of the patients' ≥ 65 years old. PSD is recorded in 65.4% of the patients ≥ 65 years old. In 50.9% a mild depression was recorded.

**Table 2: Hamilton Depression Ranking Scale**

| Finding / control | First | | Second | |
|---|---|---|---|---|
| | N° | % | N° | % |
| 0-7 normal | 19 | 19.0 | 32 | 32.0 |
| 8-13 (mild depressive reaction) | 55 | 55.0 | 43 | 43.0 |
| 14-18 (moderate depression) | 14 | 14.0 | 16 | 16.0 |
| 19-22 (severe depression) | 11 | 11.0 | 5 | 5.0 |
| >23 (very severe depression) | 1 | 1.0 | 1 | 1.0 |
| Lethal outcome | 0 | | 3 | 3.0 |
| Total | 100 | 100.0 | 100 | 100.0 |

The percentage difference between the normal score and the mild depression versus the severe depression is statistically significant for p <0.05 (p = 0.01, Difference test). On the second examination, using the GDS, a normal score is recorded in 49.1% of the patients, while 50.9% of the ≥65-year-old patients showed PSD. Mild depression is mostly recorded-32.7%. The percentage difference between the normal score and the mild depression versus the severe depression is statistically significant for p < 0.05 (p = 0.00, Difference test).

**Table 3: The Geriatric Depression Scale-GDS**

| Finding / control | First | | Second | |
|---|---|---|---|---|
| | N° | % | N° | % |
| 0-10 normal | 19 | 34.6 | 27 | 49.1 |
| 11-20 (mild depression) | 28 | 50.9 | 18 | 32.7 |
| 21-30 (moderate depression) | 8 | 14.5 | 9 | 16.44 |
| Lethal outcome | 0 | 0.0 | 1 | 1.8 |
| Total | 55 | 100.0 | 55 | 100.0 |

The average age of the patients from the study is 64.8 ± 9.0, ranging from 32 to 75 years old (Table 4).

**Table 4: The average age of the patients**

| | N° | Average | Minimum | maximum | Std.Dev |
|---|---|---|---|---|---|
| *AGE* | 100 | 64.8 | 32.0 | 75.0 | 8.997328 |

The average age of the patients with PSD on the first examination is 65.0 ± 8.3, whereas on the second it is 64.5 ± 9.2. The average age of the patients without PSD on the first examination is 63.7 ± 11.6, while on the second it is 65.5 ± 8.7 (Table 5). According to the Mann-Whitney U test the difference between the average age on both examinations is statistically insignificant for p > 0.05 (U = 767.5, Z = 0.013180, p = 0.989485; U = 967.5, Z = 0.552431, p = 0.580653).

**Table 5: The average age of the patients with or without PSD**

| First | Average - psd | Without – avg. | N-psd | Without - n | Std.dev. - psd | Without - std.dev |
|---|---|---|---|---|---|---|
| | 65.0 | 63.7 | 81 | 19 | 8.341163 | 11.60384 |
| Second | Average - psd | Without – avg. | N-psd | Without - n | Std.dev. - psd | Without - std.dev |
| | 64.5 | 65.5 | 65 | 32 | 9.236607 | 8.710336 |

In the investigated group 59.0% of the patients are men and 41.0% are women. The percentage difference is statistically significant for p < 0.05 (p = 0.0117, Difference test).

**Table 6: The presence and absence of PSD about gender**

| Control/gender/PSD | First | | Second | |
|---|---|---|---|---|
| | Without | With | Without | With |
| Male | 13 | 46 | 21 | 37 |
| Female | 6 | 35 | 11 | 28 |
| Total | 19 | 81 | 32 | 65 |

In male patients, PSD was diagnosed on both examinations: 78.0% and 62.7% of the patients. In female patients, PSD was diagnosed in 85.4% on the first examination and in 68.3% on the second examination.

On both examinations a statistically significant dependence of p > 0.05 between PSD and the gender is not recorded (Pearson Chi-square: 0.860660, p = 0.353556; Pearson Chi-square: 0.675418, p = 0.411171) (Table 6).

On both examinations a statistically significant dependence of p > 0.05 is not recorded between PSD and the occupational status (Pearson Chi-square: 1.09193, p = 0.579281; Pearson Chi-square: 0.710394, p = 0.701035) (Table 7). The highest percentage of PSD is recorded among unemployed patients on both examinations (43.2% and 43.1%), followed by retired patients (34.6% and 33.8%) and employed patients (22.2% and 23.1%). The percentage difference is statistically insignificant.

**Table 7: Number of patients with or without PSD about the occupational status**

| Control/working status/PSD | First | | Second | |
|---|---|---|---|---|
| | Without | With | Without | With |
| Retiree | 7 | 28 | 13 | 22 |
| Employee | 6 | 18 | 8 | 15 |
| Unemployed | 6 | 35 | 11 | 28 |
| Total | 19 | 81 | 32 | 65 |

On both examinations, a statistically significant dependence of p>0.05 between PSD and education is not recorded (Pearson Chi-square: 3.38223, p=.336360; Pearson Chi-square: 2.53304, p=.469350) (Table 7). The highest percentage of PSD is diagnosed in patients who completed high school, on examinations, (44.4% and 40.0%), followed by those who completed elementary education (37.0% and 41.5%), illiterates (12.3% on both examinations) and higher education-6.2%. The percentage difference is statistically insignificant.

**Table 8: Number of patients with or without PSD about the education**

| Control/education/PSD | First | | Second | |
|---|---|---|---|---|
| | Without | With | Without | With |
| Illiterate | 5 | 10 | 6 | 8 |
| Primary | 7 | 30 | 9 | 27 |
| Secondary | 7 | 36 | 16 | 26 |
| High | 0 | 5 | 1 | 4 |
| Total | 19 | 81 | 32 | 65 |

More than half of the patients, 53.0%, have two children and the range is from 1 to 8 (Table 9).

**Table 9: Number of children of the patients**

| Number of children | N$^0$ | % |
|---|---|---|
| 1 | 2 | 2.0 |
| 2 | 53 | 53.0 |
| 3 | 21 | 21.0 |
| 4 | 10 | 10.0 |
| 5 | 3 | 3.0 |
| 6 | 3 | 3.0 |
| 7 | 1 | 1.0 |
| 8 | 1 | 1.0 |
| No children | 1 | 1.0 |
| Total | 95 | 100.0 |

## Discussion

Our study confirmed a high prevalence of early-stage PSD with a decreasing rate after 5 months according to both scales that were applied. Compared to the results from previous scientific research the prevalence of early stage PSD is higher, but it presents same dynamics [1] [8].

The risk for depression in the general population increases with age [2] [9]. The average age of the patients is 64.8, which confirms that stroke is a condition with a higher risk after the sixth decade. However, the results do not point outage as a statistically significant risk factor for the occurrence of PSD.

According to our study male patients carry a higher risk of stroke, yet gender did not present itself as a statistically significant risk factor for PSD.

The research sample in our study can be regarded as representative considering the specific structure of the population in the municipality of Tetovo. Namely, 74% of the patients were Albanian, while 26% were Macedonian which corresponds with the nationality structure in the municipality. Their level of education and occupational status did not show a statistically significant risk factors for PSD, even though they are important for the poor health culture and bad quality of life.

In conclusion, the socio-demographic characteristics of the patients are not a significant risk factor for PSD. There are some other significant factors that contribute to the occurrence of the disease whose definition requires additional studies.

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# Evaluation of the Efficacy of Combined Therapy of Methotrexate and Etanercept versus Methotrexate as a Mono-Therapy

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**Abstract**

**AIM:** This study aims to evaluate the efficacy of Methotrexate (MTX) alone and combined therapy with Etanercept (ETN) and Methotrexate in patients with active rheumatoid arthritis (RA).

**METHODS:** In the randomised control study, conducted in the period from March 2014 until March 2016, we evaluated the efficacy of the treatment of patients with RA with MTX as monotherapy and combination treatment with MTX and ETN. In the Clinic of Rheumatology in Prishtina, 90 adult patients with RA were treated in combination with ETN (doses of 50 mg subcutaneously/weekly), with oral MTX (doses up to 20 mg weekly), and MTX alone (doses up to 20 mg weekly) during this period of two years. Clinical response was assessed using European League against Rheumatism (EULAR)/American College of Rheumatology (ACR) Criteria and the Disease Activity Score (DAS28). Radiographic changes were measured in the beginning and at the end of the study using Larsen's method.

**RESULTS:** Of the cohort groups of 90 patients, mean age of 55.63, 15 patients, (16.6 %) were treated with combined therapy (ETN plus MTX) and 75 patients (83.3%) with monotherapy (MTX). After two years of treatment the group with combined therapy resulted with improvement of acute phase reactants as erythrocyte sedimentation rate (ESR) for the first hour (41.1 vs. 10.3 mm/hour) and C - reactive protein (CRP) (40.8 vs. 6 mg/liter), and compared to the group treated with monotherapy, there were no significant changes (ESR: 45.7 vs 34.3 mm/hour; CRP: 48 vs 24 mg/liter). Before the treatment, the severity of the disease was high, wherein the group with combined therapy DAS28 was 5.32, compared to the monotherapy group whom DAS28 was 5.90. After 2 years of treatment, we had significant changes in the results of DAS28, wherein the group treated with ETN plus MTX DAS28 was 2.12 ± 0.15, while in the group of patients treated with MTX DAS28 were 3.75 ± 0.39 ($t = 13.03$; $df = 58$; $p < 0.0001$). The group with combined therapy showed no evidence of radiographic progression comparing to the group of patients with monotherapy.

**CONCLUSIONS:** Based on our results achieved during 2 years we can conclude that ETN in combination with MTX reduced disease activity, slowed radiographic progression and improved clinical manifestations more effectively than MTX alone. No serious adverse events were noticed in the group with combination treatment.

## Introduction

Rheumatoid arthritis (RA) is an autoimmune disorder with unknown aetiology, characterised by symmetric, erosive synovitis and, sometimes multisystem involvement. The prevalence is 1 - 2% worldwide. Both, incidence and prevalence of rheumatoid arthritis, are two to three times higher in women than in men [1] [2]. The treatment goal is achieving the lowest level of activity of the disease and longest remission, minimization of the joint damage, maintaining physical function and quality of life. Treatment of RA contains a program that includes medical, social and emotional support for the patients. ETN is effective in reducing the signs and symptoms of RA, as well as in slowing or halting the radiographic damage when used either as a monotherapy or in combination with MTX. ETN binds TNF in circulation and in the joint, preventing interaction with cell surface TNF receptors, thereby reducing TNF activity [3] [4] [5].

This study aims to evaluate the efficacy of Methotrexate (MTX) as a monotherapy versus combined therapy with Etanercept (ETN) and Methotrexate (MTX), in patients with active rheumatoid arthritis (RA).

## Patients and Methods

In this randomised controlled study conducted during a period of two years, from March 2014 to March 2016, we have evaluated the efficacy of treatment of patients with RA treated with MTX alone and the combination of MTX and ETN.

Patients were diagnosed with RA fulfilling criteria of European League against Rheumatism (EULAR)/American College of Rheumatology (ACR) and disease duration of at least one year [6]. Patients included in the study were treated with DMARDs such as Sulfasalazine and Hydroxychloroquine and they did not have a satisfactory response to the therapy. The youngest patient included in the study was aged 29 years, with function class I - III (ACR), with 8 swollen and 10 painful joints; erythrocyte sedimentation rate (ESR) of 25 mm/hour, C - reactive protein (CRP) level of 12 mg/liter, and morning stiffness more than 30 minutes. Pain on a visual analog scale (VAS: 0 - 100 mm) was evaluated for each patient, clinical response was assessed using American College of Rheumatology (ACR) criteria for 20% improvement ACR20, the ACR50 and the ACR70 in RA, and the Disease Activity Score in 28 joints (DAS28) [7][8][9]. Patients with a history of previous hepatitis, tuberculosis and active infectious disease were excluded from the study.

Study protocol Included patients treated in combination with ETN and MTX (doses of 50 mg subcutaneously weekly), oral MTX (doses up to 20 mg weekly), and alone MTX (doses up to 20 mg weekly) in two years, in the Clinic for Rheumatology in Prishtina. Patients received a low dose of corticosteroids at the beginning of the treatment during 3 months (initial dose of 20 mg and the maintenance dose was 7.5 mg). Radiographic changes were measured at the beginning and the end of the study with Larsen's methodology 1995, using a scoring system that attributes 0 to 5 points of each synovial joint evaluated on a radiograph [10] [11]. Safety assessments were based on reported adverse events, laboratory tests and physical routine examinations. The study was approved by the Ethical Committee of the University Clinical Center of Kosovo, and a written informed consent has been obtained from each patient before he/she entered the study.

All data were expressed as the mean ± standard deviation (SD) and percentages. The significant difference from each group was analysed by t-test of proportion, Superman's Correlation-test for calculation the radiographic changes. Statistical analyses were performed using the statistical SPSS. Significance was set up at $p < 0.05$.

## Results

Out of the total number of 90 patients included in the study, 15 were males, and 75 were females, with a mean age of 55.64 years. Fifteen patients or 16.6 % were treated with combined therapy (ETN plus MTX) and 75 patients or 83.3% with monotherapy (MTX). The group of combined therapy showed improvement of acute phase reactants compared to the group treated with MTX alone. ESR (normal values < 10 mm/hour), of the first group (ETN plus MTX) in the first hour of ESR was 41.1 vs. 10.3 mm/hour and CRP (normal value < 6 mg/liter) was 40.8 vs. 6 mg/liter ($p = 0.001$) compared to the second (MTX alone) showed no significant changes (ESR: 45.7 vs 34.3 mm/hour; CRP: 48 vs 24 mg/liter), $p = 0.17$.

Patients treated with combined therapy achieved ACR20, ACR50 and ACR70 better response rather than patients treated with monotherapy (MTX). ACR20 responses were achieved at the level of 90% in patients with combined therapy group (ETN plus MTX) vs 84% of the monotherapy group (MTX) $p = 0.63$. ACR50 responses were achieved by 70% of the combination therapy group vs 46% of the monotherapy group $p = 0.17$. ACR70 responses were achieved by 40% of the combination group vs 16% of the monotherapy group $p = 0.089$. The severity of the disease, measured before treatment by DAS Score was high. The group that was treated with combined therapy (ETN plus MTX) had DAS28 of 5.32, whereas the group with monotherapy of MTX had DAS28 of 5.90. After 2 years of treatment, we had significant changes in the results of DAS28. The group treated with ETN plus MTX had DAS28 of 2.12 ± 0.15, while the group of patients treated with MTX had DAS28 of 3.75 ± 0.39 ($t = 13.03$; $df = 58$; $p < 0.0001$) (Table 1).

**Table 1: Characteristics of treatment of RA with Methotrexate and combined therapy with Etanercept and Methotrexate**

| | Methotrexate (MTX) | Etanercept and Methotrexate (ETN + MTX) | T-test of proportion t-test p-value |
|---|---|---|---|
| | N (%) | N (%) | |
| | 75 (83.3%) | 15 (16.6%) | |
| Female | 63 (84%) | 9 (60%) | |
| Male | 12 (16%) | 6 (40%) | |
| American College of Rheumatology (ACR) criteria | | | |
| ACR20 | 84% | 90% | t=0.49; df=58; p=0.63 |
| ACR50 | 46% | 70% | t=1.39; df=58; p=0.17 |
| ACR70 | 16% | 40% | t=1.73; df=58; p=0.089 |
| Disease Activity Score (DAS28) | | | |
| Before treatment | 5.9 ± 0.49 | 5.32 ± 0.26 | |
| After treatment | 3.75 ± 0.39 | 2.12 ± 0.15 | t=13.03; df=58; p<0.0001 |

Radiography of hands and feet were done at the beginning and the end of the study. The scoring was done by total Larsen's score in 32 joints (total Larsen score range is 0 - 160). Mean value of total Larsen's score in the first group of patients, treated with ETN + MTX at the beginning of the study was 2.58, whereas at the end of the study was 3.54. In the second group of patients treated with MTX, at the beginning of the study, the mean value of Larsen's score was 2.84 whereas at 24 months 3.95. According to the Larsen's method, in the group of combined therapy showed no evidence of radiographic progression comparing to the group of monotherapy ($p < 0.010$).

During the treatment, in the group of patients with monotherapy 8 cases were reported for transitory adverse effects such as nausea, vomiting, and gastrointestinal ache. In the group of combined therapy, there is no evidence of serious adverse events, infectious or noninfectious that was noticed. During the study, none deaths were reported.

## Discussion

Our study is based on the comparison of results from the treatment of RA with combined therapy (ETN plus MTX) and monotherapy (MTX). Results confirm the significant advantage of combined therapy over monotherapy. DMARD medications are utilised much earlier in the treatment of RA, due to their efficacy in retarding erosive damages. Biologics produce rapid and sustained amelioration of the signs and symptoms of RA, retard radiological progression, and improve quality of life more effectively than DMARDs [12].

Our study showed that combined therapy had a statistically significant benefit compared to the monotherapy for ACR20, ACR50 and ACR70 response. It also shows the significant advantage of combined therapy over monotherapy in controlling the disease activity measured by DAS28 Score. DAS28 was obviously lower in the group treated with combined therapy compared to the group treated with monotherapy.

Different authors showed the improvement of RA using combined therapy (ETN + MTX), e.g. Weinblatt ME et al., (1999). A trial of Etanercept, a recombinant tumour necrosis factor receptor: Fc fusion protein, in patients with rheumatoid arthritis receiving MTX. The authors show that in 89 RA's, patients treated with ETN showed more efficacy of ETN when used a combination treatment with MTX over 6 months of treatment. Our results are similar to recent studies [13].

The reason we conducted this study was to improve the care of our patients. Our study confirmed that combined therapy had given better improvement. Clinical studies suggest that etanercept is safe and effective as a long-term therapy for the treatment of RA, and the risk and benefit ratio of continuous etanercept treatment remains beneficial [14] [15] [16].

Limitations of the study include the small number of patients in the group of combined therapy, because of the economic limitation conditions of applying etanercept to other patients with RA. Furthermore, studies with higher number of patients need to be done.

According to our results, we can conclude that etanercept in combination with methotrexate reduced disease activity, slowed radiographic progression and improved clinical manifestations more effectively than methotrexate alone within 2 years. During the treatment, no serious adverse events were noticed with a combination treatment of etanercept and methotrexate.

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# *IGF1R* Gene Alterations in Small for Gestational Age (SGA) Children

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**Keywords:** SGA born children; Microcephaly; Short stature; Exon 2 of IGF1R gene; Gene analysis

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**Abstract**

**BACKGROUND:** Small for gestational age children (SGA) is born on term with BW and or BL of -2.0 standard deviation score (SDS). SGA children have an increased risk of being short, developing DM, and cardiovascular and cerebrovascular disease. Often defects of *IGF1R* are the cause of SGA. Most frequently affected part of the IGF1R gene is the exon 2.

**AIM:** To investigate whether the exon 2 of the *IGF1R* gene is affected in the SGA children.

**PATIENTS AND METHODS:** A cohort of 100 SGA children born in term was evaluated for alterations in *IG1R* gene. Their anthropometric parameters, IGF1 serum concentrations and IGF1 SDS values were analysed. The molecular analysis of IGF1R gene was performed by PCR restriction-site analysis and followed by direct sequencing of conspicuous fragments.

**RESULTS:** Within our cohort, 64 SGA children were with short stature (height SDS -3.25 ± 0.90 SDS), and 36 were with normal height for their age and sex, (H SDS was 0.20 ± 1.1 SDS). None of these children had microcephaly (occipitofrontal circumference -0.70 ± 1.01 SDS vs 0.06 ± 0.56 SDS in SGA children with normal height) or dysmorphic features. The IGF1 serum concentrations and IGF1 SDS values of all children were within normal range. Only one child had lower normal serum IGF1 concentration. No alterations in exon 2 of *IGF1R* gene were detected.

**CONCLUSIONS:** The genetic analysis of the exon 2 of the *IGF1R* gene did not detect any gene defects in the analysed patients. The putative genetic defect in those children affects other parts of the *IGF1R* gene or another gene (s), or yet unidentified factors.

## Introduction

SGA children comprise 3% of all births. Those are the children with birth weight (BW) and/or birth length (BL) less than 2.0 standard deviation score (SDS). Only 10% of children do not attain normal height until the age of four or five years. In fact, that 10 % have a higher risk to remain short, as well as increased prone to diabetes mellitus, cerebrovascular and cardiovascular diseases in adulthood [1].

The aetiology of SGA is heterogeneous. Genetic factors are a culprit in some of them. Defects in *IGF1R* gene are reported as a cause of SGA [2] [3] [4]. The most affected part of the *IGF1R* gene is its exon 2.

Insulin-like growth factor 1 receptor (IGF1R) is a heterotetrameric (α2β2) transmembrane glycoprotein with intrinsic kinase moiety. It contains 2 alfa and two beta subunits synthetized by one mRNA precursor (α2β2). IGF-1R and IR (insulin receptor) are parts of the same family together with IGF-2R and their ligands IGF-1 and IGF-2 and at least 6 IGF-binding proteins, so-called protein kinase superfamily or tyrosine protein kinase family and insulin receptor subfamily [5] [6] [7] [8]. GF1R and IR receptors are found in skeletal muscles, heart, kidneys, fat tissue, liver, spleen, fibroblasts and placenta.

We investigated the exon 2 of the *IGF-1R* gene, as this gene part is the often affected by nucleotide alterations. 100 SGA children were analysed.

## Patients and Methods

The cohort of 100 (M:F = 40:60) children born in term (≥ 37 GW), but small for gestational age is composed of two groups: a group of 64 (M:F = 31:33) SGA born children who did not achieve catch-up growth after the 2nd year and remained short and a group of 36 (M:F = 13:23) SGA born children (older than 4 years) with normal growth spurts for their age and sex.

Anthropometric data and IGF-1 concentrations were evaluated. IGF1-BP3 was not available. Clinical birth data include children's birth weight (BW) in kilograms, BW standard deviation score (SDS), birth length (BL) in centimetres, BL SDS and gestation week (GW). Also, the height SDS, weight SDS, occipitofrontal circumference SDS, body mass index (BMI), BMI z-score and target height (TH) SDS were also analysed.

The serum concentrations of Insulin-like growth factor 1 (IGF1) were determined by chemiluminescence immune assay method on IMMULITE 2000 Siemens, Immunoassay System apparatus.

The molecular analysis of *IGF1R* gene was performed on ("Biometra"-T3 Thermocycler PCR apparatus) in Laboratory for Molecular medicine in University Children's Hospital Skopje. Start DNA material was isolated from leukocytes with high concentrated 5 M NaCl solution. This genomic DNA material of particular exon 2 was amplified using the polymerase chain reaction (PCR) to perform the restriction-site analysis. We used following primers: 5'TCGACATCCGCAACGACTATC3' as *the* forward primer and the 5'CGAAGATGACCAGGGCGTAG3' as reverse primer. PCR products were digested with *Dde* I (Sigma Aldrich), and the resulting fragments were characterized by 1% agarose gel horizontal electrophoresis and staining with ethidium bromide. PCR products of coding *IGF1R* exon 2 were screened by direct sequencing of conspicuous fragments in Molecular Laboratory of Faculty of Pharmacy.

The Kolmogorov-Smirnov test (KS test) is used to check whether the values have a normal (Gaussian) distribution. A confidence interval (CI) is calculated for the mean of each of the quantities. For the comparison of 2 groups, Fisher's test is used to check the equivalence of variances. The result of this test is relevant for applying the test for equivalence of means. If the 2 samples have a size greater than 30, a z-test is used, otherwise a t-test. All tests are with a 99% significance or α = 0.01.

## Results

The mean birth weight (BW) and BW standard deviation score (BW SDS), birth length (BL) and BL SDS were: in 64 short children BW 2284.37 gr ± 433 SDS and BW SDS -2.71 ± 1.05 SDS, BL 47.06 ± 2.09 SDS and BL SDS **-**1.33 ± 1.03 SDS and in 36 children with normal height BW 2502.5 gr ± 317.8 SDS and BW SDS -2.15 ± SDS and BL 46.63 cm ± 2.12 SDS and BL SDS -1.61 ± 1.12 SDS (Table 1).

**Table 1: Birth parameters: Birth weight (BW), BW SDS, birth length (BL), BL SDS and Gestation Week (GW) in 2 groups of children: a group of 64 short SGA born children and group of 36 SGA born children with normal height**

| Groups | | Short SGA Children | | SGA children with Normal height | | Comparison Between Two groups |
|---|---|---|---|---|---|---|
| Parameters | | Mean | P-value From Ks test | Mean | P-value From Ks test | P-value |
| BW | Gr | 2284.37±433 SDS | 0.18 | 2502.5±317.8 SDS | 0.97 | 0.0095 |
| BW SDS | - | -2.71±1.05 SDS | 0.78 | -2.15± 0.56SDS | 0.48 | 0.0009 |
| BL | Cm | 47.06±2.09 SDS | 0.56 | 46.63±2.12 SDS | 0.87 | 0.33 |
| BL SDS | - | -1.33±1.03 SDS | 0.34 | -1.61±1.12 SDS | 0.93 | 0.22 |
| GW | W | 39.17±0.94 SDS | 0.46 | 39.34±0.90 SDS | 0.61 | 0.37 |
| Patients | M | N=31 | | N=13 | | |
| | F | N=33 | | N=23 | | |

Short children had measured height SDS (H SDS) (-3.25 ± 0.90 SDS), weight SDS (W SDS) (-2.72 ± 1.39 SDS), BMI z-score (0.88 ± 1.78 SDS) and occipitofrontal circumference SDS (OFC SDS) (-0.70 ± 1.01 SDS) at the time of entrance the study. Growth parameters of children with caught up growth were: H SDS (0.20 ± 1.1 SDS), W SDS (0.29 ± 1.53 SDS), BMI z-score (0.01 ± 1.68 SDS) and OFC SDS (0.06 ± 0.56 SDS) (Table 2).

**Table 2: Auxology parameters: height SDS (H SDS), weight SDS (W SDS), BMI and BMI z-score, occipitofrontal circumference SDS (OFC SDS), Target Height SDS (TH SDS) in 2 groups of children: a group of 64 short SGA born children and group of 36 SGA born children with normal height**

| Groups | | Short SGA Children | | SGA children with Normal height | | Comparison Between Two groups |
|---|---|---|---|---|---|---|
| Parameters | | Mean | P-value From Ks test | Mean | P-value From Ks test | P-value |
| H SDS | - | -3.25±0.90 SDS | 0.19 | 0.20±1.1 SDS | 0.62 | 0.024 |
| W SDS | - | -2.72±1.39 SDS | 0.45 | 0.29±1.53 SDS | 0.65 | 3.30e-16 |
| BMI | $Kg/m^2$ | 15.25±2.15 SDS | 0.41 | 17.28±4.39 SDS | 0.14 | 0.012 |
| BMI Z-SCORE | - | -0.88±1.78 SDS | 0.31 | 0.01±1.68 SDS | 0.70 | 0.015 |
| OFC SDS | - | -0.70±1.01 SDS | 0.91 | 0.06±0.56 SDS | 0.95 | 3.82e-06 |
| TH SDS | - | -1.00±0.87 SDS | 0.52 | 0.15±0.91 SDS | 0.98 | 6.98e-9 |

None of our SGA born children had dysmorphic features.

Only in one boy with short stature (-2.63 SDS) had low normal IGF1 serum concentration (52.3

ng/ml, N = 50-286) and IGF1 SDS (-1.57 SDS) for his age and sex. The PCR restriction-site analysis in all SGA born children did not show any genetic alteration in exon 2 of *IGF1R* gene. The screened PCR products coding *IGF1R* exon 2 by direct sequencing of conspicuous fragments were uneventful.

## Discussion

Several alterations (point mutations and deletions) in the *IGF1* and insulin-like growth factor 1 receptor (*IGF-1R*) genes have been demonstrated last decade. Alterations have been identified that affect IGF1R biosynthesis, signal reception and receptor kinase activity [9] [10] [11] [12] [13] [14] [15] [16] [17] [18] [19].

Homozygous defects in the *IGF1* gene have been found in patients with major developmental impairments and severe intrauterine and postnatal growth retardation. On the other hand, mutations in *IGF1R* gene that have been predominantly heterozygous are 3 found in patients with mild phenotype and clinically heterogeneous presentation [20].

Since 2003y Abuzzahab et al., [10] described 2 point mutations in exon 2 of *IGF1R* gene: the first was compound heterozygosity with the exchange of arginine by glutamine at amino acid 108 (p. R108Q) and exchange for lysine by asparagine in the amino acid 115 (p. K115N) in SGA born girl with delayed motor development, psychiatric problems and growth retardation. The authors also described another heterozygous mutation in the éxon 2 (p. R89X) in an IUGR born boy with microcephaly, short stature and delayed motor and speech development.

Kawashima et al., [11] in 2005 described missense mutation in heterozygosity at 11 éxon the *IGF1R* (p. R739Q) in a SGA born patient with significant mental retardation and postnatal growth. In SGA born 35 y old patient with microcephaly and elevated IGF1 values, Walenkamp et al., [12] 2006y found a mutation in the éxon 16 (p. E1050K). Inagaki et al., [13] 2007y found heterozygous point mutation resulting in R481Q in a girl with short stature and elevated IGF1 values. Kruis et al., 2010y [14] in 7 members of the same family with low BW, microcephaly and normal mental development, reported similar mutation which resulted in G1125A protein.

Wallborn et al., [15] found a mutation of *IGF1R* gene p. V599E in SGA born patient with microcephaly, mental retardation and elevated IGF1 levels. Fang et al., 2009 [16] reported novel heterozygous 19 nucleotides duplication within 18 éxon of *IGF1R* gene and consequently to haploinsufficiency of IGF1R protein in 4 short statured family members with normal IGF1 levels. Mohn et al., 2011 [17] described 4 SGA family members with short stature and impaired glucose metabolism with a novel mutation (p.Tyr387X).

Labarta et al., 2013 [21] described novel heterozygous *IGF1R* missense mutation in exon 7 (c.A1549T, p.Y487F) in 3 IUGR born females from the same family with short stature and microcephaly. Juanes et al., [22] 2015 identified three novel heterozygous missense mutations in 3 patients with microcephaly and growth retardation, de novo p.Arg1256Ser, de novo p.Asn359Tyr and p.Tyr865Cys.

We investigated 100 SGA born children, 64 children were with short stature -H SDS (-3.25 ± 0.90 SDS) and 36 children with normal height -H SDS (0.20 ± 1.1 SDS). They were born with low BW SDS (-2.71 ± 1.05 SDS) vs (-2.15 ± 0.56 SDS) and/or BL SDS (-1.33 ± 1.03 SDS) vs (-1.61 ± 1.12 SDS). No alterations in exon 2 of the IGF1R gene were found.

The IGF1 serum concentration only in one patient of our cohort was in the lower normal range for his age and sex at the time of diagnosis. The defects of IGF1R usually result in elevated IGF1 serum concentrations [2] [12] [13] [15].

In conclusion, within a cohort of 100 SGA born children without microcephaly or dysmorphic features we did not find alterations in the exon 2 of the *IGF1R* gene. Exon 2 of the *IGF1R* gene might not be a hotspot for alterations. Investigating most or the whole of the *IGF1R* gene together with other genes implicated in SGA might yield an answer on the SGA cause of a particular child.

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# Immunopathological and Modulatory Effects of *Cag A*$^{+}$ Genotype on Gastric Mucosa, Inflammatory Response, Pepsinogens, and Gastrin-17 Secretion in Iraqi Patients infected with *H. pylori*

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**Keywords:** pepsinogens; gastrin-17; gastric mucosa; H. pylori; CagA; Iraq

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**Abstract**

**OBJECTIVE:** To determine the immunopathological correlation between Cag A+ *H. pylori*-specific IgG; pepsinogen I&II (PI&PII); gastrin-17 (G-17); status of gastric and duodenal mucosa and inflammatory activities on different gastroduodenal disorders.

**METHODOLOGY:** Eighty gastroduodenal biopsies were taken from patients with gastroduodenal disorders for histopathological evaluation and *H. pylori* diagnosis. Serum samples were used for evaluation of gastric hormones and detection of *H. pylori*-specific IgG antibodies. The tissue expression of *H. pylori Cag A* gene was detected by in situ hybridisation.

**RESULTS:** *H. pylori* IgG antibodies were detected in (88.8%) of enrolled patients. According to *Cag A* gene expression, Significant difference (P value < 0.05) was detected in levels of PG I; PGII, PG I/PG II among patients with gastric disorders. Serum G-17 level was negatively correlated with *Cag A* gene expression (P-value = 0.04). There was a significant correlation between *H. pylori* IgG and PG I; PG II; G-17. The current study revealed that corpus atrophic gastritis was diagnosed histologically with (5%) gastric ulcer cases; (3.75%) of duodenal ulcer cases; (3.75%) of duodenitis cases; (1.25%) of gastropathy cases and (8.75%) of gastritis cases. At the same time *H. pylori* gastritis diagnosed concurrently with (8.75%) of gastric ulcer cases; (11.25%) of duodenal ulcer cases; (17.5%) of gastropathy cases; (3.75%) of duodenitis cases and (2.5%) of prepyloric ulcer cases. A significant correlation was reported between the Immunopathological status of gastric mucosa and endoscopic mucosal finding among duodenal ulcer cases and gastritis cases only. A positive correlation was reported between serum levels of PGI; PGII; PGI/PGII; G-17; PMNs grade and Immunopathological status of the gastroduodenal mucosa of *H. pylori* Infected patients. A significant difference was reported in lymphocyte grades among gastric disorders without correlation with immunohistopathological changes in the mucosa (P-value = 0.002). A significant difference was reported in lymphocyte grades among different disorders according to *H. pylori* IgG. A significant difference was reported in serum level of PG I; PG II; PG I/PG II; G-17 according to PMN and lymphocyte grades (P-value < 0.01). PMNs grades positively correlated with gastric Cag A expression; *H. pylori* IgG; PG II; G-17 levels. PG I; PG I/ PG II correlated with lymphocyte grades (P-value < 0.05); while PGII has a negative correlation (P-value = 0.039).

**CONCLUSION:** Endoscopic mucosal finding does not reflect exactly the actual immunopathological changes of gastric mucosa during *H. pylori* infection. Secretion of gastrin was not affected by the presence of *Cag A* in gastric tissue. Instead, the fluctuation in the hormone level appears to be due to the presence of *H. pylori* infection in gastric tissue. Gastric tissue infiltration with PMNs & lymphocytes inflammatory infiltrates has a direct effect on PGs and gastrin levels in serum of infected patients. The level of PG I; PG II; G-17 secretion correlated with the development of immune response against *H. pylori* and production of specific *H. pylori* IgG. Finally, *H. pylori* can modulate gastric secretions through *Cag A* dependent and independent pathways.

## Introduction

There are studies with evidence indicating that Helicobacter pylori play a role in the pathogenesis of various gastroduodenal disorders [1]. *H. pylori* colonisation of gastric tissue induces recruitment of inflammatory cells to the infected gastric epithelium and releasing of virulence factors from the bacteria as opposite reaction [2]. Gastritis induces disruption of acid secretion depending on the predominant location in the stomach, antrum or corpus [3] [4]. The gastroduodenal response to chronic *H. pylori* infection is characterised by infiltration of plasma cells, lymphocytes, neutrophils, and monocytes into gastric mucosa [2]. The gastric epithelium plays an active role

in the mucosal defence. Neutrophil activation and the production of reactive oxygen metabolites are induced directly by bacterial factors and indirectly via host-derived cytokines and products of complement activation [5]. As well as stimulating specific T and B cell responses and systemic immunoglobulin (Ig) G and A antibody production, *H. pylori* infection also induces a local proinflammatory cytokine response and the development of gastric lymphoid follicles which are important in immune cells infiltration [3].

Pepsinogens (PG) are aspartic proteinases, which are mainly secreted by gastric cells. PG can be classified into two biochemically and immunologically distinct types: pepsinogen I (PGI) and pepsinogen II (PGII). PGI is secreted only from the gastric fundic mucosa by chief cells and mucous neck cells in the corpus area [6], while PGII is secreted from the cardiac, fundic, and antral mucosal epithelium of the stomach, and also from the duodenal mucosa [7]. Gastrin-17 is produced mainly by the G cells in the antrum. PGs are released into the circulation and serum PG level reflects the functional and morphologic status of the stomach mucosa. Gastrin-17 (G-17) and pepsinogen I (PGI) levels respectively reflect distal and proximal stomach, while pepsinogen II (PGII) level, reflects the status of the entire stomach and particularly inflammation [8]. Human pepsinogens and gastrin have a diagnostic value for various gastroduodenal disorders, especially for peptic ulcer, atrophic gastritis and gastric cancer [9]. The pepsinogen I/II ratio can provide even better information on the extent of chronic gastritis [10].

The aim of present study was to detect *in situ* expression of *H. pylori Cag A* gene in gastroduodenal biopsies and determination of *H. Pylori*- specific IgG antibodies in serum samples taken from patients presented with gastroduodenal disorders. The second aim was the detection of serum level of gastric hormones (PGI, PGII, PG I/II ratio, G-17) among infected cases. Study the possible correlation between levels of PGI, PGII, PG I/II ratio, G-17, serum H Pylori-IgG antibodies; expression of *Cag A* gene in gastric tissue and status of gastroduodenal mucosa as well as the possible effects of *H. pylori Cag A* gene on levels of gastric hormones and mucosal inflammatory activity.

## Subjects and Methods

This cross-sectional, hospital-based study was achieved at gastroenterology department of Baqubah teaching hospital in Diyala province-Iraq after approval of ethical review committee of Department of Pathology, College of Veterinary medicine-Diyala University-Iraq.

A total of, 80 patients presented with clinical indications for upper gastrointestinal tract endoscopy during June 2013 to January 2015 were enrolled. The age range of attended patients (16-80 years) means (47.24 ± 18.82) years. Males represent 44 (55%) versus 36 (45%) females.

This study was conducted according to the principles of Helsinki declaration. A full explanation of the purpose of this study to all patients was done before endoscopy. A signed duly filled consent form obtained from all patients that agree to participate in the study. Exclusion criteria were applied to any patient having a previous gastric surgery; recent or active gastrointestinal bleeding; under antibiotics or colloidal bismuth compounds for past one-month treatment.

### *Methods*

A sterile flexible endoscope was introduced for a full investigation of stomach and duodenum after topical pharyngeal anaesthesia for overnight fasted Patients [4]. Any congested, inflamed or erosive lesions were picked via sterile biopsy forceps. Maximum 6 biopsies were taken. *H. pylori* urease activity was detected in biopsies by placing the samples in Serim® PyloriTek® Test Kit. Each PyloriTek® strip has a built-in positive analyte control and negative control, which run concurrently with the test specimen. The PyloriTek® positive control automatically appears with every test within the normal 1-hour time. With competitive tests, the positive control is run after waiting 24 hours then inserting a urease positive control material [11].

A sterile glass slide with a drop of normal saline was used to teasing the biopsy sample with a sterile scalpel to make smaller fragments of tissue then another sterile glass slide was placed over the teased first tissue, and the tissue was crushed between the two glasses then stain by Gram's staining. The existence of Gram-negative spiral bacteria embedded in the tissue cells was diagnostic for *H. pylori* [12]. true positive results were considered if a combination of urease test and Gram stain give positive results for a single biopsy specimen [13].

*In situ* hybridisation procedure was used for detection of *H. pylori Cag A* gene expression in 5 µm thickness serial gastric mucosal sections fixed on positively charged slides using biotinylated long DNA probe for H.pylori/ Cag A Gene, Cat. No.: IH-60061(HPY-6001-B) (Maxim biotech-USA) and the DNA Probe hybridisation/Detection System - In Situ Kit (Maxim biotech-USA), according to Maxim biotech instruction manual [14]. The examination and scoring were done under a light microscope by pathologists at powerX400 according to the scoring system [15].

The intensity of gastric inflammation was detected by recording lymphocyte infiltration in gastric tissue via grading scale from 0 to 3, based on both gastric lymphocyte and plasma cell infiltration. Grade

0 considered if normal cellular finding detected. Grade 1 considered in case of low inflammation, Grade 2 for Moderate inflammation and Grade 3 indicate heavy inflammation [16]. Inflammation activity scored as following: None (Grade 0), Rare PMNs(Grade 1); 0-1 intraepithelial (IE) PMN/hpf (Grade 2), Grade (3): 1-10 intraepithelial (IE); PMN/hpf (Grade4): ≥10 IE PMN/hpf [5].

For serological assay; blood was drawn from each patient during the visit to the endoscopy unit. Separated serum samples were stored at 27˚C until analyses. H pylori-specific IgG antibodies were determined using a monoclonal enzyme immunoassay method according to BIOHIT HealthCare instructions [17]. Serum pepsinogen I (PGI) and II (PGII) and gastrin-17 (G-17) were assayed with ELISA using monoclonal antibodies to PGI and II and G-17 (BIOHIT Diagnostics, Biohit, Devon, UK). All procedures were carried out according to the manufacturer's instructions, and results of PGI and II reported in µg/l and pmol/l for gastrin-17. The pepsinogen I: II ratio was calculated and reported in fraction [17].

The frequency of variables expressed as a percentage. PG I, II and G-17 values expressed as mean±standard deviation (Mean±SD). Pearson test for correlation was used for non-categorical data. Chi-test used to compare the PG I, PGII, and G17 according to CagA gene expression. The level of significance was 0.05 (two-tail) in all statistical tests. Significant of correlations(Pearson, Spearman) also include 0.01 (two-tail). Statistical analysis was performed using SPSS for Windows TM version 17.0, and Microsoft EXCEL for windows 2010.

## Results

As shown in Table 1, the mean serum level for PGI (112.10 ± 87.73 µg/L) and (40.09 ± 50.80 µg/L) for PGII. Hypersecretion of PGI (> 160µg/L) detected in (31.3%) of patients, mainly among gastropathy; gastritis (8.75%) and duodenal ulcer (DU), (7.5%).

**Table 1: Description of Gastric secretions and *H. pylori*-specific serum IgG**

| Parameters | Minimum | Maximum | Mean±Std. Deviation | Under normal Value | Negative or normal value | Positive or higher than normal |
|---|---|---|---|---|---|---|
| Pepsinogen I (µg/L) | 4 | 400 | 112.10±87.73 | <30 µg/L 6 (7.5%) | 30-160 µg/L 49 (61.3%) | > 160 µg/L 25 (31.3%) |
| Pepsinogen II (µg/L) | 6 | 220 | 40.09± 50.80 | <3 µg/L 0 (0%) | 3-15 µg/L 19 (23.8%) | > 15 µg/L 61 (76.3%) |
| Pepsinogen I / Pepsinogen II ratio | 0.17 | 18.18 | 4.65± 4.13 | <3 µg/L 33 (41.3%) | 3-20 µg/L 47 (58.8%) | > 20 µg/L 0 (0%) |
| Gastrin 17 (pmol/l) | 1 | 400 | 9.58± 44.30 | <1 pmol/ml 0 (0 %) | 1-7 pmol/ml 70 (87.5%) | > 7 pmol/ml 10 (12.5%) |
| H.pylori IgG (EIU) | 9.29 | 250 | 107.61±52.00 | 0 (0%) | <30-EIU 9 (11.3%) | >30 EIU 71 (88.8%) |

Normal secretion of PGI was detected in gastritis (28.75%) while hyposecretion detected in (3.77%) of gastric ulcer (GU) cases. A significant difference (P-value = 0.005) was detected among gastric disorders in PG I secretion levels as shown in (Table 2).

Hypersecretion of PG II (> 15 µg/L) detected in (76.3%) patients mainly with gastritis (28.75%), gastropathy (16.25%) and DU (15%). The normal value of PG II was detected in (23.8%) of gastric disorders while hyposecretion of PGII not observed with significant difference (P value = 0.006). The mean of PG I/PG II ratio was 4.65 ± 4.13 µg/L. Hyposecretion of PG I/PG II detected in (41.3%), while hypersecretion of PGI/PG II not determined in all gastric disorders with significant difference (P value = 0.000) (Table 2).

The mean of G-17 (9.58 ± 44.30) (pmol/l). Normal range of G-17 (1-7pmol/l) detected in (87.5%) patients; Hypersecretion of G-17 detected in (12.5%) mainly among gastritis (7.5%) without significant difference (P value=0.49) among gastric disorders (Table 2). There was no correlation between serum levels of PG I; PG II; PG I/PG II or G-17 and type of gastroduodenal disorder as shown in Table 2.

As shown in Table 3, the PG I hyposecretion (7.5%), normal (32.5%) and hypersecretion level (18.75%) was significantly higher in CagA positive (P-value = 0.009) (Figure 1).

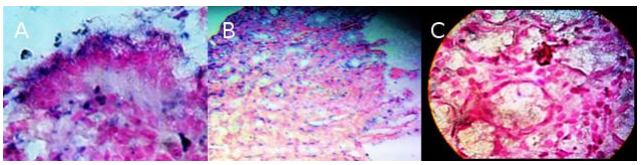


*Figure 1: In situ hybridisation for Cag A Positive H. pylori in a gastric tissue section, staining by BCIP/NBT (bluish purple) counterstained with nuclear fast red. Bar size = 50 µm; A) Gastric epithelia; B) Cag A expression extended to gastric pits; C) negative expression*

Significant difference was detected between CagA positive and CagA negative cases in PGII (P value = 0.005); PG I/PG II, (P value = 0.003). No significant difference was detected between patients in G-17 serum level; (P value=0.479). There was no correlation between CagA gene expression and serum levels of PG I; PGII; PG I/PGII but only for Gastrin17 (P value = 0.04). Significant difference and correlation between specific *H. pylori* IgG; PGI (P value = 0.000; P value = 0.004); PG II (P value = 0.000; P value = 0.003); G-17 (P value = 0.000; P value = 0.05). Significant difference without correlation was detected between CagA positive and negative cases in PG I/PG II (P value = 0.000; P value = 0.215) as shown in Table 4.

One of the most interesting points in the current study was that the endoscopic and microscopic examination of gastric mucosa revealed different findings as shown in Table 5.

**Table 2: Correlation of Gastric Secretions with Gastroduodenal Disorders**

| Parameter | | Gastric ulcer | Duodenal ulcer | Gastropathy | Gastritis | Duodenitis | Prepyloric ulcer | χ2 | P value | r | P value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | <30 µg/L | 4 (3.77%) | 1 (1.25%) | 0 (0%) | 0 (0%) | 1 (1.25%) | 0 (0%) | | | | |
| *Pepsinogen I* | 30-160 µg/L | 8 (10%) | 5 (6.25%) | 8 (10%) | 23 (28.75%) | 5 (6.25%) | 0 (0%) | 157.97 | 0.005 | -0.016 | 0.887 |
| | > 160 µg/L | 3 (3.75%) | 6 (7. 5%) | 7 (8.75%) | 7 (8.75%) | 0 (0%) | 2 (2.5%) | | | | |
| | <3 µg/L | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | | | | |
| Pepsinogen II | 3-15 µg/L | 8 (10%) | 0 (0%) | 2 (2.5%) | 7 (8.75%) | 2 (2.5%) | 0 (0%) | 144.50 | 0.006 | -0.044 | 0.698 |
| | > 15 µg/L | 7 (8.75%) | 12 (15%) | 13 (16.25%) | 23 (28.75%) | 4 (3.77%) | 2 (2.5%) | | | | |
| | <3 µg/L | 2 (2.5%) | 9 (11.25%) | 3 (3.75%) | 14 (17.5%) | 5 (6.25%) | 0 (0%) | | | | |
| Pepsinogen I/ Pepsinogen II ratio | 3-20 µg/L | 13 (16.25%) | 3 (3.75%) | 12 (15%) | 16 (20%) | 1 (1.25%) | 2 (1.89%) | 266.35 | 0.000 | - 0.054 | 0.637 |
| | > 20 µg/L | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | | | | |
| | <1 pmol/l | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | | | | |
| Gastrin17 | 1-7 pmol/l | 12 (15%) | 12 (15%) | 15 (18.75%) | 24 (30%) | 5 (6.25%) | 2 (2.5%) | 69.531 | 0.493 | 0.075 | 0.506 |
| | > 7 pmol/l | 3 (3.75%) | 0 (0%) | 0 (0%) | 6 (7.5%) | 1 (1.25%) | 0 (0%) | | | | |

Gastric ulcer was diagnosed endoscopically in 15 (18.75%) of patients; 4 (5%) of them have normal gastric mucosa and atrophic corpus gastritis equally, while 7 (8.75%) have H.pylori gastritis without atrophy. A total of 14 (17.5%) have inflamed mucosa during endoscopic examination. Among gastric ulcer cases, no significant correlation was reported between the Immunopathological status of gastric mucosa and endoscopic mucosal finding (P-value = 0.820).

**Table 3: Correlation of Gastric secretions with *Cag A* genotype and *H. pylori*-specific serum IgG**

| Parameter | | CagA positive | CagA Negative | χ2 | P value | r | P value |
|---|---|---|---|---|---|---|---|
| | < 30 µg/L | 6 (7. 5%) | 0 (0%) | | | | |
| Pepsinogen I | 30-160µg/L | 26 (32.5%) | 23 (28.75%) | 41.900 | 0.009 | 0. 085 | 0.451 |
| | >160 µg/L | 15 18.75%) | 10 (12.5%) | | | | |
| | <3 µg/L | 0 (0%) | 0 (0%) | | | | |
| Pepsinogen II | 3-15 µg/L | 11 13.75%) | 8 (10%) | 41.55 | 0.005 | 0. 187 | 0.097 |
| | >15 µg/L | 36 (45%) | 25 (31.25%) | | | | |
| Pepsinogen I/ | < 3 µg/L | 17 (21.25%) | 16 (20%) | | | | |
| Pepsinogen II | 3-20 µg/L | 30 (37.5%) | 17 (21.25%) | 64.52 | 0.003 | 0.003 | 0.980 |
| ratio | >20 µg/L | 0 (0%) | 0 (0%) | | | | |
| | < 1 pmol/l | 0 (0%) | 0 (0%) | 13.613 | 0.479 | -0.147 | 0.194 |
| Gastrin 17 | 1-7 pmol/l | 44 (55%) | 26 (32.5%) | | | -0.229* | 0.04* |
| | >7 pmol/l | 3 (3.75%) | 7 (8.75%) | | | | |

The duodenal ulcer was diagnosed endoscopically in 12 (15%) of patients; 3 (3.75%) of them have atrophic corpus gastritis, while 9 (11.25%) have *H. pylori* gastritis without atrophy. Endoscopy mucosal finding revealed that a total of 9 (11.25%) have normal mucosa during endoscopic examination while 1 (1.25%) suffered from severe erosion and 2 (2.5%) suffered from severe inflammation.

**Table 4: Correlation of Gastric secretions with *H. pylori*-specific Serum IgG**

| Parameter | | H. pylori IgG positive | H. pylori IgG Negative | χ2 | P value | r | P value |
|---|---|---|---|---|---|---|---|
| | < 30 µg/L | 3 (3.75%) | 3 (3.75%) | | | | |
| Pepsinogen I | 30-160 µg/L | 44 (55%) | 5 (6.25%) | 1352.800 | 0.000 | 0.317 | 0.004 |
| | >160 µg/L | 24 (30%) | 1 (1.25%) | | | | |
| | <3 µg/L | 0 (0%) | 0 (0%) | | | | |
| Pepsinogen II | 3-15 µg/L | 11 (13.75%) | 8 (10%) | 1204.127 | 0.000 | 0.211 | 0.06 |
| | >15 µg/L | 60 (75%) | 1 (1.25%) | | | 0.328* | 0.003* |
| Pepsinogen I/ | < 3 µg/L | 32 (40%) | 1 (1.25%) | 1914.333 | 0.000 | 0.140 | 0.215 |
| Pepsinogen II | 3-20 µg/L | 39 (48.75%) | 8 (10%) | | | | |
| ratio | >20 µg/L | 0 (0%) | 0 (0%) | | | | |
| | < 1 pmol/l | 0 (0%) | 0 (0%) | | | | |
| Gastrin 17 | 1-7 pmol/l | 65 (81.25%) | 5 (6.25%) | 593.539 | 0.000 | -0.220 | 0.05 |
| | >7 pmol/l | 6 (7. 5%) | 4 (5%) | | | | |

* Spearman Correlation.

Among duodenal ulcer cases, a significant difference (P value = 0.027) and the correlation were reported between the Immunopathological status of gastric mucosa and endoscopic mucosal finding (P-value = 0.012) as shown in Table 5.

Gastropathy was diagnosed endoscopically in 15 (18.75%) of patients; 1 (1.25%) of them have atrophic corpus gastritis, while 14 (17.5%) have *H. pylori* gastritis without atrophy. Endoscopy mucosal finding revealed that all have inflamed mucosa during endoscopic examination. No statistics are computed because endoscopy mucosal finding is a constant as shown in Table 5.

Gastritis was diagnosed endoscopically in 30 (37%) of patients; 2 (2.5%) of patients have normal mucosa; 7 (8.75%) of them have atrophic corpus gastritis, while 21 (26.25%) have *H. pylori* gastritis without atrophy. Endoscopy mucosal finding revealed that a total of 1 (1.25%) have normal mucosa during endoscopic examination while 3 (3.75%) suffered from severe erosion and 26 (32.5%) suffered from severe inflammation.

Among gastritis cases, a significant difference (P value = 0.001) and the correlation were reported between the Immunopathological status of gastric mucosa and endoscopic mucosal finding (P-value = 0.004).

Duodenitis was diagnosed endoscopically in 6 (7.5%) of patients; 3 (3.75%) of them have atrophic corpus gastritis, while 3 (3.75%) have *H. pylori* gastritis without atrophy. Endoscopy mucosal finding revealed that a total of 2 (2.5%) during endoscopic examination suffered from severe erosion and 4 (5%) suffered from severe inflammation. Among duodenitis cases, no significant difference (P value = 0.083) nor correlation (P-value = 0.116) was reported between the Immunopathological status of gastric mucosa and endoscopic mucosal finding as shown in Table 5. Prepyloric Ulcer was diagnosed endoscopically in 2 (2.5%) of patients; all have *H. pylori* gastritis without atrophy. Endoscopy mucosal finding revealed that patients suffered from severe inflammation.

No statistics are computed because endoscopy mucosal finding is a constant. Status of gastroduodenal mucosa significantly differs and correlated with serum levels of PG I (P-value = 0.0000); PG II (P-value = 0.029); PG I/PG II (P-value = 0.008); G-17 (P-value = 0.004) (Table 6). PMNs grades significantly correlated with (P-value = 0.02) status of gastroduodenal mucosa.

**Table 5: Correlation of Clinical Diagnosis According To Endoscopy, Immunopathological Status of gastroduodenal mucosa and Endoscopic Mucosal Findings according to *H. pylori* infection**

| Clinical Diagnosis According To Endoscopy | Immuno-pathological Status of gastroduodenal mucosa | Endoscopy Mucosal Finding | | | Total | $\chi^2$ | P value | r | P value |
|---|---|---|---|---|---|---|---|---|---|
| | | Normal | Sever Erosion | Inflammation | | | | | |
| Gastric Ulcer | Normal Mucosa (No Infection) | 0 (0%) | 0 (0%) | 4 (5%) | 4 (5%) | 2.946 | 0.229 | 0.064 | 0.820 |
| | Atrophic Corpus Gastritis | 1 (1.25%) | 0 (0%) | 3 (3.75%) | 4 (5%) | | | | |
| | *H. pylori* Gastritis Without Atrophy | 0 (0%) | 0 (0%) | 7 (8.75%) | 7 (8.75%) | | | | |
| | Total | 1 (1.25%) | 0 (0 %) | 14 (17.5 %) | 15 (18.75%) | | | | |
| Duodenal ulcer | Normal Mucosa (No Infection) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 7.259 | .027 | -0.697 | 0.012 |
| | atrophic corpus gastritis | 1 (1.25%) | 0 (0%) | 2 (2.5%) | 3 (3.75%) | | | | |
| | *H. pylori* gastritis without atrophy | 8 (10%) | 1 (1.25%) | 0 (0%) | 9 (11.25%) | | | | |
| | Total | 9 (11.25%) | 1 (1.25%) | 2 (2.5%) | 12 (15%) | | | | |
| Gastropathy | Normal Mucosa (No Infection) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | ND | ND* | ND | ND* |
| | atrophic corpus gastritis | 0 (0%) | 0 (0%) | 1 (1.25%) | 1 (1.25%) | | | | |
| | *H. pylori* gastritis without atrophy | 0 (0%) | 0 (0%) | 14 (17.5%) | 14 (17.5%) | | | | |
| | Total | 0 (0%) | 0 (0%) | 0 (0%) | 15 (18.75%) | | | | |
| Gastritis | Normal Mucosa (No Infection) | 1 (1.25%) | 0 (0%) | 1 (1.25%) | 2 (2.5%) | 17.866 | .001 | 0.507 | 0.004 |
| | atrophic corpus gastritis | 0 (0%) | 2 (2.5%) | 5 (6.25%) | 7 (8.75%) | | | | |
| | *H. pylori* gastritis without atrophy | 0 (0%) | 1 (1.25%) | 20 (25%) | 21 (26.25%) | | | | |
| | Total | 1 (1.25%) | 3 (3.75%) | 26 (32.5%) | 30 (37.5%) | | | | |
| Duodenitis | Normal Mucosa (No Infection) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 3.000 | 0.083 | 0.707 | 0.116 |
| | atrophic corpus gastritis | 0 (0%) | 2 (2.5%) | 1 (1.25%) | 3 (3.75%) | | | | |
| | *H. pylori* gastritis without atrophy | 0 (0%) | 0 (0%) | 3 (3.75%) | 3 (3.75%) | | | | |
| | Total | 0 (0%) | 2 (2.5%) | 4 (5%) | 6 (7.5%) | | | | |
| Prepyloric Ulcer | Normal Mucosa (No Infection) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | ND | ND* | ND | ND* |
| | atrophic corpus gastritis | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | | | | |
| | *H. pylori* gastritis without atrophy | 0 (0%) | 0 (0%) | 2 (2.5%) | 2 (2.5%) | | | | |
| | Total | 0 (0%) | 0 (0%) | 2 (2.5%) | 2 (2.5%) | | | | |

*No statistics are computed because endoscopy mucosal finding is a constant. ND: not detected.

A significant difference (P-value = 0.002) in grades of mucosal lymphocyte infiltration among gastroduodenal disorders also, there was no correlation between grades of mucosal lymphocyte infiltration and histopathological changes in mucosa as shown in Table 7.

As shown in Table 8, PMNs grades significantly correlated with Cag A expression (P value = 0.0000); *H. pylori* IgG (P value = 0.003). Significant difference in PG I (P value = 0.0000); PG II; PG I/PG II ratio; G-17 according to PMN grade. Significant correlation between PG II (P value = 0.009); G 17 (P value = 0.000) and PMNs Grade.

As shown in Table 9, inflammation intensity according to lymphocyte grade do not correlate with Cag A expression and presence of *H. pylori*-specific IgG (P-value = 0.063), (P-value = 0.706). A significant difference in PG I (P-value = 0.0000); PG II; PG I/PG II ratio; G-17 according to lymphocyte grades; positive correlation between PG I (P-value = 0.007); PG I/PG II (P-value = 0.037) and lymphocyte grades. The negative correlation between PG II (P-value = 0.039) and lymphocyte grades.

## Discussion

In this study, the age and gender distribution for *H. pylori*-infected patients come in line with [2] [5] [16] [18] and counteract with recent international studies [19]. *H. pylori* infection provokes both local and systemic antibody responses. The systemic response typically comprises a transient rise in IgM, followed by a rise in specific IgA and IgG maintained throughout infection [20].

In this study, (88.8%) have *H. pylori*-specific IgG antibodies (> 30-EIU) which reflect the high level of immune response to *H. pylori* as the mean of *H. pylori*-specific IgG antibodies (107.61 ± 52) EIU, which come in agreement with [20] and higher than [21] [22], reporting *H. pylori* seropositivity of 56.3%; 57% of Indian and Saudi Arabia patients respectively. The negative *H. pylori*-specific IgG (< 30-EIU) was detected in (11.3%) but, histologically the infection proved through detection of Cag A gene expression in gastric tissue.

**Table 6: Correlation Of Gastric Secretions And Status Of Gastroduodenal Mucosa according to *H. pylori* Infection**

| *Parameter* | | Immunopathological Status of gastroduodenal mucosa | | | Total | $\chi^2$ | P value | Correlation | |
|---|---|---|---|---|---|---|---|---|---|
| | | *H. pylori* Associated atrophic corpus gastritis | *H. pylori* Gastritis without atrophy | Normal mucosa (no infection) | | | | r | P value |
| Pepsinogen I | < 30 µg/L | 6 (7.5%) | 0 (0%) | 0 (0%) | 6 (7.5%) | 116.251 | 0.000 | 0.408 | 0.000 |
| | 30-160 µg/L | 12 (15%) | 32 (40%) | 5 (6.25%) | 49 (61.25%) | | | | |
| | >160 µg/L | 0 (0%) | 24 (30%) | 1 (1.25 %) | 25 (31.25%) | | | | |
| Pepsinogen II | <3 µg/L | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 94.710 | 0.000 | 0.244 | 0.029 |
| | 3-15 µg/L | 5 (6.25%) | 9 (11.25%) | 5 (6.25%) | 19 (23.75%) | | | | |
| | >15 µg/L | 13 (16.25%) | 47 (58.75%) | 1 (1.25 %) | 61 (76.25%) | | | | |
| Pepsinogen I/ Pepsinogen II ratio | < 3 µg/L | 15 (18.75%) | 17 (21.25%) | 1 (1.25 %) | 33 (41.25%) | 148.229 | 0.000 | 0.095<br>0.294* | 0.403<br>0.008* |
| | 3-20 µg/L | 3 (3.75%) | 39 (48.75%) | 5 (6.25%) | 47 (58.75%) | | | | |
| | >20 µg/L | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | | | | |
| Gastrin 17 | < 1 pmol/l | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 64.856 | 0.000 | -0.317 | 0.004 |
| | 1-7 pmol/l | 17 (21.25%) | 51 (63.75%) | 2 (2.5%) | 70 (87.5%) | | | | |
| | >7 pmol/l | 1 (1.25%) | 5 (6.25 %) | 4 (5%) | 10 (12.5%) | | | | |

* Spearman Correlation.

**Table 7: Correlation of Inflammation intensity; activity and Immunopathological Status of gastroduodenal mucosa according to *H. pylori* Infection**

| Pmns grade | *Status Of Gastroduodenal Mucosa According To H. pylori Infection* | | | Total | $\chi^2$ | P value | r | P value |
|---|---|---|---|---|---|---|---|---|
| | Normal mucosa (No infection) | *H. pylori* corpus Gastritis with atrophy | *H. pylori* gastritis Without atrophy | | | | | |
| 0 | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | | | | |
| 1 | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | | | | |
| 2 | 1 (1.25 %) | 3 (3.75%) | 2 (2.5%) | 6 (7.5%) | 6.625 | 0.157 | 0.260 | 0.02 |
| 3 | 3 (3.75%) | 9 (11.25%) | 21 (26.25%) | 33 (41.25%) | | | | |
| 4 | 2 (2.5%) | 6 (7.5%) | 33 (41.25%) | 41 (51.25%) | | | | |
| Total | 6 (7.5%) | 18 (22.5%) | 56 (70%) | 80 (100%) | | | | |
| Lymphocyte grade | Normal mucosa (No infection) | Atrophic corpus gastritis | *H. pylori* gastritis without atrophy | Total | $\chi^2$ | P value | R | P value |
| 0 | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | | | | |
| 1 | 0 (0%) | 2 (2.5%) | 0 (0%) | 2 (2.5%) | | | | |
| 2 | 0 (0%) | 12 (15%) | 24 (30%) | 36 (45%) | 17.475 | 0.002 | 0.037 | 0.746 |
| 3 | 6 (7.5%) | 4 (5%) | 32 (40%) | 42 (52.5%) | | | | |
| Total | 6 (7.5%) | 18 (22.5%) | 56 (70%) | 80 (100%) | | | | |

It means recent infection with a scanty number of *H.pylori* and the time of infection less than 20 IgG seroconversion occurs in 22-23 days after infection [21].

Fluctuations of H. pylori-specific IgG antibody titer predict the variation in response of the host against *H. pylori*. This may give a great possibility for continuous exposure of local population in Iraq to *H. pylori* because of low-quality drinking water, improper sanitation for household sewage, continuous exposure to *H. pylori* from other sources like raw vegetables. All these factors may act in development of the high level of humoral immune response in pre-exposed persons [4].

One of motivating results in the current study was the hypersecretion of PGI (> 160 µg/L), in (31.3%) of patients while hyper secretion of PG II (> 15 µg/L) in (76.3%), both of them among gastropathy; gastritis and duodenal ulcer (P value < 0.05) indicating that the density of the pathogens distributed gradually to be pangastric and even duodenal region stimulating intracellular nitric oxide and calcium production inducing sever inflammatory response due to *H. pylori* that subsequently induce PGs hypersecretion [23] [24]

Cag A expression in gastric tissue appears to play a role in hyposecretion of PGI by fundic gland that was detected in (7.5%) mainly in gastric and duodenal ulcers. All patients infected with Cag $A^+$ *H. pylori* strain and have a positive association with anti-*H. Pylori* IgG response and histologically gastric and duodenal ulcers associated with corpus atrophic gastritis (table 2, 3 and 4) which explain the main reason for hyposecretion of PGI. Hyposecretion of PGI/PGII detected in (41.3%) among them (21.25%) infected with Cag $A^+$ *H. pylori* associated with (40%) anti-*H. Pylori* IgG response mainly among Duodenal ulcer, gastritis and duodenitis.

Also, these cases associated with atrophic changes (Tables 2, 3 and 4), the main factor for such disturbance; besides heavy inflammation belongs to PGI because PGII which mainly secreted by pyloric glands and proximal duodenal mucosa still within normal range. These finding supported by others [25] [27] and come in agreement with [26] [28] [29], indicating that serum PG I/PG II ratio decreased when *H. pylori* infection occurs, but the ratio increased after eradication of the bacterium.

In the current study, a normal range of G-17 (1-7 pmol/l) was detected in (87.5%) of patients compared with (12.5%) associated with hypergastrinemia mainly among gastritis (7.5%). A significant correlation (P-value = 0.04) between Cag A expression and serum G-17 level was reported. A negative correlation was recorded between anti-*H. pylori* IgG and serum G17 which come in line with [3] [27]. The current study proved that no correlation between serum levels of PG I; PG II; PG I/PG II or G-17 and type of gastroduodenal disorder that comes in line with [30].

**Table 8: Correlation of gastric secretions; inflammatory activity according to PMNs grade; Cag A genotype; *H. pylori*-specific IgG**

| Parameters | | Inflammatory activity according to PMNs grade | | | | | Total | $\chi^2$ | P value | r | P value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | | | | | |
| *Cag A Genotype* | Negative | 0 (0%) | 0 (0%) | 2 (2.5%) | 24 (30%) | 7 (8.75%) | 33 (41.25%) | 23.536 | 0.000 | 0.381 | 0.000 |
| | Positive | 0 (0%) | 0 (0%) | 4 (5%) | 9 (11.25%) | 34 (42.5%) | 47 (58.75%) | | | | |
| *H.pylori Igg* | <30 EIU | 0 (0%) | 0 (0%) | 4 (5%) | 3 (3.75%) | 2 (2.5%) | 9 (11.25%) | 99.232 | 0.001 | 0.329 | 0.003 |
| | >30 EIU | 0 (0%) | 0 (0%) | 2 (2.5%) | 30 (37.5%) | 39 (48.75%) | 71 (88.75%) | | | | |
| *Pepsinogen I* | <30 µg/L | 0 (0%) | 0 (0%) | 3 (3.75%) | 0 (0%) | 3 (3.75%) | 6 (7.5%) | 90.265 | 0.000 | 0.196 | 0.081 |
| | 30-160 µg/L | 0 (0%) | 0 (0%) | 3 (3.75%) | 21 (26.25%) | 25 (31.25%) | 49 (61.25%) | | | | |
| | >160 µg/L | 0 (0%) | 0 (0%) | 0 (0%) | 12 (15%) | 13 (16.25%) | 25 (31.25%) | | | | |
| *Pepsinogen II* | <3 µg/L | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 92.322 | 0.000 | 0.290 | 0.009 |
| | 3-15 µg/L | 0 (0%) | 0 (0%) | 5 (6.25%) | 6 (7.5%) | 8 (10%) | 19 (23.75%) | | | | |
| | >15 µg/L | 0 (0%) | 0 (0%) | 1 (1.25%) | 27 (33.75%) | 33 (41.25%) | 61 (76.25%) | | | | |
| *Pepsinogen I / Pepsinogen II* | <3 µg/L | 0 (0%) | 0 (0%) | 0 (0%) | 15 (18.75%) | 18 (22.5%) | 33 (41.25%) | 131.843 | 0.000 | -0.64 | 0.573 |
| | 3-20 µg/L | 0 (0%) | 0 (0%) | 0 (0%) | 18 (22.5%) | 23 (28.75%) | 47 (58.75%) | | | | |
| | >20 µg/L | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | | | | |
| *Gastrin 17* | <1 pmol/l | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 40.236 | 0.05 | -0.107<br>-0.426* | 0.347<br>0.000* |
| | 1-7 pmol/l | 0 (0%) | 0 (0%) | 4 (5%) | 25 (31.25%) | 41 (51.25%) | 70 (87.5%) | | | | |
| | >7 pmol/l | 0 (0%) | 0 (0%) | 2 (2.5%) | 8 (10%) | 0 (0%) | 10 (30%) | | | | |

**Table 9: Correlation of gastric secretions; inflammatory intensity according to lymphocytes grade; *Cag A* genotype; *H. pylori*-specific IgG**

| Parameters | | Lymphocyte grade | | | | Total | $\chi 2$ | P value | r | P value |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | | | | | |
| *Cag A* In situ | Negative | 0 (0%) | 2 (2.5%) | 17 (21.25%) | 14 (17.5%) | 33 (41.25%) | 4.465 | 0.107 | 0.209 | 0.063 |
| | Positive | 0 (0%) | 0 (0%) | 19 (23.75%) | 28 (35%) | 47 (58.75%) | | | | |
| *H. Pylori* Antibodies | <30 EIU | 0 (0%) | 0 (0%) | 3 (3.75%) | 6 (7.5%) | 9 (11.25%) | 83.222 | 0.017 | -0.043 | 0.706 |
| | >30 EIU | 0 (0%) | 2 (2.5%) | 33 (41.25%) | 36 (45%) | 71 (88.75%) | | | | |
| Pepsinogen I | <30 µg/L | 0 (0%) | 0 (0%) | 6 (7.5%) | 0 (0%) | 6 (7.5%) | 90.860 | 0.000 | 0.302 | 0.007 |
| | 30-160 µg/L | 0 (0%) | 2 (2.5%) | 23 (28.75%) | 24 (30%) | 49 (61.25%) | | | | |
| | >160 µg/L | 0 (0%) | 0 (0%) | 7 (8.75%) | 18 (22.5%) | 25 (31.25%) | | | | |
| Pepsinogen II | >3 µg/L | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 64.346 | 0.015 | -0.232 | 0.039 |
| | 3-15 µg/L | 0 (0%) | 0 (0%) | 8 (10%) | 11 (13.75%) | 19 (23.75%) | | | | |
| | >15 µg/L | 0 (0%) | 0 (0%) | 2 (2.5%) | 28 (35%) | 31 (38.75%) | | | | |
| Pepsinogen I/ Pepsinogen II | <3 µg/L | 0 (0%) | 2 (2.5%) | 24 (30%) | 7 (8.75%) | 33 (41.25%) | 154.153 | 0.000 | 0.233 | 0.037 |
| | 3-20 µg/L | 0 (0%) | 0 (0%) | 12 (15%) | 35 (43.75%) | 47 (58.75%) | | | | |
| | >20 µg/L | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | | | | |
| Gastrin 17 | < 1 pmol/l | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 50.834 | 0.005 | 0.107 | 0.346 |
| | 1-7 pmol/l | 0 (0%) | 2 (2.5%) | 33 (41.25%) | 35 (43.75%) | 70 (87.5%) | | | | |
| | >7 pmol/l | 0 (0%) | 0 (0%) | 3 (3.75%) | 7 (8.75%) | 10 (12.5%) | | | | |

The interaction between *H. pylori* and gastrin was shown to be specific, essential and depends on a defined gastrin sequence. In the current study, *H. pylori* cause hypergastrinemia in (12.5%) of infected patients. Enhancement of gastrin secretion in the majority of *H. pylori*-infected patients might be due to several factors. First, increase in leptin production that may be induced after meal or *H. pylori* infection due to direct effect of cholecystokinin (CCK) secretion [30]; reduction of somatostatin secretion as a results of *H. pylori* infection [31] which leads to disruption of the inhibitory effect of somatostatin on the G cell [32]. Mucosal cytokines as a result of *H. pylori* infection, mainly TNFα and IL1β increase gastrin production via G cells [33]. Increased gastrin level reflect the activity of *H. pylori* CagA positive strains in the induction of G cells to increase gastrin mRNA expression in gastric mucosa [31] which give support for present findings that all gastric hormones significantly affected by CagA production in situ [27] [34].

One of the most interesting points in the current study that the endoscopic examination of *H. pylori*-infected gastric mucosa comes with different findings when further assessments take place via histopathology and serological evaluation of GI, GII, G17 (Table 4). This fact reflects the needs for further evaluation of endoscopically *H. pylori* positive cases via histopathological and serological gastric biomarkers for identification of numerous lesions that occurs concurrently in a single patient.

One of the most valuable points in the present study, which revealed by the endoscopic and microscopic examinations of gastric mucosa was different findings. The endoscopic results not exactly reflect the immunopathological changes in gastric tissue. The current study revealed that corpus atrophic gastritis was diagnosed histologically in (5%) gastric ulcer cases; (3.75%) of duodenal ulcer cases; (3.75%) of duodenitis cases; (1.25%) of gastropathy cases and (8.75%) of gastritis cases.

At the same time *H. pylori* gastritis diagnosed concurrently with (8.75%) of gastric ulcer cases; (11.25%) of duodenal ulcer cases; (17.5%) of gastropathy cases; (3.75%) of duodenitis cases and (2.5%) of prepyloric ulcer cases. A significant correlation was reported between the Immunopathological status of gastric mucosa and endoscopic mucosal finding among gastritis cases in which only (1.25%) has normal mucosa and (3.75%) have severe erosion while (32.5%) suffered from severe inflammation. Among duodenal ulcer cases (11.25%) have normal mucosa during endoscopic examination while (1.25%) suffered from severe erosion and (2.5%) suffered from severe inflammation.

These results come in line with [1] [35] and give assumption of the heavy intensity of Cag A positive (58.75%) *H. pylori* colonization leads to severe inflammatory response and finally to reduction of PGI; PGI/PGII ratio and level of gastrin-17 increased significantly in subjects with atrophic gastritis, which affect the morphology and function of gastric mucosa [3].

A significant correlation was detected between gastric secretions (PGI and PGII; G-17) and status of the gastroduodenal mucosa, whether normal, atrophic or inflamed. Hyposecretion of PGI was reported in (7.5%) of *H. pylori*-associated atrophic corpus gastritis cases, due to the loss of mucosal glands and cells which come in line with [3] [36]. Reasonable hypersecretion of PGI (> 160 µg/L ) was detected in (30%) of cases with *H. pylori* mucosal gastritis which may be progressed to ulcers due to hyperchlorhydria [7]. Hypersecretion of PGII was detected in (58.75%) of *H. pylori* mucosal gastritis which gives an obvious indication of pangastric inflammatory pattern compared with (16.25%) in *H. pylori*-associated atrophic corpus gastritis, that may indicate a starting of damage to PGII producing chief cells, which come in accordance with other studies [8]. Hypergastrinemia detected among (6.25%) of *H. pylori*-associated gastritis without atrophy and in (5%) of normal mucosa which explains the role of *H. pylori* infection in limitation of inhibitory activity of D cells producing somatostatin against gastrin production via G cells which come by others [3] [31].

In the current study, a significant correlation (P-value = 0.02) between the status of gastroduodenal

mucosa whether associated with atrophic changes or not and grade of PMNs infiltrated in lamina propria associated with *H. pylori* Infection. No correlation between grades of mucosal lymphocyte infiltration and histopathological changes in mucosa was reported. These results come in line with others, stated that gastric inflammation with *H. pylori* has a considerable impact on the gastric morphology and acid secretion [3]. The present study finding has support from previous studies stated a significant correlation between atrophic changes in the gastric mucosa of Iraqi patients and the activity of lymphocytes and PMNs infiltrated [16].

Reasonable significant correlation between PMNs grades infiltration; specific *H. pylori* IgG and Cag A genotype in situ expression among different disorders were reported, which come by others [5] [16] [25]. The present study reported no correlation (P-value = 0.063) between Cag A in situ expressions; lymphocyte grades infiltration and specific *H. pylori* IgG among different disorders. This may attribute to the fact that numerous virulence factors associated with induction of inflammatory response in infected patients like iceA1, vac A and oip A [37].

A significant difference (P-value = 0.0000) was detected in serum level of PG I; PG II; PG I/PG II ratio; G-17 according to PMNs grade and lymphocyte grades. Significant correlation was detected between PG II (P-value = 0.009); G-17 (P-value = 0.000)and PMNs Grade, which come in agreement with [6] [38], they proved that serum levels of PG II and G 17 increased when gastric mucosa is infiltrated with neutrophils and mononuclear cells in antrum as a result of *H. pylori* infection and its extension into the upper stomach. Others stated that gastrin levels were related to *H. pylori* density and acute/chronic inflammation scores in the corpus mucosa but not in the antral mucosa [39].

The present study recorded a positive correlation between PG I; PG I/PG II ratio and lymphocyte grades infiltrated in lamina propria. While negative correlation was detected between PG II and lymphocyte grades. This finding was supported by previous studies which recorded a correlation between *H. pylori* infection, inflammatory activity in-situ and gastric hormones fluctuation before and after eradication, suggesting that the H. pylori-induced heavy inflammation is a strong stimulus for the synthesis of these biomarkers [6] [40].

In conclusion, the endoscopic mucosal finding does not reflect exactly the actual immunopathological changes of gastric mucosa during *H. pylori* infection. Secretion of gastrin was not affected by the presence of Cag A in gastric tissue. Instead, the fluctuation in the hormone level appears to be due to the presence of H.pylori infection in gastric tissue. Infiltration of gastric tissue with inflammatory infiltrates mainly PMNs and lymphocytes has a direct effect on PGI; PGII and gastrin levels in serum of infected patients. The level of PG I; PG II; G-17 secretion correlated with the development of immune response against *H. pylori* and production of specific *H. pylori* IgG. Finally, *H. pylori* have the ability to modulates gastric secretions through CagA dependent and independent pathways.

Current results recommend the need for further Intensive studies to determine the network of other virulence factors that play a destructive role on the level of gastric hormones and lead to tissue damage that may alter the clinical pathway from simple inflammation to the tumour**.**

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# Maternal and Neonatal Outcomes in Pregnant Women with Gestational Diabetes Mellitus Treated with Diet, Metformin or Insulin

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**Abstract**

**AIM:** Aim of the study was to compare outcomes of pregnancy in gestational diabetes mellitus (GDM) treated with metformin, insulin, or diet.

**MATERIAL AND METHODS:** The study included 48 women with GDM treated with metformin, 101 with insulin, and 200 women on a diet from the Outpatient Department of Endocrinology and University Clinic of Obstetrics and Gynecology in Skopje.

**RESULTS:** The groups were comparable in age, smoking cigarettes and positive family history of diabetes. Mean glycosylated haemoglobin (HbA1c) at 37 gestation week, mean fasting, postprandial glycaemia, and gestational age at delivery were lower in diet and metformin than insulin group. No differences in mode of delivery were observed between the metformin and insulin group. Women in metformin group had a significantly lower incidence of LGA newborns than diet and insulin groups. The percent of SGA new-borns was higher in insulin group than diet and metformin groups. The incidence of neonatal hypoglycemia was statistically significantly higher in the insulin group than in the metformin and diet group.

**CONCLUSION:** Metformin in women with GDM can improve maternal and neonatal outcomes compared with those treated with diet or insulin.

## Introduction

Pregnancy itself is characterised by insulin resistance [1]. Gestational diabetes mellitus (GDM) develops if there is inadequate insulin secretion to compensate insulin resistance.

GDM increases the risk of pregnancy complications and adverse neonatal outcomes. Excessive mother to fetus glucose transfer increases the risk for large or small for gestational age newborn, neonatal hypoglycaemia and neonatal respiratory distress syndrome, as well as increased risk for preeclampsia, cesarean, preterm delivery and higher risk for development of type 2 diabetes mellitus after pregnancy in women with GDM. Meta-analysis of several randomised trials has shown that appropriate therapy can decrease maternal and fetal morbidity [2] [3]. An effective treatment regimen consists of diet alone for most patients and the administration of insulin if target blood glucose concentrations are not met with diet alone.

Prospective randomised studies demonstrated that effective treatment of hyperglycemia in women with GDM could reduce adverse perinatal outcomes [2]. The treatment is with diet, metformin or insulin. Insulin treatment is safe and effective for pregnant women, but the disadvantages of insulin are: need to give injections, the risk of hypoglycemia, the risk of excessive weight gain, and cost [1]. Therefore, oral metformin is a logical option

for pregnant women with GDM. It does not induce hypoglycemia, and it is not associated with increased weight gain. Also, metformin improves insulin sensitivity probably by activating AMP kinase and reduces hepatic gluconeogenesis, which could be beneficial for preservation of β–cell function [1]. But it has been found that metformin has a maternal to the fetal transfer rate of 10-16% which might be associated with fetal anomalies or potential adverse effects for mother and newborns [4].

The randomised trials and observational studies observed that maternal glucose levels did not differ between pregnant women treated with insulin versus those treated with an oral glucose-lowering agent such as metformin. There is a small number of studies reporting on the use of metformin during GDM pregnancy. They provide conflicting information about the safety of metformin use in GDM pregnancies or type 2 diabetes and pregnancy [1] [10] [17].

The study aimed to compare maternal and neonatal outcomes in patients with gestational diabetes mellitus (GDM) treated with metformin versus those with insulin, or diet alone.

## Material and Methods

Three hundred and forty-nine women with GDM that have consulted the Outpatient Department of University Clinic of Endocrinology Diabetes and Metabolic Disorders were enrolled. From them, 48 women were treated with metformin, 101 with insulin, and 200 received no pharmacological treatment, were treated only with the dietary regimen. All were with singleton pregnancies and gave informed consent to participate in the study.

The diagnosis for GDM was made with 75 gr OGTT (normal values: a fasting level < 5.1; 1-hour level < 10.0 and 2-hour level < 8.5 mmol/L), according to The International Association for Diabetes and Pregnancy Study Group (IADPSG). Only one abnormal plasma glucose level was sufficient for the diagnosis of GDM4

The studied outcome measures were: glycemic control, maternal, and neonatal outcomes.

All women were asked to perform a daily glucose profile (fasting, pre-prandial and 1-h postprandial measurements) twice a week from the diagnostic moment of GDM until delivery, using a home glucometer (OneTouch Basic 200-200; LifeScan, Milpitas, California, USA). Multiple daily measurements (at least four a day) allowed recognition of women who should begin an anti-hyperglycemic agent. The desirable target glucose levels were: fasting glycaemia between 3.8 to 5.0 mmol/l and one-hour postprandial blood glucose concentration < 7.8 mmol/l (American Diabetes Association). At 37 week of gestation after overnight fasting, HbA1c was taken and was measured by anion-exchange HPLC instrument (DS5; Drew, USA) with a reference range of 4.2–6.5%.

The mode of treatment (diet, metformin or insulin), based on self-monitored plasma glucose values, was determined within a week after starting monitoring. Women with GDM on a diet were educated regarding an individualised diabetic diet based on pre-pregnancy weight (30 kcal/kg/day) with a caloric restriction for overweight and obese women (25 kcal/kg/day). Metformin was given at a dose of 500 mg three times a day to a maximum of 2000 mg/day based on the glycemic profile. Adjustments in the insulin doses were made if two or more glycaemic values were consistently higher than the target blood glucose concentrations, in a two-week interval. Insulin therapy in the regime of multiple injections of short (apart) and long-lasting analogue (detemir) was introduced, starting from 0.3 IU/kg of body weight. According to blood glucose profile, the insulin doses were changed by 2 to 4 units at a time.

At the first visit all patients were asked about their age, weight before pregnancy, gestational week, smoking habits and familial history of diabetes. Body mass index before pregnancy was calculated retrospectively. Weight before delivery was measured again in all patients wearing clothes without shoes in the morning. Height was measured to the nearest 1 cm with a stadiometer.

At every visit, blood pressure was measured twice in a supine position. In a case of hypertension (>145/90 mmHg), the measurement was repeated after five minutes. Preeclampsia was registered if blood pressure was >140/90 mmHg with proteinuria >0.3 g/24 h.

Mode of delivery was noted as spontaneous, assisted or caesarean section. Birth weight and the proportion of LGA (defined as birth weight > 90th percentile for local population after adjusting for gestational age and sex) and SGA (defined as a birth weight < 10th percentile for local population after adjusting for gestational age and sex) were determined. Prematurity was defined as born before 37 gestational weeks. The gestational age of newborns was estimated from the date of the last menstrual period. Neonatal serum glycaemia was measured after delivery and values lower than 2.6 mmol/l were considered as hypoglycemia. Apgar score was measured at 1’ and 5’ after delivery, but we used only values at 5’.

All neonatal outcomes were performed in University Clinic of Gynecology and Obstetrics.

Statistical analyses were performed using SPSS software for Windows, version 14.0. Dates are given as mean ± standard deviation and percent. We used a t-test for independent samples to compare the

numeric variables between each of the groups; for categorical variables, Chi-square test was used. Values of $p < 0.05$ were considered statistically significant.

## Results

From 349 GDM pregnancies, 200 were treated with diet alone, 101 with insulin, and 48 with metformin.

Baseline characteristics of the women enrolled in the study are given in Table 1. It can be seen that women treated with diet have lower BMI before pregnancy, but higher weight gain during pregnancy, then the other two groups. The weight gain was lowest in the metformin group. Patients treated with insulin enrolled earlier in the study than patients from other groups. Between the three groups, no significant differences in the incidence of smoking cigarettes and familial history of diabetes were noted.

**Table 1: Maternal Characteristics at Baseline**

| | Diet (N = 200) | Metformin (N = 48) | Insulin (N = 101) | Metfom vs. diet P | Metfom vs.insuli P | Diet vs. insulin P |
|---|---|---|---|---|---|---|
| Age (years) | 31.5 ± 5.2 | 32.2 ± 4.7 | 32.7 ± 5.7 | NS | NS | NS |
| Pre-pregnancy BMI (kg/m$^2$) | 26.7 ± 5.3 | 28.8 ± 5.3 | 27.5 ± 4.9 | < 0.05 | NS | NS |
| Weight gain (kg) | 10.9 ± 6.1 | 8.1 ± 4.9 | 8.7 ± 6.1 | < 0.01 | NS | < 0.01 |
| Gestational week at enrolment (g.w.) | 29.5 ± 5.8 | 28.6 ± 5.6 | 24 ± 7.8 | NS | < 0.01 | < 0.01 |
| Smoking cigarettes (%) | 20 (10%) | 5 (10.4%) | 11 (10.8%) | NS | NS | NS |
| Familiar history for diabetes (%) | 105 (52.5%) | 24 (50%) | 62 (62%) | NS | NS | NS |

Mean glycosylated haemoglobin (HbA1c) at 37 gestation week was statistically significantly lower in diet and metformin groups than in insulin group (Table 2). Mean fasting (FPG) and postprandial glycaemia (PPG) was statistically significantly lower in diet and metformin group than in insulin group (Table 2). The percent of preeclampsia was higher in the metformin group but without statistical significance between metformin and insulin groups; only between diet and other two groups.

Women treated with insulin had delivery earlier than those treated with metformin or diet alone. This difference was statistically significant. Caesarean deliveries were more likely in women treated with insulin and metformin than in the diet group (Table 2). The percent of LGA newborns was statistically significantly lower in metformin-treated group versus diet and insulin groups. The percent of prematurity was statistically significantly higher in the insulin group than in the diet and metformin groups. The percent of SGA was statistically significantly higher in the insulin group than in the diet and metformin groups. Mean birth weight in insulin group was statistically significantly lower than in diet and metformin groups. The incidence of neonatal hypoglycemia was statistically significantly higher in the insulin group compared with those treated with metformin or diet. There were no differences in Apgar scores in 5' between the three groups (Table 3).

**Table 2: Maternal primary outcomes**

| | Diet (N = 200) | Metformin (N = 48) | Insulin (N = 101) | Metfom vs. diet P | Metfom vs.insulin P | Diet vs. insulin P |
|---|---|---|---|---|---|---|
| HbA1c at 37 g.w. (mean) | 5.4 ± 0.9 | 5.3 ± 0.7 | 6.2 ± 1.8 | NS | < 0.01 | < 0.01 |
| Fasting glycaemia mmol/l | 5.1 ± 0.9 | 5.3 ± 0.7 | 5.8 ± 1.4 | NS | <0.05 | <0.01 |
| Postprandial glycaemia (PPG) mmol/l | 6.9 ± 1.6 | 7.0 ± 1.2 | 7.9 ± 1.9 | NS | <0.05 | < 0.05 |
| Preeclampsia | 1 (0.5%) | 4 (8.3%) | 6 (6%) | < 0.01 | NS | < 0.01 |
| Gestational age at delivery (g.w.) | 38.9 ± 1.9 | 38.9 ± 1.4 | 37.5 ± 2.2 | NS | < 0.01 | < 0.01 |
| Mode of delivery<br>-spontaneous<br>- assisted<br>- caesarean section | 86/130 (66.1%)<br>3/130 (2.3%)<br>41/130 (31.5%) | 22/46(47.8 %)<br>0<br>24/46(52.2 %) | 34/100 (34%)<br>0<br>66/100 (66%) | < 0.05<br>NS<br>< 0.05 | NS<br>NS<br>NS | < 0.05<br>NS<br>< 0.05 |

There were no major complications or perinatal deaths in this study. One neonate of a mother treated with insulin had asphyxia. There were no cases of diabetic ketoacidosis or lactic acidosis.

**Table 3: Neonatal primary outcomes**

| | Diet (N = 200) | Metformin (N = 48) | Insulin (N = 101) | Metfom vs. diet P | Metfom vs.insulin P | Diet vs. insulin P |
|---|---|---|---|---|---|---|
| Birth weight (gr) | 3631 ± 650 | 3496 ± 480 | 3348 ± 739 | NS | NS | < 0.01 |
| Prematurity | 13 (6.5%) | 2 (4.2%) | 20 (19.8%) | NS | < 0.01 | < 0.01 |
| LGA (> 2SD/%) | 59 (29.5%) | 6 (12.5%) | 22 (21.7%) | < 0.05 | < 0.05 | NS |
| SGA (< 2SD/%) | 8 (4%) | 3 (6.2%) | 14 (13.8%) | NS | NS | < 0.01 |
| Neonatal glycaemia (mean, % with hypoglycae-mia) | 3.3 ± 1.2 (24%) | 2.8 ± 1.1 (35.4%) | 2.6 ± 1.1 (51.5%) | < 0.05<br>< 0.05 | < 0.05<br>< 0.05 | < 0.01<br>< 0.01 |
| Apgar score at 5 | 8.9 ± 0.7 | 8.6 ± 0.7 | 8.6 ± 0.8 | NS | NS | NS |

LGA-large for gestational age; SGA-small for gestational age; SD-standard deviation.

Correlation analysis found a statistically significant positive correlation between preeclampsia and pre-pregnancy BMI. Statistically significant positive correlations were found between baby birth weight and weight gain and between baby birth weight and HbA1c at 37 g.w. Also, there was statistically significant positive correlation between HbA1c and incidence of LGA newborns. Fasting plasma glucose values had a statistically significant positive correlation with pre-pregnancy BMI.

## Discussion

Our study has shown that women with GDM treated with metformin had similar, even better outcomes than those treated with diet or insulin alone.

Metformin reduces hyperglycemia by suppressing hepatic glucose output (hepatic gluconeogenesis), increasing insulin sensitivity and enhancing peripheral glucose uptake [6]. The weight gain was lower in metformin and insulin groups compared to the diet group. These effects are potentially useful during pregnancy when glucose control deteriorates with changes in insulin resistance [7].

Mean HbA1c at 37 gestation week, mean FPG and PPG were statistically significantly lower in diet and metformin groups than in the insulin group. Better glycemic profiles in the metformin group in comparison to the insulin group can be explained by reducing insulin resistance in GDM pregnancies which is the main pathophysiologic way for developing gestational diabetes in pregnancy. Similar results have been noted in other studies [8]. In this study, HbA1c was shown as an important factor for increased incidence of delivery LGA newborns. The better glycemic control means lower risk for LGA, which can be achieved with metformin.

We noted a high incidence of preeclampsia in the metformin group, identically as Hellmuth et al9. But contrary studies found that metformin may reduce preeclampsia in GDM women by reducing the endothelial activation and maternal inflammatory response to insulin resistance [8][10]. However, the percent of preeclampsia in the metformin group was not statistically significantly greater than the percent of preeclampsia in the insulin group. Fluctuating glucose levels have a stronger effect on endothelial function, which is more important in the pathogenesis of preeclampsia, than sustained hyperglycaemia [11] [12]. This can be the explanation for our findings. Unrelated to metformin use, other increased risk factors for pre-eclampsia, such as older age or overweight may exist. In our study, the groups were matched for age, but the percentage of obese woman in the metformin group was higher than in the insulin and diet groups. Also, their antihyperglycemic medication was started four weeks later than the initiation of insulin.-

Average gestational age at delivery was significantly lower in the insulin group, and consequently, the percent of prematurity and SGA newborns was higher in the insulin group. Contrary there is a number of studies with opposite findings10, [13] [14] [15] [16] [17]. Only Balani et al., [14] and Goh et al., [18] presented identical results as ours. In their studies, the percent of prematurity was lower in women treated with metformin compared with insulin group. Also, they found the higher percent of caesarean delivery in insulin group than metformin. Probably, a higher percent of LGA newborns in insulin group was responsible for higher incidence of caesarean section in those patients.

Surprisingly, although mean glycemic values were higher in the insulin group, the percent of SGA newborns was higher. It can be explained by a high incidence of prematurity in the insulin group. Similar results were presented by Lavanya et al., [19].

We found significantly fewer macrosomic neonates in the metformin group than in the diet and insulin groups. Unlike our experience, in the MiG9 trial, there was no significant difference in the proportion of LGA newborns in metformin versus insulin group. The addition of supplementary insulin in metformin group in the above study may be responsible. Similar results as ours were presented in the study of Gandhi et al., [20].

In correlation with the previous studies [9] [17] [18] [21], the incidence of neonatal hypoglycemia was reduced in the metformin group in comparison to the insulin group.

Mean Apgar scores at 5’ were almost identical in the metformin and insulin groups, higher than in the diet group, but without statistical differences. This is consistent with other studies [9] [21].

The study has several limitations. It was not randomised, and small number women were included. Baseline differences between the groups might have influenced the outcomes.

The percentage of GDM patients needing pharmacological treatment varies from 20% to 60% in various studies [22]. That’s not a small number. Insulin has several disadvantages including multiple daily injections, the risk of hypoglycemia and maternal weight gain [23]. On the other hand, metformin is more acceptable to women with GDM, it’s safe, with no significant maternal or neonatal outcomes, and has low cost. If metformin had any unanticipated adverse effect on fetal growth or well-being, there would be more iatrogenic preterm births [1]. But, the frequency of preterm births in our study was higher in the insulin group than in the metformin group. Metformin use in our study was not associated with increased perinatal complications. Even more, metformin treatment resulted in better glycemic control and improved neonatal outcomes compared with insulin. Because metformin crosses the placenta, Glueck et al., [24] assessed long-term effects of metformin on the children. They presented that growth, motor and social development in the offspring of mothers who conceived and continued on metformin did not differ from that of control babies over the first 18 months of life. So, metformin may have its place as first line GDM therapy, especially in a subgroup of patients that are overweight but not obese, but between one third and one half of women will need insulin to achieve glycemic targets. However, further clinical long-term follow-up studies are needed to determine the role of metformin as an alternative treatment to insulin in GDM patients.

In conclusion, according to the current knowledge, metformin is effective and safe in the treatment of GDM, because women with GDM treated

with metformin had less weight gain and improved neonatal outcomes compared with those treated with diet or insulin. But it is not known whether fetal exposure to an insulin-sensitizing agent, such as metformin is beneficial or harmful, and thus caution is warranted in its use in pregnancy.

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# Absent $^{99m}$Tc-MIBI Uptake in the Thyroid Gland during Early Phase of Parathyroid Scintigraphy in Patients with Primary and Secondary Hyperparathyroidism

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**Keywords:** Parathyroid scintigraphy; Diminished thyroid MIBI uptake; Primary hyperparathyroidism; Chronic renal failure

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**Abstract**

**BACKGROUND:** Thyroid uptake of technetium-99m methoxyisobutylisonitrile ($^{99m}$Tc-MIBI) during parathyroid scintigraphy can be affected by various conditions.

**AIM:** To evaluate the frequency of absent $^{99m}$Tc-MIBI uptake by the thyroid gland in the early phase of dual-phase parathyroid scintigraphy.

**METHODS:** The early planar images of dual phase Tc$^{99m}$ MIBI parathyroid scintigraphy from 217 patients performed between 2014 and 2017 were retrospectively analysed. Patients were divided into two groups. The first group included 147 patients with primary hyperparathyroidism and the second group included 70 patients with chronic renal failure. Patient records, laboratory and ultrasonographic data were analysed in all patients. Descriptive statistic was used for data analysis.

**RESULTS:** Out of all patients in the first group, 18 patients (12.24%) showed absent thyroid uptake. Thyroidectomy was performed in 44.4% of these patients, and the rest of them had some thyroid disease. Only one patient had no thyroid or another chronic disease. In the second group, 8 patients (11.42%) presented with absent thyroid uptake of MIBI. Among them, 5 patients had no history of thyroid disease and had been on hemodialysis programme, and 3 patients had hypothyroidism.

**CONCLUSION:** Absent $^{99m}$Tc-MIBI uptake in the thyroid during the early phase of parathyroid scintigraphy is most frequently related to thyroid disease. A small proportion of patients with chronic renal failure can present with absent $^{99m}$Tc-MIBI uptake in the thyroid as well. The mechanism for this alteration is still unclear and needs further investigation.

## Introduction

Parathyroid scintigraphy is an imaging modality used in the diagnosis and preoperative localisation of parathyroid adenomas or hyperplastic parathyroid glands in patients with hyperparathyroidism. Several protocols are used for this purpose: Dual-phase single isotope imaging, single phase dual-isotope subtraction method or a combination of the two [1] [2]. Most commonly used method in clinical practice nowadays is Dual-phase technetium-99m methoxyisobutylisonitrile ($^{99m}$Tc-MIBI) scintigraphy because of its high specificity and simplicity, despite the low sensitivity for detection of very small adenomas.

MIBI is a lipophilic, monovalent, cationic complex. The uptake by parathyroid tumours was first published in 1989 by Coakley et al., [3]. MIBI is distributed proportionally to blood flow in the body and is sequestered intracellularly within the mitochondria by passive diffusion in cells with negative transmembrane potential. The slower MIBI washout from the parathyroid adenomas and hyperplastic glands compared with the normal thyroid and parathyroid tissue provides the possibility for the use of this radiopharmaceutical for this kind of imaging. The main pathophysiological mechanism for slower MIBI washout from the parathyroid adenomas lies within the increased number of mitochondria in hyperactive parathyroid cells [4]. Several factors can influence the normal distribution of $^{99m}$Tc-MIBI in the early phase of the scanning in the thyroid and parathyroid glands. Awareness about variations in the

biodistribution of MIBI is important for the proper interpretation of the scan results. Diminished MIBI uptake in the thyroid and parathyroid adenomas is reported in several studies [5] [6]. Our study aimed to evaluate the frequency of absent $^{99m}$Tc-MIBI uptake by the thyroid gland in the early phase of dual-phase parathyroid scintigraphy in patients with primary hyperparathyroidism and patients with secondary hyperparathyroidism due to chronic renal failure.

## Material and Methods

Retrospective analysis of 278 parathyroid scintigraphies from 217 patients (151 females 66 males, aged 16 to 82 years, and mean age 54.7 ± 14.13) from the archive of the Institute of Pathophysiology and Nuclear Medicine at the Medical Faculty in Skopje was performed. The scintigraphies were performed under the same protocol in the period from January 2014–June 2017. Patients were divided into two groups. The first group included 147 patients, 119 females 28 males, age range 16-78 years (mean age 55.9 ± 12.75), referred for investigation of primary hyperparathyroidism and the second group included 70 patients, 32 females 38 males, age range 16-82 years (mean age 53 ± 14.58), referred with diagnosis of secondary hyperparathyroidism due to chronic renal failure. The parathyroid scintigraphy was performed after IV injection of 740 MBq $^{99m}$Tc-MIBI. Early scans of the neck and the upper thorax were obtained 10 minutes after injection with a planar gamma camera in AP view (MEDISO-DHV) with low energy, general purpose collimator (LEGP) and 128 x 128 matrix. Late planar scans were obtained 2 hours after injection.

Some patients had SPECT/CT (OPTIMA NM/CT 640, GE Healthcare) performed in the delayed phase. Thirty 15-second images were taken by each head over 180-degree stepwise rotation and stored in a 128*128 matrix of a frame mode. All patients had thyroid ultrasound performed at our Institution with a high-resolution broadband linear array transducer (LN 12-3, Philips HD6) to verify the presence/ absence of parathyroid adenoma and to exclude the presence of thyroid nodules or other focal lesions in the thyroid gland.

All scans were evaluated for $^{99m}$Tc-MIBI uptake in the thyroid gland in the early phase (thyroid phase) qualitatively by 2 nuclear medicine specialists blinded to patient data. The patients' groups were further subdivided according to these findings. The patients from the first group with a normal distribution of $^{99m}$Tc-MIBI were classified as group 1a, and the patients with absent thyroid $^{99m}$Tc-MIBI uptake as group 1b. The patients in the second group were subdivided in the same manner. Anamnestic and laboratory data from the patients' files were analysed in all patients. The laboratory tests were not performed at our institution. The data were evaluated with the methods of descriptive statistics.

## Results

Thyroid $^{99m}$Tc-MIBI uptake in the early phase of parathyroid scintigraphy was absent in 26 patients from both groups. The rest of the patients, 129/147 in the first group with primary hyperparathyroidism and 62/70 from the second group with secondary hyperparathyroidism had normal thyroid uptake of $^{99m}$Tc-MIBI in the early phase and complete washout of the tracer in the delayed planar images of parathyroid scintigraphy. Figure 1 presents a patient with normal uptake in the thyroid during parathyroid scintigraphy.

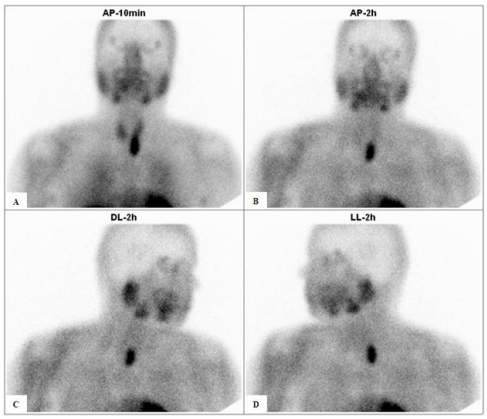


*Figure 1: Early-phase (A) and delayed phase: anterior view (B), right lateral (C), left lateral (D) planar images with $^{99m}$Tc-MIBI in a 42-year-old male patient referred for investigation of primary hyperparathyroidism. The PTH level was measured 890 pg/ml; plasma ionised calcium level was elevated 3.87 mmol/L. The patient had no history of thyroid disease, TSH level was within the normal range*

Early-phase (A) image shows normal uptake of $^{99m}$Tc-MIBI in the thyroid gland and focus of increased activity in the lower pole of the left lobe. Delayed phase images (B; C; D) show normal clearance of $^{99m}$Tc-MIBI from the thyroid and persistence of the focal increased activity, a finding consistent with parathyroid adenoma.

Concomitant thyroid disease was observed in 35/129 patients (27.1%) with suspicion for primary hyperparathyroidism and 6/62 patients (9.7%) with chronic renal failure. The data regarding early thyroid $^{99m}$Tc-MIBI uptake, and the frequency of thyroid disease in both patients' groups are presented in Table 1.

The most frequent thyroid disease in patients with primary hyperthyroidism and normal $^{99m}$Tc-MIBI thyroid uptake (group 1a) was nodular goitre in 19 patients followed by hypothyroidism due to chronic

autoimmune thyroiditis (CAD) in 9 patients and euthyroid CAD in 5 patients.

**Table 1: Number of patients with absent and normal early $^{99}$Tc MIBI thyroid uptake in both groups with data on the presence/absence of thyroid disease**

| Primary hyperthyroidism | | | | Secondary hyperthyroidism | | | |
|---|---|---|---|---|---|---|---|
| 147 patients | | | | 70 patients | | | |
| Absent thyroid uptake | | Normal thyroid uptake | | Absent thyroid uptake | | Normal thyroid uptake | |
| 18 (12.2%) | | 129 (87.8%) | | 8 (11.4%) | | 62 (88.6%) | |
| TD | NTD | TD | NTD | TD | NTD | TD | NTD |
| 17 (94%) | 1 (6%) | 35 (27.1%) | 94 (72.9%) | 3 (37.5%) | 5 (62.5) | 6 (9.7%) | 56 (92.3%) |

TD thyroid disease; NTD no thyroid disease

Only 2 patients had a history of Graves' disease but were in remission when scintigraphy was performed. The cause of thyroid disease in group 2a was nodular goitre in 5 patients and CAD with hypothyroidism in 1 patient. The data presenting the type of thyroid disease in all patients' groups are shown in Fig. 2.

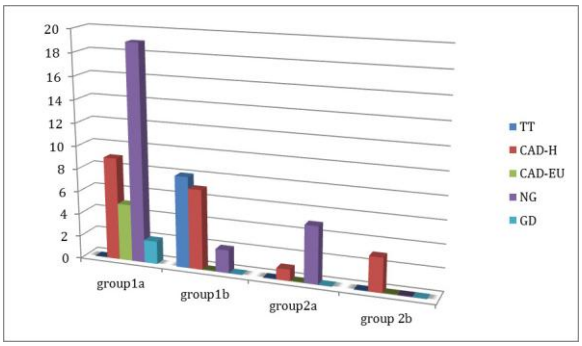


*Figure 2: Thyroid diseases in all patients' groups. TT-Total thyroidectomy; CAD-H Hypothyroid chronic autoimmune disease; CAD-EU Euthyroid chronic autoimmune disease; NG Nodular goitre; GD Graves disease*

The scan analysis showed that in the group of patients with primary hyperparathyroidism 18/147 patients (12.24%) showed absent thyroid uptake (group 1b). Further analysis showed that 8 out of 18 patients (44.4%) had previously performed thyroidectomy due to various reasons and their ultrasound revealed minimal to absent residual tissue in the thyroid bed. The rest of the patients except one had thyroid disease. Out of these 9 patients, 7 patients had hypothyroidism due to chronic autoimmune thyroiditis. All 7 patients were on L-thyroxine substitution therapy and had TSH level within normal range. This was the most frequently encountered thyroid disease 7/9 (77.77%), and the other patients had nodular goitre 2/7 (22.22 %). The ultrasound report in patients with autoimmune thyroiditis confirmed normal sized thyroid with non-homogenous structure in 4 patients, while 3 patients had a small atrophic thyroid gland. Only one patient presented with completely normal thyroid ultrasound, thyroid pertechnetate scan and normal values for thyroid hormones and antiperoxidase antibodies and absent thyroid uptake of MIBI. The patient didn't have a history of another chronic disease, nor was taking any medications or nutritional supplements. The characteristics of the patients in this group are summarised in Table 2. The scintigraphy results showed parathyroid adenoma or hyperplasia in 10 out of 18 patients. The laboratory tests collected from the patients' files were not performed at the same institution and were frequently missing. Therefore, statistical analysis was not performed for these data.

**Table 2: Clinical, biochemical, ultrasonographic and scintigraphic data of patients with primary hyperthyroidism with diminished thyroid uptake**

| Patient no. | Gender | Age (years) | iPTH (pg/ml) | Ca++ (mmol/L) Total Ca* (mmol/L) | Thyroid US report | TD | Scintigraphy report |
|---|---|---|---|---|---|---|---|
| 1 | F | 38 | 140.1 | 1.43 | Normal sized thyroid with the isoechoic structure | No TD | Positive |
| 2 | F | 40 | 170.7 | 1.49 | Normal sized thyroid with isoechoic structure and an anechoic nodule in the right lobe with dimension 21 mm | NG | Positive |
| 3 | F | 60 | 3295 | 3.64* | Normal sized thyroid with isoechoic structure and a hypoechoic nodule in the right lobe with dimension 25 mm | NG | Positive |
| 4 | F | 60 | 82.2 | NA | Small sized thyroid with the hypoechoic and inhomogeneous structure | CAT-H | Positive |
| 5 | F | 63 | 105.5 | 1.44 | Small sized thyroid with isoechoic and inhomogeneous structure with fibrotic septae | CAT-H | Negative |
| 6 | F | 66 | 331 | 2.9* | Normal sized thyroid with hypoechoic and inhomogeneous structure with fibrotic septae | CAT-H | Positive |
| 7 | F | 52 | 183 | NA | Normal sized thyroid with hypoechoic and inhomogeneous structure with fibrotic septae | CAT-H | Positive |
| 8 | F | 57 | 520.2 | 1.61 | Normal sized thyroid with isoechoic and inhomogeneous structure with fibrotic septae and small circumscribed hypoechogenic lesions | CAT-H | Positive |
| 9 | F | 33 | 263 | 1.5 | Small sized thyroid with the isoechoic and inhomogeneous structure | CAT-H | Negative |
| 10 | F | 72 | 304 | 2.9* | Normal sized thyroid with the isoechoic and inhomogeneous structure | CAT-H | Positive |
| 11 | F | 42 | 140.4 | 1.12 | Absent thyroid gland | TT | Negative |
| 12 | F | 66 | 119 | 1.23 | Residual thyroid parenchyma in the right thyroid bed with dimension 7 mm | TT | Negative |
| 13 | F | 58 | 134 | NA | Absent thyroid gland | TT | Negative |
| 14 | F | 68 | 391.5 | 1.2 | Absent thyroid gland | TT | Positive |
| 15 | F | 43 | NA | NA | Residual thyroid parenchyma in the right thyroid bed | TT | Negative |
| 16 | F | 40 | NA | NA | Absent thyroid gland | TT | Positive |
| 17 | F | 62 | NA | NA | Residual thyroid parenchyma in the left thyroid bed | TT | Negative |
| 18 | F | 66 | NA | NA | Absent thyroid gland | TT | Negative |

m: male; f: female; iPTH: intact parathyroid hormone; $Ca^{++}$: plasma ionized calcium; * total plasma calcium level; NA: not available; US: ultrasound; TD: thyroid disease; NG: Nodular goiter; CAT-H: Hypothyroidism due to chronic autoimmune thyroiditis; TT: Total thyroidectomy; Scintigraphy report-Positive: presence of parathyroid adenoma/hyperplasia; Negative: absence of parathyroid adenoma/hyperplasia.

Normal ranges for laboratory results: iPTH: 12.0-65.0 pg/ml, $Ca^{++}$: 1.12-1.32 mmol/L, Total plasma Ca: 2.10-2.16 mmol/L. In the group of patients with secondary hyperparathyroidism due to chronic kidney failure, 8 patients presented with absent thyroid uptake of MIBI in the thyroid phase of the scanning (Figure 3).

From these patients (group 2b), 5 patients (7.1%) had normal thyroid ultrasound and no history of thyroid disease. The laboratory tests for thyroid function were within normal range. One of the patients had repeated scans, and both times the thyroid uptake was absent during the early phase. The other 3 patients had hypothyroidism and were on L-thyroxine substitution therapy with TSH level within normal range. All 5 patients had been on hemodialysis programme for more than 5 years (range 7-19 years, mean 12 ± 5.5), and had high levels of PTH (>1000pg/ml, mean 1889 ± 750.76). The data for

serum calcium and phosphate levels were not available in all patients. The patients' data are presented in Table 3.

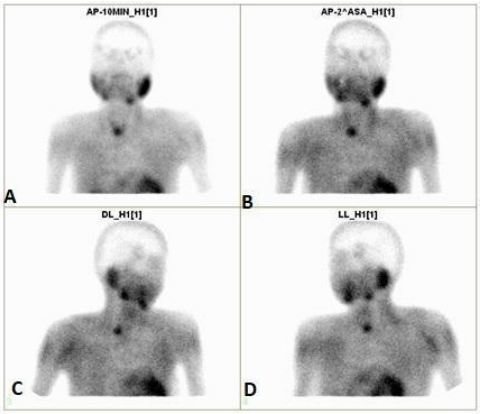


*Figure 3: Early-phase (A) and delayed phase: anterior view (B); right lateral (C); left lateral (D) planar images with $^{99m}$Tc-MIBI in a 45-year-old female patient with chronic renal failure who has been on hemodialysis treatment for 9 years. The PTH level was measured 2500 pg/ml, and the patient had no history of thyroid disease. In both the early and the delayed images it was not possible to see any activity in the thyroid region, only higher uptake in the lower pole of the right lobe suggestive of parathyroid adenoma*

Data analysis showed that in our patients with chronic kidney failure 7.1% of patients had absent thyroid MIBI uptake in the early phase and no history of thyroid disease. The uptake on the early and late scans in the parathyroid lesions was visible in 4 patients, while in one patient there was no uptake in thyroid or parathyroid lesion. After surgery in this patient, parathyroid hyperplasia was confirmed on histopathology.

## Discussion

The early uptake and washout of MIBI in the thyroid gland can be affected by several factors. The most frequently encountered reason is the pathology of the thyroid gland. The increased uptake and slow washout in some thyroid nodules are well-established fact. This scintigraphy pattern was related with a higher probability of malignancy in several studies [7], but can also lead to difficulties in the interpretation of parathyroid scintigraphy especially in the departments where SPECT/CT is not available. Thyroid nodules can also have diminished uptake, although there are not many studies that were studying the early uptake 5-10 minutes after injection. This pattern, found in 2 patients in our study, is most probably due to degenerative changes in the nodule, although histology data was not available. The diminished thyroid uptake can also be attributed to other thyroid pathologies. There are several reports that connect the chronic thyroiditis with diminished MIBI uptake [8][9]. The study of Santos et al. was designed to estimate the thyroid MIBI uptake at a 5$^{th}$ minute after injection in patients with different thyroid pathologies. The thyroid MIBI uptake was correlated to euthyroid patients referred for cardiac perfusion scintigraphy. This study reports reduced thyroid MIBI uptake in patients with atrophic Hashimoto thyroiditis, but higher uptake compared with euthyroid control group in patients with hypertrophic Hashimoto disease. The authors concluded that this kind of findings is probably due to glandular destruction and fibrosis in chronic atrophic thyroiditis [9]. Our study confirmed diminished uptake in 7 patients with hypothyroidism due to chronic autoimmune thyroiditis in patients with primary hyperparathyroidism, and in 3 patients with secondary hyperparathyroidism who had been diagnosed with hypothyroidism. The ultrasound was in concordance with normal sized or atrophic thyroid with signs of diffuse changes in the parenchyma. All patients were hypothyroid, with L-thyroxine substitution therapy at the time of MIBI scintigraphy. The administration of L-thyroxine to suppress the thyroid was claimed by some authors to improve the sensitivity of parathyroid scintigraphy in primary hyperparathyroidism [10].

**Table 3: Clinical, biochemical, ultrasonographic and scintigraphic data of patients with chronic kidney failure with diminished thyroid uptake in the early phase of parathyroid scintigraphy**

| Patient no. | Gender | Age (years) | iPTH (pg/ml) | Ca++ (mmol/L) Total Ca* (mmol/L) | P (mmol/L) | Thyroid US report | TD | Scintigraphy result | HD duration (years) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | M | 55 | 2830 | NA | NA | Normal sized thyroid with the isoechoic structure | No TD | Positive | 7 |
| 2 | F | 59 | 1016 | 2.58 | 1.76 | Normal sized thyroid with isoechoic structure | No TD | Positive | 8 |
| 3 | F | 45 | 2500 | 1.10 2.18* | 1.26 | Normal sized thyroid with isoechoic structure | No TD | Positive | 9 |
| 4 | M | 61 | 1500 | NA | NA | Normal sized thyroid with the isoechoic structure | No TD | Positive | 19 |
| 5 | M | 53 | 1602 | 2.18 | 1.33 | Normal sized thyroid with the isoechoic structure | No TD | Negative | 17 |
| 6 | F | 65 | 88 | 2.5* | 2.00 | Small sized thyroid with the isoechoic and inhomogeneous structure | H | Positive | 18 |
| 7 | F | 63 | 474.4 | 2.2 | 1.32 | Small sized thyroid with isoechoic, inhomogeneous structure and fibrotic septae | H | Positive | 6 |
| 8 | F | 66 | 1347 | NA | 2.2 | Small sized thyroid with isoechoic, inhomogeneous structure and fibrotic septae | H | Positive | NA |

**Abbreviations:** m: male, f: female, iPTH: intact parathyroid hormone, $Ca^{++}$: plasma ionized calcium, * total plasma calcium level, P: phosphorus, NA: not available, US: ultrasound, TD: thyroid disease, H: Hypothyroidism, HD: hemodialysis, Scintigraphy report: Positive: presence of parathyroid adenoma/hyperplasia; Negative: absence of parathyroid adenoma/hyperplasia Normal ranges for laboratory results: iPTH: 12.0-65.0 pg/ml, $Ca^{++}$: 1.12-1.32 mmol/L, Total plasma Ca: 2.10-2.16 mmol/L, P: 0.80-1.40 mmol/L.

The TSH in our patients was in normal range; therefore we concluded that the reduced thyroid uptake was due to the parenchymal destruction rather than the L-thyroxin administration. Diminished thyroid MIBI uptake is well documented in patients with amiodarone-induced hyperthyroidism type 2 [11]**.** The possible pathophysiological mechanism for reduced MIBI uptake in this type of thyroiditis is the presence of apoptotic or necrotic processes involving mitochondrial membrane potential collapse in these patients. However, there were no patients with this kind of thyroid disruption in our patients.

Thyroid diseases were almost the only cause of absent early thyroid MIBI uptake during parathyroid scintigraphy in our patients with primary hyperparathyroidism. Thyroid disease also caused for absent early thyroid MIBI uptake in 3 patients with secondary hyperparathyroidism. However, thyroid disease was also present in patients with normal early thyroid uptake (Table 1). The level of the damage to the thyrocytes and their mitochondrial content by thyroid disease is probably the factor that determines the $^{99m}$Tc-MIBI thyroid uptake.

To the best of our knowledge, this is the first study that reports absent thyroid uptake in patients with chronic renal failure without thyroid disease during the early phase of parathyroid scintigraphy. This kind of altered MIBI distribution was reported in 7.1% of patients in this group. Few studies have aimed to evaluate MIBI uptake in the thyroid gland during dual-phase parathyroid scintigraphy in patients with chronic renal failure. The study of Kiratli et al. evaluated the ratio between the ROI over thyroid and ROI over mediastinum in patients on hemodialysis with and without calcitriol supplementation, and in patients without renal disease but with high PTH levels and calcitriol supplementation. These study groups were compared with a control group of patients without parathyroid or renal disease referred for myocardial perfusion scintigraphy. All three groups of patients demonstrated significantly lower thyroid/mediastinum ratio compared to control group of patients [5]. The authors suggested the role of calcitriol supplementation considering that vitamin D receptors and its ligands have been recognised as factors that can influence the expression of P-glycoprotein [12]. P -glycoprotein is well described cellular efflux pump for lipophilic compounds like MIBI. However, the level of P-glycoprotein in the normal thyroid of healthy individuals is not very high and is not considered a factor that influences the faster washout of MIBI from the thyroid compared with parathyroid adenomas [13]. Although it is possible that therapy with calcitriol in patients with chronic renal failure can up-regulate P-glycoprotein and induce very fast washout from the thyroid, the study of Kiratli et al., demonstrated lower MIBI thyroid uptake in hemodialysis patients that were not receiving calcitriol as well [5]. Therefore, lower thyroid MIBI uptake in this group of patients can also be attributed to the other factors that can influence the mitochondrial and plasma membrane potentials and mitochondrial content and function in the thyroid follicular cells. Although this study reported lower early thyroid MIBI uptake, they do not report absent uptake in their patients as we did in a small proportion of our patients. The semiquantitative evaluation was not performed in our study. Therefore it is possible that thyroid uptake was lower than in healthy patients in a larger percentage of patients in our study.

However, not all studies in the literature report diminished thyroid MIBI uptake during the early phase of the parathyroid scintigraphy. Kandeel et al. report normal thyroid uptake in the early scan and appropriate wash out in their series of patients with the end-stage renal disease, although the number of patients was small and the thyroid uptake during parathyroid scintigraphy was not the aim of their research [14]. Our study found normal thyroid MIBI distribution during parathyroid scintigraphy in 88.6% of patients with chronic renal failure. Our study was retrospective and is missing a lot of information on laboratory results and therapy regimens in these patients. Therefore the mechanism underlying this altered distribution cannot be explained. Larger prospective studies might offer better insight on the biodistribution of $^{99m}$Tc-MIBI in patients with secondary hyperparathyroidism due to chronic renal disease.

The most common cause of altered biodistribution of MIBI in the thyroid during parathyroid scintigraphy is thyroid disease. Thyroid surgery or chronic autoimmune thyroiditis with glandular destruction are the most common, although nodular goitre can alter the thyroid uptake of MIBI as well. The thyroid MIBI uptake can be very low in a small proportion of patients with chronic renal failure without any sign of thyroid disease. The causes for these findings in this group of patients are still unclear and need further investigation. Discovering the factors that influence the distribution of this radiopharmaceutical in the thyroid in these patients can lead to better understanding of the pathophysiology of the thyroid cell during chronic renal failure.

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# The Spectrum of Kidney Diseases in Children Associated with Low Molecular Weight Proteinuria

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**Keywords:** Proteinuria; Children; Kidney diseases; SDS-PAGE; Outcome

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**Abstract**

**BACKGROUND:** Proteinuria, in addition to haematuria, is the most important laboratory parameter in patients with nephro-urological diseases. Low molecular weight proteinuria (LMWP) is of particular importance because some diseases genetic and tubulointerstitial are diagnosed based on its presence.

**AIM:** The purpose of this study is to describe the clinical features, the course and outcome of pediatric patients with a renal disease associated with LMWP.

**MATERIAL AND METHODS:** This retrospective observational study included 250 pediatric patients with various kidney diseases in which the type of proteinuria was defined by 4-20% gradient gel sodium dodecyl sulphate polyacrylamide gel (SDS-PAG) electrophoresis.

**RESULTS:** Isolated LMWP was detected in 12% of patients, while mixed glomerulotubular proteinuria was detected in 18% of patients. It was detected in all patients with the Dent-1/2 disease, Lowe's syndrome and secondary Fanconi syndrome. Transient LMWP was also detected in a series of 12 patients with distal renal tubular acidosis. In patients with nephrotic syndrome, it was associated with corticoresistence and unfavourable clinical course.

**CONCLUSION:** This study contributes to the understanding of the clinical spectrum of various kidney diseases associated with LMWP, their natural course, and the effect of therapy.

## Introduction

Proteinuria in addition to haematuria is the most important laboratory parameter in patients with nephro-urological diseases. It is of paramount importance in the diagnosis, monitoring of the effect of therapy and follows up of these patients. It is defined as excretion of proteins in the urine above the upper limit of normal. Because most of the excrement is due to albumin, values above 30mg/L are taken for an upper limit of normal [1]. The remainder belongs to the Tamm-Horsfall protein, whose function is not completely clarified and is conceived in the distal tubule.

A problem in younger children who are not toilet trained is the inability to determine proteinuria in a 24-hour sample. The protein/creatinine index is then determined in a single urine sample. This mg/mmol ratio is normally < 20. In everyday work, proteinuria is tested with urinary sticks and is graded as negative, trace (+/-), 1+, 2+, and 3+. The sticks primarily detect albumin and are less sensitive to low molecular weight proteins, Bence Jones proteins or gamma globulins.

A false positive finding is when the urine is extremely concentrated, while a false negative finding is when the urine is maximally diluted. But the ideal way is to determine proteinuria with a more precise method, such as, for example, with sulphosalicylic acid.

Before the treatment of a child with proteinuria, functional (orthostatic) proteinuria should be excluded [2] [3] [4] [5]. It is characterised by excretion of proteins only in postural position. It is typical for older children, usually occurs in puberty and tends to resolve itself. Renal biopsy does not show specific signs and is not indicated in this condition. Some patients have "nutcracker phenomenon" or so-called aortomesenteric pliers, with the left renal vein entrapped between the aorta and the upper mesenteric artery in an upright position.

In the treatment of a child with orthostatic proteinuria, it is necessary to measure blood pressure, examine the sediment for the presence of haematuria, quantify proteinuria (allowed up to 1.0 g/day) and measure the C3 complement. If this processing shows normal results, only annual audits are sufficient. SDS-PAG electrophoresis is an elegant method where the presence of Apolipoprotein A1 fraction (APO-A1) with a molecular mass of 28 KD is a pathognomonic sign for orthostatic proteinuria [2] [3].

The next step in treating a child with proteinuria is the classification of the same. The following classification is the most common: (i) Glomerular proteinuria: proteinuria with a molecular weight greater than 60 KD, with the presence of albumin, haptoglobin, IgG and transferrin (ii) Tubular proteinuria-proteins with a mass lower than 60 KD, which are alpha-1-microglobulin, beta-2-microglobulin, retinol binding protein (RBP), light chains, and post-gamma globulin (iii) Mixed proteinuria - a combination of glomerular and nonglomerular fractions. Glomerular proteinuria can then be divided into selective (albumin with or without transferrin) and non-selective (albumin, transferrin, and IgG). Tubular proteinuria can be classified into incomplete and complete tubular proteinuria according to the size of the fractions.

Low molecular weight proteinuria (LMWP, i.e., tubular proteinuria) is the subject of this study. Many diseases as primary (hereditary) are characterized by the presence of tubular, ie, low molecular weight proteins [6] [7] [8] [9] [10] [11] [12] [13] [14] [15]. As already mentioned in healthy individuals, the intact proximal tubular cell completely absorbs proteins with a small molecular mass. If transport systems are damaged as a result of a genetic defect (recycling of cubilin and megalin, defect in endosomal acidification), as in the case of Fanconi syndrome or Dent's disease, then LMWP is present [16].

It can be isolated as in Imerslund Grasbeck syndrome [14] or be accompanied by outbursts in other tubular functions (electrolyte, phosphate, bicarbonate, glucose, and amino acid loss). Low molecular weight proteinuria is often also found in other metabolic diseases with the affection of the proximal tubular cell such as tyrosinaemia, fructoseemia, galactosaemia, Wilson's disease.

For the detection of tubular (low molecular weight proteinuria, single markers such as alpha-1-microglobulin, beta-2-microglobulin or retinol binding protein alone or in combination may be used [17] [18] [19] [20]. Beta-2-microglobulin, which is one of the first and most popular markers, is unstable in an acidic environment so that one can gain unreliable results by using it.

Sometimes the interpretation of these markers can be problematic and hence the so-called urinary protein expert system has been introduced in clinical practice [21]. This system is computerised and allows to doctors without experience in this field to interpret the findings from determining urinary protein markers.

In this study, low molecular weight proteinuria was determined by SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis). It is a method that was developed during the 90s of the last century. The advantage is that a panoramic view of all proteins is obtained, so a clear classification of the type of proteinuria is possible [1] [2] [3] [17] [18] [19] [20]. It is relatively fast and inexpensive method; it is possible to analyse multiple samples, especially using the Phast system [21]. The method is refined, by providing a computerised analysis of the resulting fractions and their relative estimation using laser densitometry.

This study aimed to describe the clinical features, the course and outcome of pediatric patients with renal disease in whom the LMWP has been diagnosed.

## Material and Methods

The study was designed as a retrospective-prospective study. Pediatric patients aged 1-18 years who have been treated as out- or in-patients at University Children’s Hospital Skopje were included in the study. In all patients, standard clinical and laboratory workup was performed. It included a detailed personal and family history of renal disease, as well as a physical examination including measurement of the blood pressure.

Proteinuria was determined by urinary stix and sulphosalicylic acid qualitatively and quantitatively. The glomerular function was assessed through the values of degradation products (urea, creatinine and uric acid depending on the reference values). Glomerular filtration rate was calculated according to the new Schwartz formula. Tubular functions have been investigated by determining low molecular weight proteinuria, glucosuria, phosphaturia, uricosuria, aminoaciduria, the presence of tubular acidosis (proximal, distal, mixed).

The serum values of bicarbonate, sodium, potassium, calcium, phosphorus, magnesium,

chloride, uric acid, total proteins and albumin, hepatic enzymes, alkaline phosphatase, and creatinine phosphokinase were also determined. Tubular reabsorption of phosphate (TRP) and fractional excretion of urate was calculated according to the reference formulas. Depending on the clinical picture, other studies have been performed-e.g. imaging of the kidneys and urinary tract to assess the gross morphology. In some patients, especially those with glomerulonephritis, the biopsy findings obtained with a punctured renal biopsy were also analysed.

Determination of low molecular weight (tubular proteinuria) with Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) was performed at the Laboratory for Protein Chemistry at the Institute for Clinical and Experimental Biochemistry at the Faculty of Medicine in Skopje, with the procedure for separation and identification performed in several stages : linear gel preparation (4-22%), treatment of urinary samples before their application on the gel, electrophoresis, gel fixation, coloring with Coomassie blue, and identification of the separated protein fractions based on standards with exactly known molecular weight.

This study was approved by the Ethical Committee at Medical School Skopje. Informed consent was obtained from the participants' parents/legal guardians.

## Results

This study included 250 pediatric patients with the various nephro-urological disorders in whom the type of proteinuria was defined by 4-20% gradient gel sodium dodecyl sulphate polyacrylamide gel (SDS-PAG) electrophoresis. There was a mild predominance of the female sex (n = 132, 52.8%). The average age of the patients was 7.6 years (ranging from 2 to 23 years). Regarding the ethnicity, subjects from the Macedonian nationality predominate, which corresponds to the national level in the Republic of Macedonia (n=158, 63.2%).

The most common indications for performing SDS-PAGE electrophoresis were following: haematuria, urinary infection, proteinuria, nephrolithiasis, and congenital anomalies of the urinary tract.

Isolated LMWP was detected in 12% of patients, while mixed glomerulotubular proteinuria was detected in 18% of patients (Figure 1). It has been detected in all patients with Dent-1 and Dent-2 disease, Lowe's oculocerebrorenal syndrome and secondary Fanconi syndrome. LMWP was also detected in a series of 12 patients with distal renal tubular acidosis, and it was transient. The presence of LMWP in patients with nephrotic syndrome was associated with corticoresistence and unfavourable clinical course.

The value of SDS-PAGE electrophoresis in children with OCRL mutations was also evaluated. Three children were phenotypically characterised as Lowe's oculocerebrorenal syndrome and 3 children as Dent-2 disease. Low-molecular-weight proteinuria was detected in all 6 children with SDS-PAGE. It is noteworthy that only one child with Lowe syndrome had a complete Fanconi syndrome and this patient had a fatal outcome.

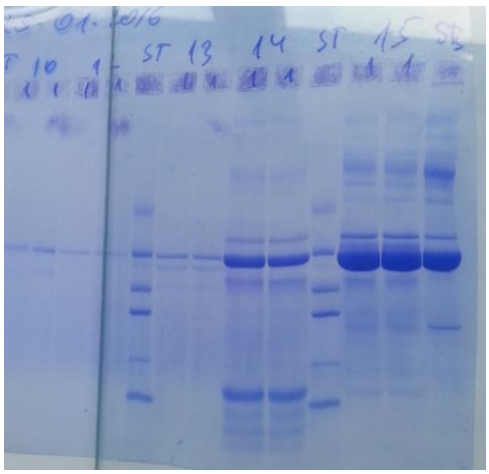


*Figure 1: St-standard; Track 13, incomplete tubular proteinuria; Track 14, complete tubular proteinuria; Track 15, Mixed glomerulotubular proteinuria*

An interesting case is a 12-year-old boy who presented with nephrotic range proteinuria at the age of 3 years. Because serum biochemistry was normal, as well as the values of C3 complement, the biopsy was postponed, but the child was lost to follow up. At the age of 13, he was hospitalised for persistent proteinuria of nephrotic range but without oedema. The biopsy did not show abnormalities on light-microscopic and immunofluorescent examination. SDS-PAG electrophoresis was then performed showing the presence of complete tubular proteinuria (Figure 2). Finally, 24-hour urinary excretion of calcium was determined (11.0 mg/kg/d) leading to the establishment of a clinical diagnosis of Dent's disease. The diagnosis of the Dent-2 disease was confirmed by genetic analysis which showed the presence of a pathogenic OCRL mutation.

The outcome of patients with LMWP: in children with acute tubular-intestinal nephritis (drugs in three cases), complete normalisation of renal function has occurred. Two children had the fatal outcome (one with Lowe syndrome and one with a secondary Fanconi syndrome- eczema, enteropathy, IPEX syndrome). A complete resolution of the Fanconi syndrome ensued in two children (one cisplatin nephrotoxicity and one with Fanconi anaemia treated with a chelating agent)

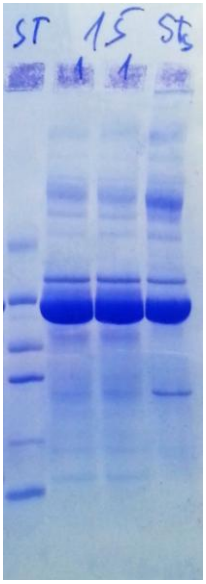


*Figure 2: Patient with the Dent-2 disease, Eleferogram shows massive proteinuria, mixed (glomerulo-tubular)*

## Discussion

In clinical practice, LMWP is detected in patients with acute tubulointerstitial nephritis. In addition to tubular proteinuria, glycosuria, acidosis and various degrees of reduction of glomerular filtration rate are present. Typically it is a non-oliguric acute renal failure. TINU syndrome is tubulointerstitial nephritis with uveitis. LMWP can be the first sign of toxic damage to the tubular cell (antibiotics in particular tetracyclines, heavy metals, mercury, cadmium, lead, etc.).

Some drugs such as cisplatin, ifosfamide, valproic acid can also lead to tubular cell damage with a consecutive occurrence of tubular proteinuria [22] [23]. In this study, in two children the incriminated agent was cisplatin, while the other was a chelating agent for the treatment of Fanconi anaemia (Fanconi in Fanconi). Low molecular weight proteinuria may be the first sign of diabetic nephropathy [24] [25]. Patients with long-standing cystic fibrosis may also have proteinuria [26]. Tubular proteinuria may also be the first sign of nephropathy in Henoch Schonlein disease [22].

There are many studies that indicate the presence of tubular proteinuria in patients with glomerular disease and may be a poor prognostic marker (steroid-resistant nephrotic syndrome, focal segment glomerulosclerosis, IgA nephropathy) [27] [28] [29] [30] [31] [32] [33] [34]. In our study, the unfavourable course of focal glomerulosclerosis and steroid-resistant nephrotic syndrome was also associated with LMWP.

In addition to the acquired diseases, LMWP is found in a number of genetic tubulointerstitial diseases (nephronophthisis, Dent-1, Dent-2 disease, Lowe syndrome, cystinosis, tyrosinaemia, fructosamine, Wilson's disease) [35] [36] [37] [38] [39] [40]. We had an interesting observation for the presence of LMWP in patients with distal renal tubular acidosis [41]. This can lead to differential diagnostic difficulties with the Fanconi syndrome. Tubular proteinuria was present only at the onset of the disease, while the children were decompensated. With metabolic compensation, a complete resolution of proteinuria ensued.

In conclusion, this study contributes to the understanding of the clinical spectrum of various diseases associated with LMWP, their natural course, and the effect of therapy. SDS-PAG electrophoresis is a sensitive, inexpensive and well-established method for the detection of LMWP in children with renal disease.

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# Transfusion of Fresh Frozen Plasma in Critically Ill Patients: Effective or Useless?

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**Keywords:** Fresh frozen plasma; Transfusion; Increased INR; Invasive procedure

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**Abstract**

**BACKGROUND:** Fresh frozen plasma (FFP) is widely used in critically ill patients to correct the deficiency of coagulation factors or increased INR.

**AIM:** In the present study we aimed to evaluate the outcome of the freshly frozen plasma use as prophylaxis in ICU patients before an invasive procedure.

**METHODS:** The study was conducted at Central Anaesthesiology and Intensive Care Service UHCT "Mother Theresa", Tirana. 136 patients were enrolled with coagulopathy with no bleeding before the invasive procedure, from June 2016 to December 2016. A group of 75 patients underwent a median volume of 12.5 ml/kg FFP given, and 61 had no transfusion. Data were collected on demographics, the severity of illness measured by APACHE III scores, INR, medication use, hemodynamic data.

**RESULTS:** From 136 patients with coagulopathy with no bleeding who underwent planned invasive interventions, 75 [55%] received FFP, vs 61 [45%] p = 0.04 who did not receive. Overall, the median FFP dose was 12.5 ml kg-1. Median INR level in FFP and non-FFP groups was respectively 3.1 (1.9-4.8) and 3.5 (1.8-5.2). INR was corrected in 24 of 75 (32%) of those who received a transfusion. The frequency of minor bleeding episodes was 9.3% in transfused patients vs 4.9% in the non-transfused group. Patients who developed an onset of acute lung injury were more frequent in the FFP group. No allergic transfusion complications were observed. Also, the median length of hospital stay [LOS] was 3.05 days vs 2.91 days and mortality rate 8.2% vs 6.5% with no significant difference between two groups.

**CONCLUSIONS:** Freshly frozen plasma transfusions are often unnecessarily administered during an inadequate correction of the deficiencies of coagulation factors. When comparing a liberal FFP transfusion strategy vs restrictive other clinical trials are required to asses which one is the best to adopt in intensive care settings.

## Introduction

The use of FFP has significantly increased in the past 10 years, and its usage continues to increase. There are certain situations where FFP is indicated, such as in patients with coagulopathy resulting from DIC who are undergoing invasive procedures or having active bleed, in patients with liver failure with active bleed and patients with thrombotic thrombocytopenic purpura (TTP) [1].

Fresh frozen plasma is widely used in ICU patients and prescribed for the treatment of bleeding or the prevention of bleeding in critically ill patients. However, there are few detailed, prospective, descriptive data from large studies describing these patterns of use in the critically ill and clinical evidence to help aid the critical care clinician make decisions on whether to transfuse or not is at present limited [2] [3].

It is now usually used in cases of excessive bleeding or to prevent bleeding in those patients with abnormal coagulation tests that are undergoing an

invasive procedure. Its use has been extended to patients with a coagulopathy but who are not bleeding (for instance, in the ICU) [4].

In the last decade, use of FFP has expanded to include prophylactic administration of FFP. However, there are concerns about the efficacy of FFP to prevent bleeding. Evidence from randomised controlled trials that support FFP transfusion to correct coagulopathy before an invasive procedure is limited, including commonly performed procedures on the ICU, such as insertion of a central venous catheter, a chest drain or a percutaneous tracheotomy [5]. Moreover, retrospective studies suggest that the risk of bleeding after an invasive procedure is low and relevant bleeding requiring blood transfusion or intervention is less than 1% [6].

Furthermore, FFP contains antibodies capable of causing complications like hemolytic reactions and transfusion-related acute lung injury. It is also capable of transmitting viruses like human immunodeficiency virus, hepatitis B virus, and C virus [7]. Hence, the use of FFP is not without potential danger, and it should be used only if indicated.

Most clinical uses of FFP, currently recommended by practice guidelines, are not supported by evidence from randomised trials. In particular, there is little evidence for the effectiveness of the prophylactic use of FFP [5].

## Material and Methods

This prospective observational study was conducted at Central Anaesthesiology and Intensive Care Service UHCT "Mother Theresa", Tirana. Data were collected prospectively from 136 patients admitted to ICU with coagulopathy with prolonged International Normalized Ratio (INR) ≥ 1.5 at any time during their ICU stay; with no bleeding before the invasive procedure, from June 2016 to December 2016.

*Inclusion criteria were:* > 18 years old, prolonged International Normalized Ratio (INR) ≥ 1.5, and undergoing any invasive procedure.

*Exclusion criteria:* thrombocytopenia <5 x $10^3$μ/L, hemodynamic instability, active bleeding; patients on warfarin, heparin and other anticoagulants treatment. All patients are carefully evaluated for next 24 hours from admission time, and all data were collected on demographics, the severity of illness measured by APACHE III scores, INR, medication use, hemodynamic data. The level of INR prompting FFP transfusion was recorded for patients who received FFP transfusion and the highest level of INR during their ICU stay, compared with patients that were not transfused with FFP.

Repeated post-invasive procedure measurements were made after 1 and 24 hours. The outcomes measured are relevant bleeding and correction of International Normalized Ratio. Standard tests were used for comparisons of proportions and means. Acute lung injury was recorded if it developed within 72 hrs after FFP administration or within 72 hrs after the highest recorded INR value in patients who did not receive FFP. Independent variables, such as warfarin or heparin anticoagulation, INR level, RBC transfusion and invasive procedure were used in the final model. Categorical outcome variables were compared between two groups based on the chi-square test. Clinical outcomes including hospital mortality and ICU length of stay among survivors were also recorded. Continuous outcome variables were compared using Student's t-test. To determine the clinical characteristics associated with FFP transfusion, logistic regression analysis was performed with FFP transfusion as the dependent variable. All P values are one-tailed, and the result is significant at $p \leq 0.05$.

## Results

From a total of 518 patients that were admitted to the ICU during the study period, 189 patients (36.5%) met our criteria. A total of 53 patients with active bleeding were excluded. In a total of 136 patients with elevated INR, coagulopathy and no bleeding, 75 (55%) received FFP vs. 61 [45%] who had no transfusion. Median INR levels in FFP and non FFP groups was respectively 3.1 (1.9-4.8) and 3.5 (1.8-5.2). INR was corrected in 24 of 75 (32%) of those who received transfusion.

Transfusion was administered before an invasive intervention was required. There was no difference in age, sex, median APACHE III scores, or INR level between the FFP and non-FFP groups. It was a difference seen in patients who developed an onset of acute lung injury which was more frequent in the FFP group: 12% vs. 3% in non-transfused group. No allergic transfusion complications were observed.

In addition, the median length of hospital stay [LOS] was 3.05 days vs. 2.91 days and mortality rate 8.2% vs. 6.5% p = 0.707 with no significant difference between two groups.

## Discussion

In our study, we saw that exists a significant variation in the use of FFP in critically ill patients with

coagulopathy but with no active bleeding. New bleeding episodes were very rare and did not differ between the groups that took FFP transfusions, and those who did not and the use of FFP was associated with the development of acute lung injury.

In our findings, in only 24 of our 75 (32%) transfused patients was INR corrected after the FFP transfusion. This result confirms some other observations that the standard recommended dose of FFP fails to correct coagulation deficit in a majority of critically ill patients [13]. According to the studies, INR level is a poor predictor of subsequent bleeding in the critically ill patients, and in lots of patients, specific factor concentrations remain adequate to prevent microvascular bleeding [13]. A significant number of patients with coagulopathy received FFP transfusion without any demonstrated efficacy [5] [13]. Other sources, such as Abdel-Wahab OI et al., have concluded that whatever the volume of transfused plasma, plasma transfusions did not correct moderate coagulopathy [8]. In another observational study by Holland et al., authors showed that plasma transfusions did not correct INR levels <2.0–2.5 [9]. So, the current practice of FFP transfusion is likely to expose the patients to transfusion risks with little or no documented benefit. During recent years new guidelines have been promoted to educate the hospital personnel [10].

It is important to emphasise that recommendations in the current guidelines are based on expert opinion, as no randomised studies are available. In the current study, FFP was commonly used before an invasive procedure. Although there is a little evidence for the effectiveness of the prophylactic use of FFP, previous studies have shown that invasive procedures can be done safely in patients with disorders of hemostasis by skilled physicians who frequently perform these procedures [5] [11]. Although some published guidelines currently define an invasive procedure as one of the indications for FFP transfusion, our data do not support this practice for the common critical care procedures [12]. We also found the considerable use of FFP in patients who had recent bleeding but no active ongoing bleeding. FFP transfusion was primarily aimed for reversal of warfarin effect. However, the latest British Society for Hematology guidelines clearly states that FFP should not be transfused for the reversal of warfarin anticoagulation when there is no evidence of severe active bleeding [10]. Previous studies also suggested that FFP may not be particularly effective in replacing coagulation factors [13]. In our study, FFP transfusion remained significantly associated with the development of acute lung injury ($p = 0.05$). However, the present study has some circumstantial limitations, and the reported data need replication.

In conclusion, plasma transfusion is a common treatment for critical care patients, and it may bring benefits for those who are massively bleeding. In our study, we concluded that FFP transfusion in critically ill medical patients with coagulopathy but without active bleeding. Plasma transfusions may be associated with worse outcomes, so the risk-benefit ratio of liberal FFP transfusion strategy may not be favourable.

Therefore, the decision to proceed with plasma transfusion must be based on individualised indications, and most physicians suggest plasma transfusions according to their own experiences while balancing the risks and benefits. Unfortunately, no randomised, controlled trial has yet decided the appropriate plasma transfusion threshold. Freshly frozen plasma transfusions are often unnecessarily indicated because of the inadequate correction of the deficiencies of coagulation factors and so comparing a liberal FFP transfusion strategy vs a restrictive one are required other clinical trials to asses which one is the best to perform to avoid the unnecessarily exposures to the ICU patients.

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# Prevalence and Predictors of Depression after Stroke - Results from a Prospective Study

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**Abstract**

**BACKGROUND:** A depression following a stroke (Post Stoke Depression-PSD) is the most common complication of a stroke that has a negative effect on the result after the stroke. A better definition of the risk factors of the disease will provide for better prediction and treatment.

**AIM:** To research identification of the risk factors for PSD, typical for the Macedonian population, which will help in early prediction, timely diagnosis and treatment of the disease?

**MATERIAL AND METHODS:** We carried out a prospective study in order to determine the prevalence and the risk factors of PSD in 100 patients treated at the hospital in Tetovo. The severity, localisation and the functional outcome of the stroke have been examined as potential risk factors for discharge and after 5 months. The symptoms of depression were quantified using the Hamilton Depression Rating Scale (HAM-d).

**RESULTS:** On discharge, 81% of the patients were diagnosed with PSD, and 67% had PSD after 5 months. A statistically significant codependence of $p < 0.05$ was registered between PSD and the level of functional dependence for activities of daily living (ADL); PSD and the severity of the stroke; and PSD and the level of disability on both examinations. In most patients with PSD, an ischemic stroke in the right middle cerebral artery has been diagnosed; the percentage difference between the other localisations is statistically significant ($p = 0.0436$; $p = 0.0002$).

**CONCLUSION:** There is an increased risk of PSD for immobile patients, those incapable of activities of daily living (ADL), with ischemic stroke in the right middle cerebral artery. A PSD screening and additional studies for better prediction are required.

## Introduction

PSD is the most common affective disorder that occurs after an acute, focal cerebrovascular insult in the context of a clinically obvious stroke. The epidemiological studies report a widely variable prevalence of PSD that ranges between 10-64% of the patients that suffered a stroke [1] [2] [3] [4] [5] [6]. Some studies indicate highest PSD prevalence in the first 3-6 months following the stroke with a gradual decline after the first year since the stroke has occurred. This is an early, reactive stage of PSD. The depression that occurs later, after the 6th month following the stroke is considered a late stage of PSD.

The early prediction and diagnosis of PSD are important because the disease has a negative impact on survival, the success of the treatment and the medical rehabilitation, functional outcome, re-socialisation and the quality of life thus increasing the medical expenses [7] [8].

The pathophysiology of the disease remains to be explained. The predictors are not precisely defined. There is a set of studies that mostly indicate to the significant association between the extensive cerebral lesion in the frontal lobes and the occurrence of PSD without a clear predominance of the left or the right hemisphere [9] [10]. A higher level of functional disability for activities of daily living (ADL) after stroke is considered to be a risk factor most consistently associated to PSD [1] [2] [3] [4].

As a result to all of this, there was a need to research identification of the risk factors for PSD, typical for the Macedonian population, which will help

in early prediction, timely diagnosis and treatment of the disease.

## Material and Methods

We carried out a prospective, longitudinal, epidemiological study in order to identify the prevalence and the risk factors for PSD on discharge from the hospital and after 5 months following the stroke. The study was carried out at the Department of Neurology at the Clinical Hospital in Tetovo, Macedonia. The study included all the patients, which fulfilled the inclusion criteria, clinically treated at the department due to an acute stroke, clinically verified and confirmed by computed tomography of the brain in the period from 1st September 2016 to 28th February 2017. Inclusion criteria: normal Mini-Mental Score according to the patient's education, maintained verbal communication ability, maintained sensorium, age ≤ 75. The study did not include patients with another comorbidity that seriously disturbed the general somatic condition and patients that were previously diagnosed with a psychiatric disorder. All the patients gave informed consent previously approved by the Ethical committee.

First, a quantification of the depression symptoms using the Hamilton Depression Rating Scale (HAM-d) was done on all the patients, which divided them into two groups, with and without PSD. For the group with PSD, there was an analysis of stroke severity, level of functional dependence for activities of daily living (ADL) and level of disability as a result of the stroke. A new examination of all the parameters was done 5 months after the stroke. Demographic data, vascular risk factors, data about the comorbidity and the localisation of the stroke was collected from the hospital's documentation and interviews of the patients and their relatives. The study included 100 patients, 97 of them were monitored for 5 months, and three deaths were recorded.

The Hamilton Depression Rating Scale (HAM-D) for quantification of depression symptoms, a form that is consisted of 21 questions. An official Macedonian translation from the Psychiatric Clinic in Skopje was used in the research. The scale score enables ranking the subject in one of the following groups: - 0-7 normal; - 7-13 mild depression; - 14-18 moderate depression; - 19-22 severe depression; - > 23 very severe depression.

National Institutes of Health Stroke Scale (NIHSS). The score range is from 0-42. A score from 24-42 indicates a severe stroke with catastrophic consequences and a patient in a coma. Such patients were not included in the study.

The level of functional dependence for activities of daily living according to the Barthel Index (BI), a questionnaire that provides an assessment of the functional ability for performing 10 basic activities of daily living. The index has a score from 0-100.

Stroke outcome according to the modified Rankin Scale (mRS) measuring the disability after stroke. The score range is from 0-6 where 2 is a slight disability, and 6 is a dead patient.

The statistical analysis was done with statistical software: STATISTICA 7.1; SPSS 17.0, using the following statistical methods: difference test, average and standard deviation, Mann-Whitney U test, Analysis of Variance-ANOVA, multiple regression analysis, Person correlation coefficient (r) and $\chi 2$ test, Shapiro-Wilk test. A statistical significance level of 0.05 (p) was defined as a confidence interval (95% CI).

## Results

On the first examination, PSD was diagnosed in 81.0% of the patients, while on the second examination 65.0% of the patients had PSD, the percentage difference is statistically significant for $p < 0.05$ ($p = 0.0108$ Difference test) (Table 1). According to the Index of dynamics PSD in patients shows a decreasing rate of 19.8%.

**Table 1: Patients with PSD**

| *Psd* | First | | Second | |
|---|---|---|---|---|
| | N° | % | N° | % |
| *Without* | 19 | 19.1 | 32 | 32.0 |
| *With* | 81 | 81.0 | 65 | 65.0 |
| *Exitus* | 0 | | 3 | 3.0 |

According to the HAM-D score the majority of patients, 55%, had mild, early stage of PSD, with remission after 5 months in 12% of the patients (Table 2).

**Table 2: Hamilton Depression Rating Scale-HAM-D**

| *Finding / control* | First | | Second | |
|---|---|---|---|---|
| | N° | % | N° | % |
| *0-7 normal* | 19 | 19.0 | 32 | 32.0 |
| *8-13 (mild depressive reaction)* | 55 | 55.0 | 43 | 43.0 |
| *14-18 (moderate depression)* | 14 | 14.0 | 16 | 16.0 |
| *19-22 (severe depression)* | 11 | 11.0 | 5 | 5.0 |
| *>23 (very severe depression)* | 1 | 1.0 | 1 | 1.0 |
| *Exitus* | 0 | | 3 | 3.0 |
| *Total* | 100 | 100.0 | 100 | 100.0 |

According to the severity of the stroke (NIHSS score), more than a half of the patients with PSD presented a neurological deficit of moderate stroke on the first examination which improved in 5 months in 31% of the patients. A statistically significant dependence, $p < 0.05$, between PSD and the severity of the stroke was registered on both examinations (Pearson Chi-square: 9.75034, $p = 0.0017932$;

Pearson Chi-square: 10.9168, df = 2, p = 0.004260) (Table 3).

**Table 3: Presence and absence of PSD about the severity of the stroke (NIHSS score)**

| *Control/NIHSS/PSD* | First | | Second | |
|---|---|---|---|---|
| | Without | With PSD | Without | With PSD |
| *0 without* | | | 9 | 5 |
| *0-4 small* | 16 | 36 | 22 | 46 |
| *5-15 moderate* | 3 | 45 | 1 | 14 |
| *Total* | 19 | 81 | 32 | 65 |

A statistically significant dependency of p < 0.05, between PSD and the degree of disability, was registered on both examinations (Pearson Chi-square: 9.79890, p = 0.043955; Pearson Chi-square: 26.4533, p = 0.000073) (Table 4). According to the modified Rankin Scale, mostly a moderately severe disability-38.3% and a moderate disability were registered in patients with PSD on the first examination. On the second examination, a moderate disability was registered in 41.5% of the patients with PSD.

**Table 4: Presence and absence of PSD about the modified Rankin Scale**

| ***Control/Mrs/PSD*** | **First** | | **Second** | |
|---|---|---|---|---|
| | Without | With PSD | Without | With PSD |
| *0/no symptoms at all* | | | 6 | 3 |
| *1/ no significant incompetence* | 4 | 5 | 8 | 7 |
| *2/easy incompetence* | 6 | 13 | 16 | 16 |
| *3/moderate incompetence* | 7 | 30 | 2 | 27 |
| *4/ moderate severe incompetence* | 2 | 31 | 0 | 11 |
| *5/ severe incompetence* | 0 | 2 | 0 | 0 |
| *Total* | 19 | 81 | 32 | 65 |

A statistically significant dependence of p < 0.05 was recorded between PSD and the Barthel Index on both examinations (Pearson Chi-square: 14.1552, p = 0.006816; Pearson Chi-square: 18.7295, p = 0.000888) (Table 5). According to the Barthel Index, a moderate dependence for performing activities of daily living was recorded in patients with PSD (39.5% and 41.5%) on both examinations.

**Table 5: Presence and absence of PSD according to the Barthel Index**

| ***Control/Barthel index S/PSD*** | **First** | | **Second** | |
|---|---|---|---|---|
| | Without | With PSD | Without | With PSD |
| *0-20 total dependacy* | 1 | 16 | 0 | 6 |
| *21-60 severe dependency* | 2 | 27 | 0 | 18 |
| *61-90 moderate dependency* | 11 | 32 | 15 | 27 |
| *91-99 easy dependency* | 0 | 2 | 3 | 3 |
| *100 independence* | 5 | 4 | 14 | 11 |
| ***Total*** | 19 | 81 | 32 | 65 |

A strong, negative, statistically significant correlation was recorded between the change of the value of the HAM-D score and the value of the BI in 5 months (Table and Chart 6). Namely, the improvement of the PSD during the 5-month period correlates with the increase of the degree of functional ability for ADL of the patients.

**Table 6: The correlation between the change of the value of the HAM-D score and the value of BI during 5 months**

| Scales | Bi |
|---|---|
| Ham-d | -0.6969 |
| | P = 0.000 |

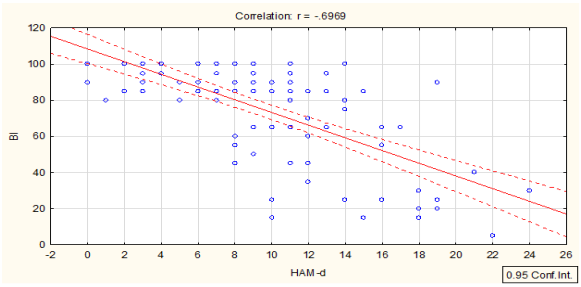


*Figure 1: Presentation of the correlation between the change of the value of the HAM-D score and the value of the BI during 5 months*

In the majority of patients with PSD, an ischemic stroke in the right middle cerebral artery was confirmed in 39.5% of the patients on the first examination and in 47.7% on the second examination. The percentage difference in relation to the other localizations is statistically significant for p < 0.05 on the first and second examination (p = 0.0436; p = 0.0002) (Table 7).

**Table 7: Patients with PSD and localisation of stroke**

*First examination*

| ***Localization*** | PSD | | Without | |
|---|---|---|---|---|
| | N° | % | N° | % |
| *IS left MCA* | 20 | 24.7 | 3 | 15.8 |
| *IS left PCA* | 7 | 8.6 | 4 | 21.1 |
| *IS right ACA* | 1 | 1.2 | 9 | 47.4 |
| *IS right MCA* | 32 | 39.5 | 2 | 10.5 |
| *IS right PCA* | 7 | 8.6 | | |
| *IS left MCA +bleeding* | 4 | 4.9 | 1 | 5.3 |
| *IS right MCA +bleeding* | 4 | 4.9 | | |
| *IS left ACA+ IS left PCA* | 1 | 1.2 | | |
| *IS left MCA+ IS left PCA* | 1 | 1.2 | | |
| *IS left PCA+ IS right PCA* | 1 | 1.2 | | |
| *IS right PCA+ IS left MCA* | 2 | 2.5 | | |
| *IS right MCA+ IS right PCA* | 1 | 1.2 | | |
| *Total* | 81 | 100.0 | 19 | 100.0 |

*IS-ischemic stroke; MCA-middle cerebral artery; ACA-anterior cerebral artery; PCA-posterior cerebral artery*

*Second examination*

| ***Localization*** | PSD | | Without | |
|---|---|---|---|---|
| | N° | % | N° | % |
| *IS left MCA* | 13 | 20,0 | 8 | 25,0 |
| *IS left PCA* | 5 | 7,7 | 6 | 18,8 |
| *IS right ACA* | 1 | 1.5 | 10 | 31,3 |
| *IS right MCA* | 31 | 47,7 | 4 | 12,5 |
| *IS right PCA* | 3 | 4,6 | | |
| *IS left ACA+ IS left PCA* | 1 | 1.5 | 1 | 3,1 |
| *IS left MCA+ IS right MCA* | 1 | 1.5 | | |
| *IS left MCA+ IS right PCA* | 1 | 1.5 | | |
| *IS left MCA + Sequel from bleeding* | 3 | 4,6 | 2 | 6,3 |
| *IS left PCA+ IS right PCA* | 1 | 1.5 | | |
| *IS right MCA+ IS left MCA* | 2 | 3,1 | | |
| *IS right MCA+ IS right PCA* | 1 | 1.5 | | |
| *Total* | 65 | 100.0 | 35 | 100.0 |

*IS-ischemic stroke; MCA-middle cerebral artery; ACA-anterior cerebral artery; PCA-posterior cerebral artery*

## Discussion

Our study confirmed high prevalence, 81%, of early, reactive stage of PSD. It is higher compared to studies with a similar design which is probably due to different depression symptoms quantification scales [11], language barrier (most of the patients were of

Albanian nationality, and Macedonian is not their mother tongue), the existence of different types of dysphasia. An early, mild stage of PSD was diagnosed in the majority of the patients, 55%, with a spontaneous remission after 5 months in 12% of the patients which corresponds with the dynamics of early PSD determined from previous epidemiological studies [3] [4] [5] [6]. In 65% of the patients, PSD was also diagnosed after 5 months and regardless of the severity of the depression symptoms they should receive treatment with antidepressants because a spontaneous remission cannot be expected. Prospective studies that observed the patients several years following the stroke indicate that if the depression symptoms emerge and are not spontaneously improved in 6 months, then PSD will most likely become chronic [6].

Certainly, the limitation of the study is the fact that the patients were only monitored for 5 months. Studies that analyze a convalescent post-stroke period of 5 and more years, such as the study conducted on 3689 patients documented in the London stroke registry, besides pointing to the variable dynamics of PSD, confirmed that the risk for the disease exists as long as the risk factors are present, or the disease can occur at any moment during the rehabilitation period of the patient [3].

The results confirm that the risk for PSD is higher in patients with a moderately severe stroke and moderate disability because, patients with symptoms of severe stroke could not be included in the study because those were patients with aphasia, disorders of consciousness and finally a fatal outcome.

In previous studies, the decreased functional ability for performing ADL is considered as the most consistent risk factor for PSD which was also confirmed in our study [3] [12]. The study demonstrated that the patients with diagnosed PSD have low BI on both examinations, i.e. in the early and the chronic stage of the disease. On the other hand, the determined, strong, negative correlations between BI and the HAM-D score indicates that the fast improvement of the depression symptoms in patients correlates to the significant improvement of the ability to perform ADL. Therefore, there is a necessity for an early prediction of PSD, but also diagnosing and treatment, which would raise the level of remission and would contribute to more rapid and more successful rehabilitation of the patients.

Our study showed that patients with an ischemic stroke in the vascular area of the right middle cerebral artery have a higher risk for developing PSD, which correlates with the occurrence of a significant motor neurological deficit and disability.

In conclusion, the results of our study confirmed the multifactorial nature of PSD. The disease presented itself as a common complication of stroke that should be taken into consideration in daily clinical practices. Thus, there is a need for preparing and introducing precise instruments for early assessment of the risk for occurrence of the disease in every patient in the rehabilitation phase. In this manner, an early prediction of PSD will be achieved, which will enable a more successful individualised treatment approach for every patient, as well as a timely education of the patient's family.

Additional studies for analysing the late and chronic stage of the disease and preparing treatment recommendations are also required.

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# The Correlation between Daily Lens Wear Duration and Dry Eye Syndrome

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**Abstract**

**AIM:** To analyze the correlation between the daily lens wear duration and dry eye syndrome.

**SUBJECTS AND METHODS:** This study was an analytic cross sectional study using consecutive sampling conducted among the students in Economy and Bussiness Faculty and Faculty of Humanities in University of Sumatera Utara aged between 17 to 23 that wore contact lens continously for at least a year and 5 days a week. The symptoms were assessed using Contact Lens Dry Eye Questionnaire-8 (CLDEQ-8) and interview about their contact lens comfort; eye drops usage, contact lens washing habit, daily circumstances, places to buy contact lens and personal experince in wearing contact lens.

**RESULTS:** The questionnaire was completed by 53 students. All of them were female and wore softlens wearers. The mean duration of daily wear was 8.19 ± 2.20 hours. The most common symptom experienced was dry eye and the least symptom experienced was removing lens. The most frequent symptom experienced was closing eyes and the least frequent symptom experienced was removing lenses. This study used Exact Test as analysis statistic method. The result was $p > 0.05$ which means there is no correlation between daily lens wear duration and dry eye syndrome.

**CONCLUSION:** This study showed that dry eye syndrome was not correlated with daily lens wear duration, but affected by many factors such as contact lens, lens care solution, eye drops usage and environment.

## Introduction

Contact lens dry eye syndrome is recognize as a growing public health problem and one of the most frequent reason for seeking ophtalmologist intervention [1] [2]. Previously conducted investigations estimate that the frequency of contact lens related dry eye is approximately 50% [1] [3]. Evidence from previous study has contributed to general consensus that contact lens discomfort is the main reason for discontinuation of wear and that the most common type of discomfort is dryness [3]. Due to changes associated with dry eyes these persons also often complain visual distrubance and blurred vision [1] [3]. Discomfort and dessication symptoms are the primary reasons for contact lens intolerance, a reduction in the length of wearing time and eventual discontinuation [1] [3]. Dumbleton et al., study on 2013 also reported dropping out rate was 23% with the primary reasons for dropping out were discomfort (24%) and dryness (20%) [4]. A previous study conducted by Rumpakis showed more than one out of 6 contact lens wearer will discontinue lens wear [2].

In some previous studies, contact lens dry eye syndrome was associated with daily wear duration. Kaštelan found a weak correlation between daily wear duration and higher OSDI values and moderate negative correlation between daily wear duration and TBUT [3]. Pili et al,. found a weak negative correlation between daily wear duration and TBUT [1]. Otherwise in Sapkota et al,. study found no correlation between daily wear duration and degree of dry eye symptoms. These inconsistent results made us interested to do a research in Indonesia.

The diagnosis of dry eye is set on the basis of patients' self reports of symptoms and clinical examination [3]. The clinical examinations that are

usually used to examine dry eye are tear film break up time test (TFBUT) to examine tear film stability and Schirmer Test to evaluate tear production, corneal and conjunctival staining to assesst ocular surface damage [6]. Whereas patient's symptoms can be assessed using some questionnaire. CLDEQ-8 is a short form of CLDEQ questionnaire that was designed to describe dry eye among contact lens wearers in particular [7].

The aim of this study was to analyze the association between daily lens wear duration and dry eye syndrome using CLDEQ-8 as the instrument.

## Subjects and Methods

The study was conducted in Economy and Bussiness Faculty and Faculty of Humanities in University of Sumatera Utara that was selected based on majority of contact lens population amongst all the faculties. Before doing this study, we had already had ethical clearance from Health Research Ethical Committee of Medical Faculty of Sumatera Utara University/H. Adam Malik General Hospital. This study was an analytic cross sectional study and sampling methods used for this study was consecutive sampling The students included in this study were recruited through direct referrals. The eligible participants had to wear contact lens continuously for at least a year and 5 days a week [8]. Those who had eye surgery history, tear gland dysfunction, eye infection, low blink rate, hormone replacement therapy, radiotherapy and chemotherapy patient, students with HIV/AIDS or diabetes were excluded from the study to avoid bias [9]. Minimal sample for this study was 45 students.

The dry eye syndrome were assessed by Contact Lens Dry Eye Questionnaire-8 (CLDEQ-8) and there are some additional questions included daily and weekly contact lens wear time, years of contact lens wear and also frequency on disposing lens. Contact Lens Dry Eye Questionnaire-8 was chosen because it was a spesific questionnaire for contact lens wearer [7]. After given out the questionnaire, the students were interviewed about their contact lens comfort, eye drops usage, contact lens washing habit, daily circumstances, places to buy contact lens and personal experince in wearing contact lens. The Contact Lens Dry Eye Questionnaire-8 results would be calculated and matched with the baseline status score (Table 1):

**Table 1: Baseline status score**

| Total Score | Eye's Condition |
|---|---|
| 0-6 | Excellent |
| 7-9 | Very Good |
| 10-14 | Good |
| 15-17 | Fair Good |
| >17 | Poor/dry |

## Results

A total of 53 students met the criterias to be participants. All of the students were female and no male student was found to wear contact lens. There was no RGP wearer. Most of student disposed their lenses every month with 30.2% and the least frequency in disposing lens is once in 5 months with 1.9% (mean 3.77 ± 2.39).

Wearing time is divided by two categories, they are daily wearing time and yearly wearing time. The daily wearing time ranged from 5 to 14 hours (mean 8.19 ± 2.20 hours) and the yearls of contact lens wear time ranged from 1 to 6 years (mean 3.38 ± 1.50 years).

The result of the students' answers showed that the most common symptom experienced by the students was dry eye and the least symptom experienced by the students was removing lens (Table 2).

**Table 2: Percentage of dry eye symptoms**

| Symptoms | Percentage |
|---|---|
| Eye Discomfort | 75.4% |
| Dry Eye | 81.1% |
| Changable, Blurry Eye | 66.0% |
| Closing Eyes | 73.6% |
| Removing Lens | 66.0% |

The most frequent symptom experienced by the students was closing eyes and the least frequent symptom experienced by the students was removing lenses (Table 3).

**Table 3: The frequencies of dry eye symptoms**

| Symptoms | Frequency | | | | |
|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 |
| Eye Discomfort | 13 | 14 | 23 | 3 | 0 |
| Dry Eye | 10 | 24 | 14 | 5 | 0 |
| Changable, Blurry Eyes | 21 | 16 | 3 | 3 | 0 |
| Closing Eyes | 14 | 14 | 18 | 7 | 0 |
| Removing lens | 18 | 15 | 12 | 8 | 0 |

Note:0=Never; 1=Rarely; 2=Sometimes; 3=Frequently; 4 = Constantly.

The questionnaires that were given to students were collected and scored. The students' scores range from 0 to 23. There were 2 students who had no score and there was 1 student whose score 23. The CLDEQ-8 scores were converted into eye's condition and most of the students had excellent eye (28.3%) and there were 7 students who had dry eyes (13.2%).

**Table 4: The students' total scores**

| Total Score | Frequency |
|---|---|
| 0-5 | 9 |
| 6-10 | 22 |
| 11-15 | 7 |
| 16-20 | 9 |
| 21-25 | 3 |

After given out the questionnaire, the students were interviewed about their contact lens comfort, eye drops usage, contact lens washing habit, daily

circumstances, places to buy contact lens and personal experince in wearing contact lens. The students usually wore their contact lenses mainly at campus. They studied at campus started 8 am to 5 pm. The classrooms didn't have air conditioner and when this study was hold the weather was rainy everyday that made the air cool and moist. For the question places to buy contact lenses, the students answered that most of them bought the contact lens online, from the optic and from a nearby marketplace. When they were asked about the lens washing habit, they usually washed the lens twice a day. Before and after using the lens using multi purpose solution.

The students also brought artificial tears to use at campus. They usually used it every 3-4 hours. An interesting story came from a student, she said that she once had a eye irritation because of one lens care solution. After changing the brand she felt relieved. There was also a question about when they felt uncomfortable while wearing contact lens and most of them would start to feel uncomfortable when the lens was almost due to dispose.

**Table 5: The condition of students' eyes**

| Eye's Condition | Frequency |
|---|---|
| Excellent | 15 |
| Very Good | 13 |
| Good | 11 |
| Fair Good | 7 |
| Dry Eye | 7 |

In this study there were 7 students with dry eye. Three students experienced dry eye syndrome due to their lens. Their lens were uncomfortable to wear because the lens would be needed to replace soon, but due to economy reason they decided to bear with it until it was really the time to replace. Other than the contact lens replacing time, one of the students had an issue with the contact lens. The new contact lens weren't comfortable to wear, but she decided not to replace the lens because of economy reason too. She had never felt uncomfortable with the old lens. The others were used to this condition and felt uncomfortable wearing contact lens, but decided not to replace the contact lens with glasses because the glasses weren't practice to wear and the lens were functioned as a daily life style to improve confidence in appearance. The contact lens also helped them to improve appearance. So they decided to wear contact lens eventhough it wasn't comfortable.

**Table 6: Statistical analysis correlation between daily lens wear duration and dry eye syndrome using Fischer's exact test**

| | | Eyes' Condition | | | | Total | P-Value |
|---|---|---|---|---|---|---|---|
| | | Non Dry Eye | | Dry Eye | | | |
| | | N | % | N | % | | |
| Daily Lens Wear Duration | ≤ 8 hrs | 32 | 69.6 | 4 | 57.1 | 36 | 0.667 |
| | > 8 hrs | 14 | 30.4 | 3 | 42.9 | 17 | |
| Total | | 46 | 100 | 7 | 100 | 53 | |

This study showed no correlation between contact lens daily wear duration and dry eye syndrome ($p > 0.05$).

## Discussion

The aim of this study was to analyze the correlation between daily lens wear duration and dry eye syndrome using CLDEQ-8 as the instrument. The result of this study is consistent with a study conducted in Nepal Eye Hospital between July 2007 and June 2012 that showed no correlation between daily lens wear duration and degree of symptoms in contact lens wearer eventhough many of contact lens wearers suffered from some symptoms. According to their study, dry eye syndrome that happened to their population was caused by dust and high polution. [5] This cause was also present in Young et al., study in 2011 among UK soft contact lens wearers. According to their study polution, dust and smoking were factors that significantly affect comfortable lens wear.[10] Chalmers and International Workshop on Contact Lens Discomfort 2013 also stated that environment factors induced dry eye [9] [11].

The other factor that affect dry eye in Sapkota et al., study was improper lens care system. The population of their study used MPS, but 14% used MPS that didn't contain appropiate disinfectant and 14.7% used unknown or solution without brand [5]. Lens care solution are intended for cleaning, disinfecting, removing protein deposit, rinsing and storing soft contact lens. To perform these functions, they have strong antibacterial properties, demonstrate compability with tear film and ocular tissue, are chemically and physically stable throughout the shelf-life and are pyshically compatible with wide range of lens materials. Multi Purpose Solution (MPS) component (buffers, surfactant, chelators) can help optimize the the physical properties of solution of solution to match those of tear film and maintain homeostatsis which may improve its biocompability with the ocular surface [12]. A randomized study by Lipener compared two different regiments of MPS. The result was regiment 1 showed statistically significant clinical difference for both corneal and conjunctival staining and reported greater comfort and tolerability to the contact lens/solution combination [13]. A randomized study by Guillon et al., also showed significant improvement in contact lens wettability and surface cleanliness with a spesific lens care solution [14]. Lens Care Solutions differently affected blink rate, subjective dry eye symptoms and visual discrimination speed. Those with wetting agents led to significant fewer eye blinks while affording better ocular comfort for contact lens wearers compared to that without. Lens Care Solutions with wetting agents also resulted in better visual

performance compared to wearing daily disposable contact lens. These presumably are because of the improved tear film quality [15]. One student told us about her experience in using lens care solution. She had eye irritation due to the use one brand lens care, but then she immediately changed to another brand lens care and her eyes were back to normal without any problems. The ability of lens care solution to clean protein deposit from the lens depends on the composition of lens care solution and contact lens material [16]. Choosing improper lens care solution can increase dry eye symptoms. Protein deposit on lens can also increase the foreign body sensation which is one of the dry eye symptoms [5]. Polymers age and and over time gather more and more deposit. Replacing the lens as often as possible can reduce problems. Daily disposable lens are the highest standard of this concept [17].

Sapkota et al., study also stated that low compliance and improper lens fitting might also contibute to the high rate of dry eye symptoms [5]. Although some lens movement is necessary to allow postlens tear exchange, loose fitting hydrogel lenses are associated with more corneal staining and bulbar and limbal hyperemia and with poorer comfort. Base curve and diameter are surrogate measures for lens sag and increasing sag reduces movement and consequently improves comfort within the limits of acceptable fitting [18]. A previous study by Wong et al., found 40% of their subjects unable to obtain acceptable fit because of loose fit due to steeper cornea [19]. Asian eyes have greater lid tightness compared with non-Asians, which could easily affect the behaviour of the lens on the eye during blink cycle, thus impacting sensations of discomfort or dryness. Minimizing lens movement would minimize its mechanical stimulus to the cornea, conjunctiva and eyelids, although too tight a fit with virtually no lens movement could result in increased dryness symptoms due to poor tear exchange [20].

Young et al., study and Sullet et al., study also found that contact lens material can increase comfort in wearing contact lens [10] [21]. The use of high water content and ionic characteristic materials are associated with greater dehydration [22] [23]. Such dehydration changes could in turn lead to ramifications such as reduced oxygen transmissibility, greater lens adherence and reduced tear exchange [22]. High water content materials have been showen to be associated with significant tear film deposition than low water content materials [17] [21]. Differences in deposition patterns of materials may impact wearer comfort and dry eye symptoms by altering the lens surface wettability and potentially tear stability/evaporation characteristics. The more polar lipids from the tear film may be attracted to the additional water to polymers (as water is polar itself and hydrogen bonds are a stronger attractive force than electrostatics bonds that might be associated with lipids binding to polymer itself). The resultant altered pre-lens tear film lipid layer and altered contact lens surface may lead to increased evaporation and/or reduced wettability, respectively [23].

Studies have revealed that continous contact lens wear can produce a reduction in corneal sensitivity, with the extent of sensation loss related to the type of contact lens, the material it is made from, and the frequency and duration of wear. Sensitivity diminishes progressively with the length of wear to a maximum after the 12 hour wear period [24]. The decrease in dry eye symptoms with more years of lens wear for non-Asians may be explained by a decrease in corneal sensitivity with contact lens wear. An alternative interpretation is that perhaps cornea becomes desensitized with long term wear leading to reduce perception of dryness [25].

According to Chalmers and Young et al., study, the use of artificial tears can also help in decreasing symptoms [10] [11]. In general, artificial tears or contact lens rewetting drops moisturize the eyes surface by simply increasing the water content of the tear film or by preventing tear evaporation and protect the ocular surface by reducing frictions between eyelids and the cornea [26]. Contact lens wear decrease the tear meniscus height especially with high water content contact lens [27]. Using lens rewetting drops can increase tear meniscus volume and reducing symptoms of dryness [11] [27] [28] [29] [30]. A reduction in the prelens tear film lipid layer and an increase in tear film evaporation are attributed contact lens wear, resulting in the precipitation of dryness symptoms [29]. A previous study that compared a lipid based eye drops with nonlipid eye drops showed that lipid based eye drops improved subjective comfort scores, increased comfortable in wearing time and reduced signs of lid wiper epitheliopathy and corneal staining compared with the use of non-lipid-containing rewetting eye drops [30].

The last factor found in this study was environmental factor. When this study was hold, the weather was always rainy almost everyday for a month. The temperature was cool with high humidity. Some studies found a connection between contact lens dry eyes and low humidity and high temperature [9] [10] [11]. The classrom had no air conditioner that was associated with dry eye due to low humidity and airflow [9]. Visual display terminal work also associated with dry eye syndrome. A previous study in Japan showed that contact lens wearers and long term visual display terminal worker had worse tear meniscus height value than non contact lens wearers and short term video display terminal workers [31]. Contact lens wearers that work in front of computer were found to be more likely to develop ocular symptoms than non contact lens wearers [32]. This study doesn’t have data about smoking, but exposure to smoke (smoker or passive smoker) tend to increase the symptoms score. This might be due to a fact that exposure to smoke increases the tear inflammatory cytokines, tear lipid peroxidations products and

decreases the mucosal defense resulting tear instability and damage the ocular surface epithelia [5].

In conclusion, this study shown that daily lens wear duration is not correlated with dry eye syndrome. Choosing the right lens material and fit for one's eye, frequent replacement of lens, using lens care solution and artificial tears and also protection from dust and smoke for comfortable lens wear can be achieved for longer comfortable duration.

There are some limitiations of the study. This study only used questionnaire as an instrument to measure dry eye syndrome without clinical examination that may give better measurement of dry eye syndrome. Second, the population in this study was only 53. Bigger population may give more differ scores that may affect the result.

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# Electrical Burns and Their Treatment in a Tertiary Hospital in Albania

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**Keywords:** Electric burns; Upper extremity; Complications; Treatment; Fasciotomy

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**Abstract**

**INTRODUCTION:** The electrical current burns represent a very aggressive pathology that leaves many functional and aesthetic consequences.

**AIM:** To evaluate the epidemiology of electrical burn injury and its associated complications and treatment.

**MATERIAL AND METHODS:** Demographic data, aetiology, burn percentage and other measures related to electrical burn injury of 33 electrical burn patients in a tertiary hospital during the years 2015-2017.

**RESULTS:** The mean age of patients is 31 (± 8.3) years old with a predominance of males (94%). The vast majority of injuries occurred at work ($p < 0.01$), superior extremities were more affected with hand (21.2%) and fingers (18.2%) being the main point of contact ($p < 0.01$). Muscular fasciotomy was performed in all patients who were treated surgically (n = 27), amputation was performed in 11 (40.7%) of cases, but amputated sites were more than the number of patients affected. Myoglobinuria (39.4%), cardio-respiratory distress (12.1%) contusion cerebri (6.1%), were the complication encountered in patients.

**CONCLUSIONS:** Electrical burn injuries are still amongst the highest accident-related morbidities. Educating the population about the dangers and hazards associated with improper use of electrical devices and instruments is imperative.

## Introduction

The electrical current burns represent a very aggressive pathology that leaves many functional and aesthetic consequences. It remains one of the most disabling accidents in the nosology of burns and plastic surgery because of the aesthetic and functional consequences in the surviving patients [1] [2]. The very aggressive and often disabling damages not so much lethal (after overcoming the first post accidental period), transform this burn in a problematic pathology and always enquiring.

Classification of electrical injuries is typically divided into low-voltage (LV < 1000 volts) and high-voltage (HV > 1000 volts), as well as by whether electrical current flows directly through the body vs a thermal injury caused by electrical flash. The electrical burn includes the tissue damages caused by the conduction of the electricity through the morphological structures of the organism.

This pathology is different from the thermal burns caused by the electric arch which because of the high temperature (around 2500ºC) causes wounds that might be misinterpreted like tissue damage from the electric current burns. The difference between these damages is significative because the thermal burns from the electric arch are not followed by debilitating consequences but are limited to the local damages. Electrical injuries are

uncommon but potentially devastating and constitute approximately 0.04 to 5% of admissions to burn units in developed countries, and up to 27% in developing countries [1] [2]. Electrical injuries in the adult population primarily affect men, are most often work-related, and are the fourth leading cause of traumatic work-related death [3]. Both morbidity and mortality in electrical injuries are relatively high and have physical and psychological short-term and long-term sequelae [4] [5]. This study was conducted to evaluate the epidemiology of electrical burn injury and its associated comorbidities in a tertiary hospital, to elucidate the burden of this type of injury and other burn-related complications for better management, prevention and treatment of patients.

## Material and the Methods

This is a retrospective study including 33 patients with electrical burns hospitalised in the intensive therapy unit in the "Burns and Plastic Service" clinic of the University Hospital Center "Mother Teresa" during the years 2015-2017.

Demographic information and the mechanism of injury (high voltage *vs* low voltage), complications, hospitalisation period, surgical interventions and severity of electrical injuries were recorded as well as acute and late complications, the degree of disability the degree of correlation of the wound surface size with the grade of the pathology and treatment strategy.

This study was accepted by the ethical committee of Tirana University of Medical Sciences, Tirana, Albania. All continuous variables were presented as means ± SD, and the frequencies of categorical variables were presented as percentages. Chi-square test was used to compare the proportions of categorical variables and student *t*-test to compare the mean of continuous variables. A P value < 0.05 was considered significant.

## Results

Sociodemographic characteristics of patients are presented in Table 1. The mean age of patients is 31 (± 8.3) years old with a predominance of males (94%). The most commonly affected were the 3-40 year age group (28.6%) and 41-50 year (30.3%) age group. The frequency of patients in other age groups was lower.

**Table 1: Sociodemographic characteristics of patients**

| Variables | N | % |
|---|---|---|
| Gender | | |
| Female | 3 | 6.0 |
| Male | 30 | 94.0 |
| Age, Mean (SD) | 31 (± 8.3) | |
| Age group | | |
| <10 | 5 | 15.2 |
| 11-20 | 3 | 9.1 |
| 21-30 | 4 | 12.1 |
| 31-40 | 7 | 21.2 |
| 41-50 | 10 | 30.3 |
| >50 | 4 | 12.1 |

Patients affected by high voltage electricity outnumbered the low voltage group (Table 2). The vast majority of injuries occurred at work ($p < 0.01$) superior extremities were more affected with hand (21.2%) and fingers (18.2%) being the main point of contact ($p < 0.01$). Foot (12.1%) was more affected in inferior extremities, and in 15.2% of patients in other regions were involved (head, thorax, abdomen).

**Table 2: Clinical characteristics of patients**

| Variables | N | % |
|---|---|---|
| Electric power | | |
| High voltage | 27 | 81.8 |
| Low voltage | 6 | 18.2 |
| Injuries | | |
| At home | 5 | 15.2 |
| At work | 28 | 84.8 |
| Point of contact | | |
| Upper extremities | | |
| Fingers | 6 | 18.2 |
| Thumb | 3 | 9.1 |
| Hand | 7 | 21.2 |
| Forearm | 3 | 9.1 |
| Arm | 2 | 6.1 |
| Lower extremities | | |
| Foot | 4 | 12.1 |
| Below knee | 2 | 6.1 |
| Above knee | 1 | 3.0 |
| Other regions | 5 | 15.2 |
| Wound surface | | |
| < 10% | 16 | 48.5 |
| 21 – 30% | 12 | 36.4 |
| >30% | 5 | 15.2 |

The average hospital stay, was higher M = 58.1 (± 24.3) in 27 (81.8%) patients with high voltage injury who underwent in surgical treatment for ectrocombustio in comparison to low voltage injury patients M = 7.5 (± 2.5) in whom the treatment was conservative for thermal burn, 6 (18.2%), ($p < 0.01$). Muscular fasciotomy was performed in all patients who were treated surgically (n = 27), amputation was performed in 11 (40.7%) of cases, but amputated sites were more than the number of patients affected (n = 15) (Table 3).

**Table 3: Surgical treatment technique**

| Surgical technique | N | % |
|---|---|---|
| Muscular fasciotomy | 27 | 100.0 |
| Amputations | 11 | 40.7 |
| Free plastic post necrosectomy | 16 | 59.3 |
| Free plastic post-amputation | 5 | 18.5 |

The most common site of amputation was the fingers. Most of these injuries occurred during spring and summer. Myoglobinuria (39.4%), cardio-respiratory distress (12.1%) contusion cerebri (6.1%), were the complication encountered in patients.

## Discussion

The electrical burns occur mainly among males 94% in our study. The cause of this high involvement of this sex implies the high mobility of the children of this sex and the risk-taking behaviour whereas among adults males are involved more in the professions such as construction and factories. Other studies have reported similar results with our study [6] [7] [8].

Also in 25% of the cases, alcohol consumption was the cause of the accident. In total, 35% of accidents occurred at night shifts. The most common cause of electrical burn injury was electrical contact. Attention has been given in this study also the differential between an electrical burn (electrocombustion) from the electric power and a thermal burn from the electric arch, which often confuses the diagnosis at the moment of the hospitalisation in the emergency department. This differentiation does have not only theoretical considerations but also practical in the further management of the pathology. Practically, the thermal burn from the electrical arch, it is characterised by a low voltage and causes a relatively superficial wound because of the short time acting on the tissue. In our study, the six patients with thermal burns are treated conservatory, *per primary*, with epithelization. On the other side, the thermal electric burns, do not manifest vital complications in the acute phase, and they do not affect the metabolic balance of the patient [9] [10]. Considering the extensive damage these burns manifest, the electric burns require careful management of the pathology since the first moment due to the serious acute complications that might follow. Myoglobinuria was one of the acutest complications encountered in our study. 39.4% of patients have manifested this symptom for 48-96 hours. Another severe complication involved by cardiac rhythm disturbances and respiratory acidosis, with pulmonary fields hypoventilation in 12.1% patients. Regarding the anatomical part, we note that the most frequent point of contact was the upper extremities, as the most active ones. In 63.6% of the cases at least one of the contact points is on the upper extremities. Theoretically, the tissue damage is greater in the entry point and smaller on the exit point [11] [12]. Nowadays we are moving more and more towards naming of these points like contact points due to the aggressiveness of the tissue damage which doesn't always discriminate which is an entry point or an exit point. Even in the clinical practice, it is difficult the morphopathological differentiation of the nature of the tissue damage to determine the entry point without a careful history of the accident to define the damage mechanism [13] [14] [15]. In this study, we found two thoracic contact points and one occipital. The thoracic cage is completely bony and presents a high resistance towards the electric current, and this is converted into a high heat production towards the tissues resulting very revitalizing. The same phenomena happen on the skull, where the cranium is very close to the surface and is covered by a very thin layer of soft of tissues, very fragile towards the heat produced by the electric current. The most susceptible to damage remain the entry points in the extremities, where the high energy produced by electric current results in severe damages in the small sections of the tissue. The cause of these lesions in the small section structures is the high resistance they display towards the electricity. Patients with high voltage injuries underwent surgical treatment. Careful management helps in preventing post-combustion complications. The sparing of damaged structures from the compartment syndrome requires surgical intervention within the first 24 hours [16]. Muscular fasciotomy for the prevention of the metabolic necrotic damage progression remained imperative and was applied in 100% of the cases. This is followed by total necrosectomy or amputation as soon the metabolic and hemodynamic equilibriums was reached. In this study, the amputation was applied in 40.7% of patients in the interval 72-120 hours after the accident. In five of the patients, the amputations were very disabling while in the rest of them the amputations were only in the level of fingers. Nevertheless, even in the cases of minor amputations, post-combustion fibrosis has contributed to the complete loss of function of the affected limb. Total necrosectomy was applied in 59.3% of the patients. The tissue damages were closed with free plastic intervention after 10-21 days, thirteen cases post necrosectomy and in three cases post-amputation in upper extremities. The high frequency of the free plastic technique, mostly on superior upper extremities increases the degree of the disability because of the aggressive fibrosis, mostly in the level of the articulations [17] [18]. The most efficient period for the above interventions was during the first week after the accident. Noteworthy in this study were the late complications of these accidents such as anaemia and haemorrhage which was treated surgically.

The surface of burn wounds varied patients from 3% to > 30% of body surface with a median of 15.8%. Also, the hospital stay varied widely, depending on the burn surface from the electric arch. The electric burn is one of the most severe and expensive pathologies, with a long hospital stay and several surgical procedures. The mean hospital stay for surgically treated patients was 58.1 (± 24.3) days, except for patients with amputations and the patient with burns in the visceral cranium, whose stay was longer. The mean hospital stay for six patients with thermal burns from the electric arch was 7.5 (± 2.5) days, hinting the mild damages of this pathology. Similar findings were reports by other authors [19]. Patients experienced significant levels of emotional

distress. Anxiety was more common in patients with high-voltage electrical injuries [20]. The treatment aims to achieve the skin cover to prevent infection and to allow early mobilisation. Adult electrical injuries usually occur as an occupational hazard, whereas children are primarily injured accidentally. The spectrum of electrical injury is very broad, ranging from minimal injury to severe multiorgan involvement, with both occult and delayed complications and death. If signs and symptoms of compartment syndrome exist, decompression is necessary. Escharotomy and fasciotomy were performed in the upper limb and trunk relieving compartment pressure in upper limb and improving ventilation. Amputations become necessary when there is damage to nerves, tendons, skin and all blood vessels. Current passing through the heart or thorax can cause cardiac arrhythmias and direct myocardial damage, whereas passing through the brain can result in respiratory arrest seizures, direct brain injury and paralysis. Current passing close to the eyes can cause cataracts [21]. Resuscitative efforts continued in the emergency department with the adequate fluid administration. A urine output of at least 1.5 ml/kg/hr was maintained. All the circuits may produce myonecrosis with myoglobinemia, and their attendant complications.

In conclusion, the awareness towards the functional and aesthetic disabling effect of the electrical burns, despite careful treatment, highlights the very important task of the prevention towards this accident. Also, it is required the perfection of tissue sparing surgical techniques that are endangered from the secondary necrosis, to minimise the tissue damages and prevent the late complications. Electrical injuries can be avoided if correct preventive measures are put in place. Work involving contact with electricity should be planned very well to ensure that anyone required to handle the various tasks has the competence to do them. Safety should be encouraged at all times and in all places from the home to the workplace. Education and compliance with safety measures, as well as common sense and respect for the potential danger of electricity, are still essential for avoiding these injuries.

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***Clinical Science***

# Is There a Place for Local Natural Treatment of Psoriasis?

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**Keywords:** Apitherapy; Psoriasis; Skin disease; Natural therapy; Propolis; Aloe vera

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**Abstract**

**BACKGROUND:** Apitherapy is the medical use of honey bee products (honey, propolis, royal jelly, bee wax, and bee venom) to relieve human ailments, propolis in particularly, rich in essential oils such as flavonoid. Propolis is derived from tree buds and plants. It is considered as one of the most well-documented products from the honeybee and has always played an important role in traditional folk medicine. Another renowned plant is Aloe vera appertaining to the Liliaceae family. Its mucilaginous gel has been extensively used in many cultures for its apparent effectiveness in treating wounds, burns, itchiness and hair loss.

**AIM:** The aim was to assess the efficacy of a mixture in an ointment form of propolis (50%) and aloe vera (3%), in the treatment of mild to moderate psoriasis.

**METHODS:** In this double-blind control study, 2248 patients with both mild to moderate cases of psoriasis were evaluated from 2012 to 2015.

**RESULTS:** In Group 1 the overall response at the end of 12 weeks was as follows: Cleared in 64.4% (excellent response), good response in 22.2%, and weak response in 5.6% and no response in 7.7%. In Group 2 (placebo group) no significant improvement was observed after 12 weeks of treatment. Also, histology also demonstrated a marked reduction in hyperkeratosis and acanthosis.

**CONCLUSION:** In comparison with Group 2 (placebo group) patients in Group 1, treated with a mixture of propolis (50%) and aloe vera (3%), in the form of an ointment have shown noteworthy improvement thus substantiating the therapeutic value of propolis and aloe vera in the treatment of mild to moderate psoriasis.

## Introduction

Psoriasis is an inflammatory and proliferative skin disease with heterogeneous genetic background and is characterised by chronic, sharply demarcated, dull-red scaly plaques on the skin and particularly on the extensor prominences and in the scalp area [1]. It is one of the most common chronic skin diseases in need of long-term therapy. Although the multi-factorial aetiology of this disease, a strong association between body mass index and psoriasis severity was shown [7]. Until now there has not been an ideal treatment for this perplexing ailment.

However, a variety of therapeutic approaches have shown limited efficacy with frequent side effects. *In vivo* and *in vitro* data prove the effectiveness of cytokines taken in low-doses [1] and antioxidants [2] Complementary therapy based on psychotherapeutic approaches has also shown to be effective [3].

The use of apitherapy (using hive products for medical and pharmacological purposes) remains a controversial matter in the treatment of dermatological diseases especially psoriasis [5].

Aloe vera is a plant that has been used in folk medicine; its mucilaginous gel is used for treating itching, hair loss and many other problems. It is also found in modern-day commercial beauty products.

Aloe vera has also proven effective on the

cutaneous burn and wound healing [6]. There have been many studies that demonstrated considerable analgesic, antipruritic, wound healing and anti-inflammatory properties [4]. These qualities justify investigating Aloe vera in the treatment of psoriasis.

This study aims to evaluate the efficacy of a combined natural topical treatment for psoriasis. The drug is mainly composed of propolis (one of the most important hive products) and Aloe vera. The ointment is used for topical treatment of mild to moderate psoriasis in all regions of the body except face and genitalia.

## Material and Methods

In a double-blind placebo-controlled study, 2248 patients of the Center of Dermatology in Heliopolis (Cairo, Egypt), with mild to moderate psoriasis have been included. Patients from 2012 to 2015 were divided into two groups. Group I was treated with an ointment containing a combination of propolis 50% and Aloe vera 3%. Group was II treated with a placebo (ointment without propolis and Aloe vera). Topical treatment was performed for 12 weeks while sparing face and genital.

*Definition of clinical outcome:*

- Excellent response: when all psoriatic lesions disappeared, and skin becomes nearly normal (no erythema, no infiltration or desquamation of skin).
- Good response: when some of the lesions disappeared.
- Weak response: when no marked improvement in erythema, infiltration or desquamation of skin was noted.
- No response: when no response occurred at all.

The outcome was also evaluated by psoriasis area and severity-score (PASI score) and skin biopsies.

## Results

In Group 1 the overall response at the end of 12 weeks was as follows: Cleared in 64.4% (excellent response), good response in 22.2%, and weak response in 5.6% and no response in 7.7% (Figures 1-6).

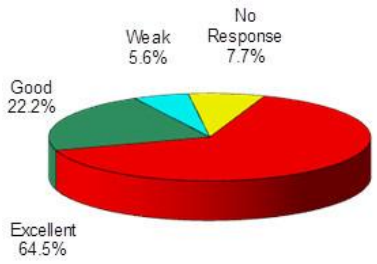


*Figure 1: Clinical results of treatment*

The formulation resulted in significant reduction of erythema and scaling. In Group 2 (placebo group) no significant improvement was observed after 12 weeks of treatment. Also, histology also demonstrated a marked reduction in hyperkeratosis and acanthosis.

The ointment worked for different types of lesions (Table 1).

**Table 1: Results of treatment I relation to the type of psoriasis lesions**

| Type | | Result | | Total |
|---|---|---|---|---|
| | | Effective | Non-Effective | |
| Guttate | Excellent | 183 | | 183 |
| | Good | 24 | | 24 |
| | Weak | | 7 | 7 |
| | No Response | | 11 | 11 |
| Total | | 207 | 18 | 225 |
| Palmo | Excellent | 246 | | 246 |
| | Good | 95 | | 95 |
| | Weak | | 12 | 12 |
| | No Response | | 38 | 38 |
| Total | | 341 | 50 | 391 |
| Plaque | Excellent | 1021 | | 1021 |
| | Good | 380 | | 380 |
| | Weak | | 107 | 107 |
| | No Response | | 124 | 124 |
| Total | | 1401 | 231 | 1632 |

We used Psoriasis Area and Severity Index (PASI score) as a method of clinical assessment of patients.

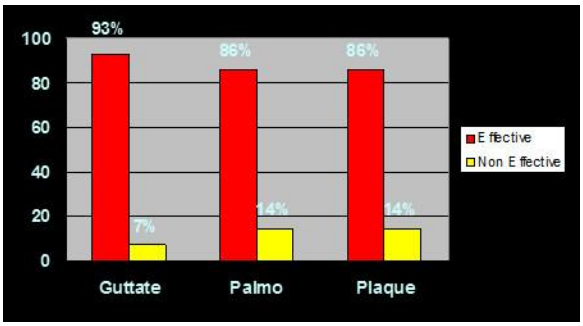


*Figure 2: Results of treatment. Percentage of PASI-reduction*

This is purely clinical rating system which assesses the area of the body affected by the intensity of the main symptoms.

**Table 2: reduction of PASI score**

| %- Reduction of PASI score | Cases | Percent |
|---|---|---|
| Effective | 1947 | 86.70% |
| Non-effective | 301 | 13.30% |
| Total | 2248 | 100% |

A punch biopsy was taken before application of the treatment and stained to be examined histologically (Figure 3).

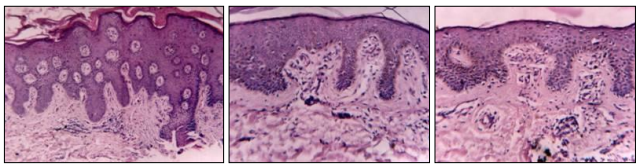

*Figure 3: Histology pre and post-treatment (Biopsy Rt.Sole; HE stain). Within the typical plaque, psoriatic epidermis shows marked epidermal acanthosis, hyperkeratosis, and elongation; the presence of Munro's microabscesses (to the left). Parakeratosis changed to orthokeratosis, acanthosis decreased, the rate ridges became shorter than before TTT; absence of Munro's microabscesses (to the right)*

## Discussion

Psoriasis is a common systemic inflammatory disorder. In this double-blind placebo-controlled study 2248 patients with mild to moderate psoriasis were enrolled. Group 1 received topical treatment with an ointment containing a combination of propolis and aloe vera. Group 2 received placebo (ointment without aforementioned active compounds. We excluded only facial and genital skin. Tolerability of the treatment was very good.

*Figure 4: Pre (left) and post (right) 3 months of treatment*

Major natural constituents have been used in this trial. The first one is propolis, a non-toxic substance which is composed of resins, waxes and fatty acids, minerals, vitamins, and flavonoids. Flavonoids, caffeic acid-phenethyl ester (CAPE), and hydroxycinnamic acid are responsible for most biological effects including with anti-inflammatory activity. CAPE has both anti-inflammatory and anti-oxidative properties. Since CAPE is lipophilic, it is capable of inhibiting the intracellular LOX and COX enzymes, and thereby indirectly the arachidonic pathway. This action prevents the release of prostaglandins and leukotrienes, and it decreases neutrophil infiltration into the skin [4] [9].

No severe side effects were noted. Minimal discomfort due to the texture of the ointment and temporal itching sensation had been observed.

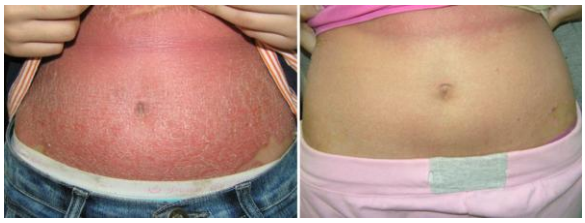

*Figure 5: Pre (left) and post (right) 3 months of treatment*

The other natural ingredients were Aloe vera. Aloe vera has moisturising qualities, which are helpful in restoring disturbed skin barrier function. Aloesin from Aloe vera positively regulated the release of cytokines and growth factors (IL-1β, IL-6, TGF-β1 and TNF-α) from macrophages and enhanced angiogenesis in endothelial cells (HUVECs). Aloesin accelerates wound closure in mice by activating Smad and MAPK signalling proteins that are of utmost importance in cell migration, angiogenesis and tissue development [10].

*Figure 6: Pre (left) and post (right) 3 months of treatment*

Topical Aloe vera was more effective in PASI reduction in moderate plaque psoriasis in a randomised, comparative, double-blind 8-weeks trial than 0.1% triamcinolone acetonide [11]. These results are supported by the present study.

In comparison with Group 2 (placebo group) patients in Group 1, treated with a mixture of propolis (50%) and aloe vera (3%), in the form of an ointment have shown noteworthy improvement thus substantiating the therapeutic value of propolis and aloe vera in the treatment of mild to moderate psoriasis.

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***Case Report***

# Unusual Signs and Symptoms in HIV-Positive Patients Coinfected with *Leishmania spp:* The Importance of Neglected Tropical Disease in Differential Diagnosis

Manuela Ceccarelli[1*], Emmanuele Venanzi Rullo[1], Fabrizio Condorelli[2], Fabrizio Vitale[3], Vincenzo Di Marco[4], Giuseppe Nunnari[1], Giovanni Francesco Pellicanò[5]

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**Keywords:** Leishmania; HIV; Visceral Leishmania; CD4: CD8 ratio; Coinfection

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**Abstract**

**BACKGROUND:** Leishmaniasis is a parasitic disease affecting both animals and humans, acquired with the bite of sand flies or, in Injection Drug Users (IDUs), with contaminated needles, still hypoendemic in Sicily and the Mediterranean basin. Even though it is responsible for 20,000 to 40,000 deaths per year, this parasitic infection is still considered a neglected tropical disease. People Living with HIV (PLWH) are considered at high-risk of developing Leishmaniasis and, despite the introduction of Highly Active Anti-Retroviral Therapy (HAART), mortality rate and relapses prevalence are still high in coinfected people.

**CASE REPORT:** We present a case of HIV-Leishmania coinfection, posing the attention on the atypical signs and symptoms and the importance of thinking about other causes than the HIV infection progression when the patient presents with a worsening of his immune status during HAART.

**CONCLUSION:** This parasitic disease has a high mortality rate, so it is mandatory to think about it in all the patients having a low CD4+ T-cell count and an inverted CD4/CD8 ratio under HAART.

## Introduction

After the introduction of Highly Active Anti-Retroviral Therapy (HAART) mean age and comorbidities related to ageing, immune suppression, coinfections and persistent inflammation increased in People Living with HIV (PLWH) [1] [2] [3] [4] [5] [6] [7] [8] [9] [10] [11] [12] [13] [14] [15] [16] [17] [18] [19] [20] [21] [22] [23] [24] [25] [26] [27].

Among the coinfections commonly observed in PLWH, there is Leishmaniasis, which is still considered a neglected tropical disease, affecting immunocompromised people, and especially PLWH, more than the immunocompetent population, being responsible for 20,000 to 40,000 deaths per year [28].

PLWH are considered at high-risk of developing Leishmaniasis because of the two infections geographical distribution: areas, where the HIV infection have a high prevalence, are usually also areas where the Leishmania infection is widespread [29].

Despite the introduction of HAART significantly reduced the coinfection prevalence, mortality rate and relapses prevalence are still high in immunocompromised people [29][31]. Prognosis is also affected by the nutritional status of the patient [29].

Treatment is difficult in immunocompromised people, because of the role of both constitutive immunodepression and Leishmania-related immunodepression on the response to the infection [28] [31] [32] [33] [34].

Here we present a case of HIV-Leishmania coinfection during which the patient developed some atypical signs before the appearance of a more typical Leishmaniasis clinical presentation allowed the diagnosis.

## Case Report

A 52-years-old HIV-positive man, followed in our outpatient clinic since 1999 for his infection, came to the Emergency Ward of our University Hospital in August 2017, complaining of diarrhoea (defined as defecation of yellow liquid faeces at least five times per day) for two months. He lived in a poor health condition setting, and he was a smoker. He always lived in Sicily and never left the island.

Until November 2015 he was on combined anti-retroviral therapy (cART) with emtricitabine/tenofovir disoproxil (FTC/TDF) and raltegravir (RAL), with successful virologic (HIV-RNA not detectable) and partially successful immunological (CD4+ 491/µl, 24%) control, but he was lost to follow-up for a year. In January 2017 he was admitted to the Thoracic Surgery Unit of our hospital for a massive left pleural effusion, drained through a chest tube. An incomplete immunological control was highlighted (CD4+ T-cells 62/ µl, 7%; CD8+ T-cells 630/ µl, 71%, CD4/CD8 0.09) at that time. Therefore, he started a cART with darunavir/cobicistat (DRV/COBI) and RAL, with a successful virologic control (HIV-RNA not detectable), and a slight improvement of his immunological control (CD4+ 82/µl, 11 %) at the last blood testing, which took place in May.

At the admission, he complained of asthenia. He reported that he had autonomously suspended cART, thinking that the diarrhoea was an adverse effect of the therapy, with no improvement. The physical examination revealed extreme dehydration and moderate hepatosplenomegaly. Moreover, a hyperemic hyperthermic painful lesion was highlighted on his left side, around the area where the chest tube was positioned in January. He was feverish (37.5 °C), while blood pressure and heart rate were normal. Blood tests showed anemia (Hb 9.9 g/dl), leukopenia (WBC 3,260/µl) and thrombocytopenia (PLTS 109,000/µl); hypoalbuminemia (1.7 g/dl); monoclonal hypergammaglobulinemia (65.58%, normal 10.5 - 19.5%). He began an intravenous (IV) therapy with albumin and IV hydration, and a cART with DRV/COBI and RAL was started again.

During the admission his conditions worsened, making it necessary to perform a blood transfusion on August, 5th. Table 1 resumes altered results of the blood tests performed on our patient during the admission.

**Table 1: Blood test results during the admission**

| | Aug, 2nd | Aug, 4th | Aug, 5th | Aug, 6th | Aug, 8th | Aug, 11th | Aug, 16th |
|---|---|---|---|---|---|---|---|
| WBC/ µl | 3.280 | 1.990 | 2.170 | 2.080 | 2.460 | 1.860 | 2.140 |
| N (%) | 55 | 56 | 52 | 56 | 50 | 61 | 54 |
| N/ µl | 1.810 | 1.114 | 1.128 | 1.165 | 1.230 | 1.135 | 1.156 |
| L (%) | 38 | 37 | 43 | 37 | 44 | 33 | 40 |
| L/ µl | 1.250 | 736 | 933 | 770 | 1.082 | 614 | 856 |
| CD3 (%) | | 87 | | | | | |
| CD3/ µl | | 641 | | | | | |
| CD4 (%) | | 8 | | | | | |
| CD4/µl | | 59 | | | | | |
| CD8 (%) | | 71 | | | | | |
| CD8/ µl | | 523 | | | | | |
| CD4/CD8 | | 0,11 | | | | | |
| CD19 (%) | | 7 | | | | | |
| CD19/ µl | | 52 | | | | | |
| CD20 (%) | | 5 | | | | | |
| CD20/ µl | | 37 | | | | | |
| CD34 gated (%) | | 0,02 | | | | | |
| CD34 vital/ µl | | 1,96 | | | | | |
| PLTS | 109,000 | 91,000 | 71,000 | 74,000 | 87,000 | 103,000 | 138,000 |
| Hb (g/dl) | 9,9 | 8,2 | 7,6 | 8,4 | 9,7 | 8,7 | 8,6 |
| CRP (mg/dl) | 12,80 | | 6,30 | | 6,70 | 6,30 | 1,50 |
| PCT (ng/ml) | | 0,44 | | | | | 0,3 |
| Albumin (g/dl) | 1,7 | | | | 2,02 | 2,34 | 2,8 |
| γ-globulin (%) | 65,58 | | | | 61,4 | 58,66 | |

As it can be seen, his immunological control had worsened, revealing a CD4+ T-cell count of 59/µl (8%), with a CD8+ T-cell count of 523/µl (71%) and a resulting CD4+/CD8+ ratio of 0.11.

Stool examinations (research of the *Clostridium difficile* toxin, parasitic infections and stool cultures, faecal occult blood) were performed, resulting in negative. Suspecting multiple myeloma, the patient underwent a bone marrow biopsy to determine the cause of the pancytopenia. No biopsy was performed on the flank skin lesion.

Indirect immunofluorescence assay (IFAT) and Polymerase Chain Reaction (PCR) for the research of a *Leishmania* infection were performed on the 4th day after the admission, resulting in positive (PCR 11,500 Leishmania/ml; IFAT 1:5120) on the 8th day after the admission.

He then began a treatment with Liposomal Amphotericin B (L-AMB) 4 mg/kg/day on days 1 to 5, according to the *Italian guidelines for the diagnosis and management of HIV infection (2016 edition)* of the Italian Society of Infectious and Tropical Diseases (ISITD), and he repeated the treatment on days 10, 17, 24, 31 and 38, completing the cycle [35]. He was discharged on the 14th day after the admission and completed the treatment as an outpatient. Diarrhoea and the hyperemic lesion on his flank completely disappeared after the fifth day of therapy with L-AMB. Figure 1 shows the Serum Protein Electrophoresis (S-PEP) trend before, during and at the end of the therapy. Secondary prophylaxis was not started, and the patient is still in follow up for the possibility of VL relapses.

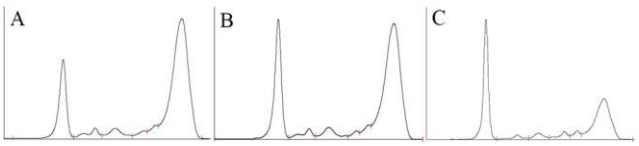


*Figure 1: S-PEP before (A); during (B) and after completing (C) the therapy with L-AMB*

## Discussion

Leishmaniasis is a parasitic disease affecting both animals and humans, acquired with the bite of sand flies or, in Injection Drug Users (IDUs), with contaminated needles, still hypoendemic in Sicily and the Mediterranean basin [34] [36].

It is a known opportunistic disease in People Living with HIV (PLWH), whose immunodeficiency promotes visceral localisation, even though the coinfection prevalence reduced after the introduction of HAART [33] [36] [37]. Moreover, *Leishmania spp* can promote viral replication and enhance progression to Acquired Immuno-Deficiency Syndrome (AIDS) [34]. As a result, despite the introduction of HAART, relapses are still common, and mortality is three times higher in HIV-Leishmania coinfection than in HIV-negative people affected by Leishmania infection [30] [31].

Cota and al [33] reported in 2017 that CD4 T-cell count at the moment of the diagnosis is not able to foresee the patient's prognosis. However, resolution of the infection depends on an efficient CD4+ T-cell response, and it was observed that a higher incidence of symptoms is related to a lower CD4+ T-cell count [31] [32].

The most frequent signs and symptoms of this infection are fever, asthenia, weight loss and splenomegaly; unspecific symptoms that could often lead to a delay in the diagnosis [31] [34]. Also, PLWH can complain of misleading non-classical symptoms [29].

In our case, the patient came to our attention in January, after more than a year of likely therapeutic vacation, with a seriously impaired immunological control. At the time he did not have any classical sign of the infection (normal S-PEP, no signs of pancytopenia, no hepatosplenomegaly), but it has been reported that Leishmaniasis can be related to pleural effusion, especially in PLWH with a very low CD4+ T-cell count [38]. Moreover, Infectious Diseases Society of America (IDSA) 2016 guidelines report that in PLWH the number of asymptomatic carriers of Leishmania seems to be higher than in the immunocompetent host [39]. Therefore, it can be supposed that our patient's disease began some time before the classical signs appeared.

An active response to Leishmania, leading to the infection control, is associated to host adaptive immunity, but, at the same time, to natural immunity [40]. The most influential factor in the immune response in Leishmaniasis seems to be the early interaction of the parasite with macrophages and dendritic cells [41].

Moreover, it has been observed an enhanced secretion of Th2 cytokines, and in particular IL-10, in Visceral Leishmaniasis associated to HIV-infection, which can promote the dissemination of both the virus and the parasite [28] [32] [40] [41] [42] [43].

Our patient presented to our attention, both in January and August, with a profoundly impaired immunological control, defined as a very low CD4+ T-cell count and an inverted CD4/CD8 ratio, despite having a suppressed VL, a common sign during visceral Leishmaniasis [42]. However, the fact the patient came from a period of therapeutic vacation made difficult to think to other causes of severe immunodepression than his HIV infection, leading to the possibility of a diagnostic delay.

The CD4/CD8 ratio is a marker of immune dysfunction leading to persistent inflammation in PLWH, and a low ratio can predict an impaired CD4+ T-cell count recovery before the start of the HAART [44]. During visceral leishmaniasis, CD8+ T-cells, and especially those expressing CD38, or activated CD8+ T-cells, increase, leading to a status of chronic inflammation which results in a T cell depletion, establishing a vicious circle that worsens the immune-depression [42].

Although both IDSA and ISITD guidelines recommend secondary prophylaxis with L-AMB in patients with a CD4+ T-cell count lower than 200/µl, our patient refused it [35] [39]. He is still in follow up for the possibility of relapses and recently completed his 5$^{th}$ month from the end of the therapy with L-AMB.

In conclusion, visceral Leishmaniasis is an important opportunistic disease in PLWH, with a complicated differential diagnosis because of its unspecific symptoms and signs. It is even more difficult because of the possibility of atypical manifestations.

However, this parasitic disease has a high mortality rate, so it is mandatory to think about it in all the patients having a low CD4+ T-cell count and an inverted CD4/CD8 ratio under HAART.

Further studies are needed to clear the pathogenesis of the infection and to establish the duration of the secondary prophylaxis.

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***Case Report***

# A Very Rare Case of Cor Triatriatum with Severe Mitral Regurgitation

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**Abstract**

**BACKGROUND:** Cor triatriatum sinister is rare congenital heart disease. It is mainly presented in childhood and often accompanied with other congenital anomalies. The cases with cor triatriatum treated surgically in adults and accompanied with severe mitral regurgitation are very rare.

**CASE REPORT:** We present a case with diagnosed cor triatriatum and severe mitral regurgitation. The diagnose was made by echocardiography. She was a female 25 years that was hospitalised with signs of heart failure NYHA II-III.

**CONCLUSION:** We performed the resection of the membrane in the left atrium and repair of a mitral valve according to Alfieri. The patient did very well after the surgery.

## Introduction

Cor triatriatum sinister is rare congenital heart disease. This pathology was first described by Church in 1868. This anomaly is mainly presented in childhood and most often is accompanied with DIA, VSD, TF, partial abnormal pulmonary return [1]. The cases treated surgically in adults and accompanied with severe mitral regurgitation are very rare. In our knowledge, there are reported some cases of surgical treatment of cor triatriatum and severe mitral regurgitation. We present a case diagnosed and treated for cor triatriatum and mitral regurgitation at the adult age. We performed the resection of the membrane in the left atrium and repair of a mitral valve according to Alfieri technique. The patient had very good progress after the surgery.

## Case Presentation

The patient of 26 years old was admitted to our clinic with the diagnosis: Cor triatriatum sinister and severe mitral regurgitation. Heart failure NYHA II-III.

The main complaints were weakness and dyspnea in minimal to moderate physical efforts with a history of about four months. The patient performed ambulatory transthoracic echocardiography and was diagnosed with cor triatriatum sinister and severe mitral regurgitation. In these conditions was hospitalised at our clinic. In electrocardiogram was normal sinus rhythm. All laboratories examinations were normal.

We performed in hospital trans-oesophagal echocardiography that resulted:

Severe mitral regurgitation, myxomatous degeneration of both leaflets of the mitral valve with prolapse of the anterior leaflet, dilated left atrium and there was a membrane that divides the left atrium into two parts. There were communication between two parts.

The left ventricle was dilated. The diameters were DTD/DTS respectively 65/45 mm. The ejection fraction of LV was estimated about 45%.

The right chambers were dilated. The pulmonary artery systolic pressure was approximately 40 mm Hg. No other structural anomalies are noticed (Fig. 1 from echocardiography).

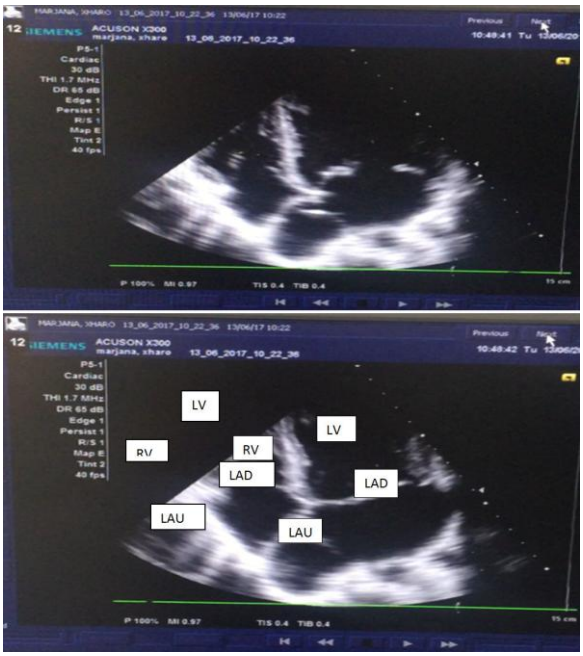


*Figure 1: LV-Left ventricle, RV-right ventricle, LAD-left atrium distal chamber, LAU-left atrium upper chamber. Between LAU and LAD, we can distinguish the membrane in the left atrium*

Fig. 2 is the schematic presentation of Cor triatriatum during the surgery [17].

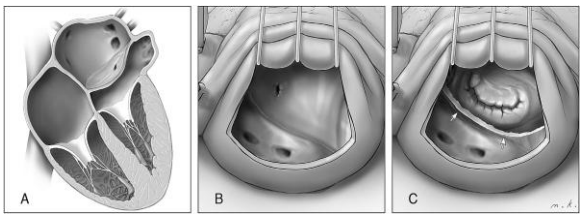


*Figure 2: (A) Cor triatriatum defect without any associated atrial septal defect; (B) Operative exposure of cor triatriatum membrane through a left atriotomy. Restrictive communication between the proximal and the distal left atrial chambers is visible; (C) Exposure of mitral valve after the excision of cor triatriatum membrane (arrows mark the line of excision Ann Thorac Surg 2014; 97:1659–63)*

The intervention was performed under extracorporeal circulation and moderate hypothermia. When the left atrium was opened, we notice a fibromuscular membrane, which divided the left atrium into two rooms, upper room, where the pulmonary veins were drained and the lower room where there were auricle and mitral valve. The hole in the membrane that made the communication between the two rooms had a diameter of about 1.2 cm (Fig. 3). It was impossible to see the mitral valve without resection of the membrane. The membrane was resected, and the mitral valve was exposed. During the examination of the valve, we noticed myxomatous degeneration of mitral valve leaflets with important prolapse of anterior leaflet macroscopically. We performed mitral valve repair according to Alfieri technique.

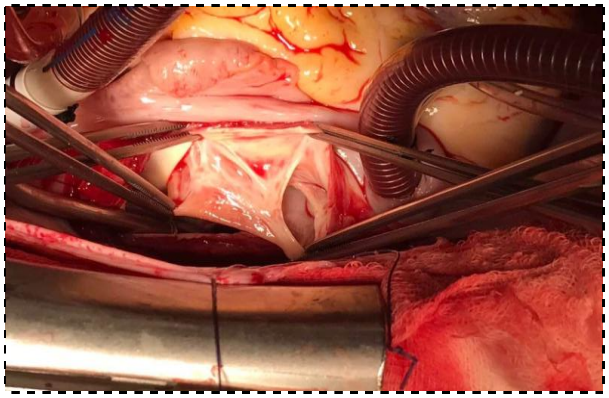

*Figure 3: After we opened LA we see the membrane that divided LA into two parts upper and distal parts. Between four forceps has located the membrane. Between the right forceps is located the hole of the membrane of Cor Triatriatum*

The patient did very well postoperatively. Postoperative echocardiography showed no leak and no transvalvular gradients of the mitral valve.

## Discussion

Cor triatriatum is a very rare congenital heart disease. The incidence ranges 0.1 to 0.4% of heart congenital heart diseases [3] [4] [5] [6] [7] [8] [9] [10] [11]. Classic (or typical) cor triatriatum, or cor triatriatum sinister, is a rare congenital cardiac anomaly in which the pulmonary veins typically enter a “proximal” left atrial chamber separated from the “distal “ left atrial chamber by a diaphragm in which there are one or more restrictive ostia. This pathology was first described by Church in 1868. The most frequent concomitants anomalies are partial anomalous pulmonary veins return, unroofed coronary sinus, VSD, CoA, AVD, TF, asplenia polysplenia [1]. The first surgical treatment is referred by Lewis [2]. The surgical treatment series referred to this anomaly are small. In the near past, the experience of surgical treatment referred about 250 cases. Mayo Clinic in 50 years’ experience refers 25 cases of surgical treatment of cor triatriatum [17]. Another reference

centre reports 28 cases in 23 years of experience [10] while the cases of surgical treatment in adults with concomitant severe mitral regurgitation are very rare. In our knowledge there are only 9 reported cases [3] [4] [8] [12] [13] [14] [15] [16].

The patients are generally presented in childhood, and symptomatic presentation depends on the size of the communicative hole. The classic clinical of the anomaly is similar to that of mitral valve stenosis. The presentation in adulthood is very rare occurred [1]. Our case is presented with signs of heart failure that may result from mitral regurgitation, congenital anomaly or both. We think that both causes resulted in diffused hypokinetic and dilatation of left ventricle.

Currently, the standard diagnostic tool for cor triatriatum is transthoracic or transesophageal echocardiography. Catheterization can be used when we are not sure about simultaneous congenital anomalies. [1] Nowadays, the use of magnetic resonance imaging also is growing up as a diagnostic tool [18] [19] [20] [21]. In our case, the diagnosis began with transthoracic echocardiography and was clear after we performed preoperatively transesophageal echocardiography.

The surgical treatment of a case with court triatriatum and severe mitral regurgitation was first reported by Wong et al. in 1989. Membrane resection and mitral valve replacement were performed [2]. Jayaprakash et al. on 2015 present a case similar to the one where an Alfieri mitral valve repair realised [16]. The authors in both cases referred that they founded anomaly of the mitral valve tensor apparatus. In our patient, we didn't notice something special except macroscopical signs of myxomatous degeneration of mitral valve. In reported of such cases there were performed 4 replacements and 5 mitral valve repairs simultaneous with resection of the membrane of cor triatriatum.

We performed a resection of fibro muscular membrane and mitral valve repair according to Alfieri. It was implanted a mitral ring Eduards Lifesciences No. 34. The period after surgery was very good.

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***Case Reports***

# Posterior Reversible Encephalopathy Syndrome as a Postpartum Complication

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**Keywords:** Posterior reversible encephalopathy syndrome (PRES); MRI; Postpartum cesarean; Epileptic seizures; Cortical blindness

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**Abstract**

**BACKGROUND:** Posterior reversible encephalopathy syndrome (PRES) is a clinical-radiological syndrome with seizures, altered consciousness, visual disturbances and headache among other symptoms. Hinchey et al. first described Pres in 1996, with two other case series published shortly after.

**CASE REPORT:** A 23-year-old women patient was emergency sent from General Hospital Tešanj due to a crisis of consciousness and repeated epileptic seizures. The patient had a second birth before 10 days (postpartum cesarean) in general endotracheal anaesthesia (two cesarean-born babies). On magnetic resonance imaging (MRI) of cranium described both sides of the symmetrically frontal, parietal (and pre-ventricular gyri) and occipitally visible T2W/FLAIR hyperintensity focuses on the cortex and the thin layer of white mass subcortically. In the projection of the lesions parts, discrete DWI hyperintensity is seen without a reliable ADC correlate. The patient improved after management with intravenous fluids, antibiotics, antiepileptics and monitoring of blood pressure. According to latest experiences delayed diagnosis and treatment may lead to mortality or irreversible neurological deficit. Aggravating circumstances are differential diagnoses that include cerebral infarction (ischemic, haemorrhage), venous thrombosis, vasculitis, pontine or extrapontine myelinolysis.

**CONCLUSION:** MRI of the brain is key to make this distinction with crucial recognition and an open mind from radiology and neurology specialist.

## Introduction

Posterior reversible encephalopathy syndrome (PRES) is a clinical-radiological syndrome with seizures, altered consciousness, visual disturbances and headache among other symptoms [1] [2]. Hinchey et al., first described Pres in 1996, with two other case series published shortly after [3] [4] [5]. Visual disturbance: blurred vision, homonymous hemianopsia and cortical blindness. Altered consciousness: mildly confused or agitated or comatose, seizures and status epilepticus are common, nonconvulsive status epilepticus (may be more frequent). Postictal confusion lasts for hours, but PRES and nonconvulsive status can both persist for several days and be mistaken for psychosis, drug intoxication, or psychogenic states [6].

## Case Report

A 23-year-old women patient was emergency sent from General Hospital Tešanj due to a crisis of consciousness and repeated epileptic seizures. The patient had a second birth before 10 days (postpartum cesarean) in general endotracheal anaesthesia (two cesarean-born babies), blood group A $Rh^+$. General condition on discharge was ($2^{nd}$ day postpartum) afebrile, TA 110/70 mm Hg, heart frequency 70/min, ordinary diuresis (mictio) and defecation, wound without signs of inflammation, leukocytes: 8.8 x $10^9$/l, erythrocytes 4.15 x $10^{12}$, platelets 195 x $10^9$/l, Hgb 132 g/l. Recommended continuation of therapy after discharge from the hospital enoxaparin 40 mg s.c. x1, cephalexin 500 mg tbl. x2, ergometrine 0.2 mg tbl. x3.

On admission to the Urgency department of Cantonal Hospital Zenica, the patient was unconscious with the presence of an epileptic attack (frozen lower jaw), it was immediately applied to airway, skin and mucous membranes were cyanotic, low frequency and shallow respiration, above the right lungs a sharp lung sound, circular-symmetrical and non-reactive pupils. TA 150/100 mm Hg, heart frequency 110/min, $spO_2$ 88%, febrile 38.2$^{o}$C, with a fixed permanent urinary catheter, urine dark (concentrated), CT cranium without pathological findings (made in General Hospital Tešanj)

At the Urgency department of Cantonal Hospital, Zenica was administered a solution of 0.9% NaCl a 500 ml (8 ml/min) i.v., diazepam 10 mg i.v. x 1, $O_2$ 3l/min. After epileptic treatment attacks stopped, with the opening of the eye on the call, the pupils symmetrical and reactive, unarticulated speech, the postictally altered state of consciousness. The wound was calm without signs of inflammation after postpartum cesarean; the breasts swarmed with no signs of mastitis. Laboratory on admission: leukocytes 17.8x10$^{9}$/l, erythrocytes 4.77 x 10$^{12}$/l, hemoglobin 142 g/l, platelets 360 x 10$^{9}$/l, MCV 89.4 fL, MCH 29.8 pg, MCHC 33.3 g/dl, MPV 8.3 fl, RDW 12% CV, glucose 6.6 mmol/l, urea 2.2 mmol/l, creatinine 83 µmol/l, sodium 143 mmol/l, potassium 3.3 mmol/l, chlorides 106 mmol/l.

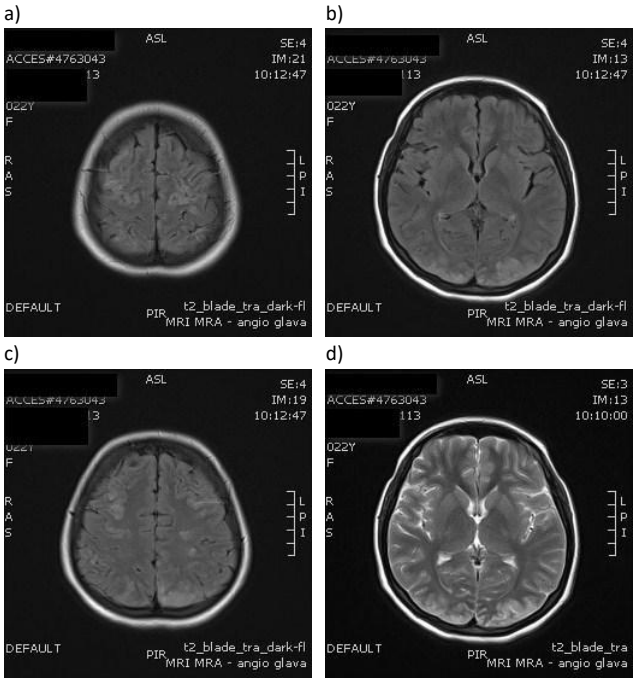


*Figure 1: MRI of the cranium*

The first day on neurological intensive care, general condition was far from stable, patient continued to febrile 38.1$^{o}$C, euphoric, dehydrated mucous membranes, cortical blindness, skin without rash and inflammation, meningeal signs negative, somnolent, febrile, cortical loss of vision, cranial nerve findings neat, on anti-gravitational position no lateralization, muscular and tapered reflexes heightened, pathological reflexes were not induced. Generalized epileptic attacks were repeated twice. Administered therapy was phenobarbitone 100 mg i.m. x 1, 0.9% NaCl a 500 ml i.v. (3 ml/min in total 1500 ml during 24 hours), mannitol 20% and 250 ml x 3 (every 8 hours) continued in next day, metamizole sodium 2.5 g i.v. x 1, diclofenac 100 mg i.v. x 1, KCl ampoules 20 ml i.v. x 2 (20 mmol/hour). Glucose 10% a 500ml (3ml/min in total 1000ml during 24 hours), amoxicillin+clavulan acid 1.2 g x 3 i.v. (every day). On ordered therapy, epileptic attacks stopped again. RTG thorax showed the bilateral Hilo-periciliary, and right Hilo-para-cardio-basal enhances the lung with gentle, murky shadings as part of infiltrative inflammatory changes.

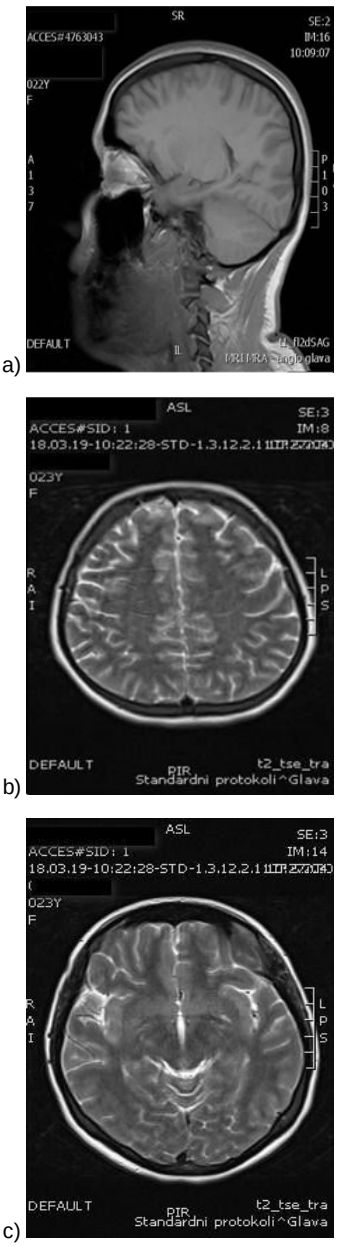


*Figure 2: Control MRI of the cranium*

On MRI of cranium described both sides of the symmetrically frontal, parietal (and pre-ventricular gyri) and occipitally visible T2W/FLAIR hyperintensity focuses on the cortex and the thin layer of white mass subcortically. In the projection of the lesions parts, discrete DWI hyperintensity is seen without a reliable ADC correlate (Figure 1).

Cerebrospinal fluid was clear, colorless, glucose 2.6 mmol/l (ref. 2.99-4.44), proteins 0.9 g/l (ref. 0.1-0.6), chlorides 125 mmol/l (ref. 115-129), cell number 1.0 x $10^6$/l (ref. 0.0-5.0).

From the second to the third day, the overall condition was stabilised without new epileptic attacks, patient aware consciousness, afebrile, sitting alone, recognised movements but still unclear images in the field of vision, in an anti-gravitational position without lateralisation, pathological reflexes were not caused.

Urine cultures were taken immediately after admission, and it was isolated Pseudomonas aeruginosa-cefotaxime-resistant, we corrected antibiotic therapy due to antibiogram with ciprofloxacin 500 mg x 2 per os, blood cultures were negative. EEG on the 6$^{th}$ day showed above the front-centre-temporal regions, complexes of the sharp wave-slow wave, sharp waves in groups of 3-4, medium-heavy to severe degree of alternating accentuation of the sides, at one time short-term paroxysm. The epileptic activity dominates the front-centre-temporal regions with the tendency of generalisation.

In the control MRI examination, infra and supratentorial cortically and subcortically were seen gentle areas of in-homogeneously elevated signals regarding almost complete regression of previously recorded changes. Changes did not show diffusion restriction. There was no sequela of haemorrhage (Figure 2).

The patient was discharged conscious, communicative, oriented; cranial nerves found neat in anti-gravity position without lateralisation or pathological reflexes. Laboratory findings were in normal ratio except potassium 3.7 mmol/l. Recommended per os therapy: Lamotrigine 50 mg tbl x 1 (after 7 days 50 mg tbl in the morning and 25 mg tbl at the evening), phenobarbital 25 mg tbl x 1 (7 days then stop), ranitidine 150 mg tbl x 1.

## Discussion

There are three proposed hypotheses of PRES pathophysiological mechanism till now: 1) cerebral vasoconstriction causing subsequent infarcts in the brain, 2) failure of cerebral autoregulation with vasogenic edema, and 3) endothelial damage with blood-brain barrier disruption further leading to fluid and protein transudation in the brain [7] [8] [9]. New reports of permanent neurological impairment and mortality reaching 15% challenge reversible nature of PRES [10] [11].

We do not have need life-sustaining treatments for PRES regarding non-available clinical studies. According to latest experiences delayed diagnosis and treatment may lead to mortality or irreversible neurological deficit [12] [13]. Aggravating circumstances are differential diagnoses that include cerebral infarction (ischemic, haemorrhage), venous thrombosis, vasculitis, pontine or extrapontine myelinolysis.

Blood transfusion may cause a rapid increase in total blood volume, which further leads to cerebral blood flow overload. Abrupt or acute cerebral hyperperfusion exceeding the capacity of auto-regulation of cerebral capillary perfusion pressure might result in vasogenic o edema found in PRES. The possibility of severe anaemia as the predisposing factor, due to an inadequate supply of oxygen to the brain may result in dysfunction of endothelial cells, further causing a functional loss or damage to the integrity of the blood-brain barrier in capillary circulation which cannot be ruled out [14].

There are published case of the patient presented with hypertensive urgency as well as stroke symptoms with hyponatraemia after regression of PRES symptoms patient was discharged with a serum sodium of 132 mmol/l [15].

Qiang Zhang and coworkers published PRES case that had unclear aetiology and they suspected and believed that aseptic meningitis might be a contributing factor. Never the less systemic infection has been linked to PRES only in one pediatric case that PRES associated with aseptic meningitis [16].

Because of that MRI of the brain is a key diagnostic method to make this distinction with crucial recognition and an open mind from radiology and neurology specialist. In the light of the absence of evidence of factors that trigger PRES as well as of the absence of sufficient medical evidence that would lead to the development of preventative and curative treatment methods, we consider that every case must be carefully and thoroughly investigate.

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***Case Report***

# Nevus Blue as a Sporadic Finding in a Patient with a Blue Toe?

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**Keywords:** blue nevus; cyanotic toe; microembolism; vasodilatation; sentinel lymph nodes; observation

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**Abstract**

**BACKGROUND:** Blue nevus is an interesting finding, which aetiology and risk of locoregional and distant metastasis have not yet been fully clarified. It may be inherited or acquired, with sporadic cases usually presented as solitary lesions. It is often localised in the area of the head and less often on the arms, legs or trunk. Blue nevi are formations with relatively low but still possible potential for switching to melanoma.

**CASE REPORT:** The patient we described was hospitalised for pronounced cyanosis of the small toe of the right foot, accompanied by painful symptoms at rest and pain symptoms for a few weeks. Using inpatient paraclinical and instrumental tests, the patient was diagnosed with cholesterol microembolism. During the dermatological examination, blue nevus on the contralaterally localised limb was also diagnosed as a sporadic finding. According to the patient's medical history, the finding had existed for many years, but in the last few months, the patient has observed growth and progression in the peripheral zone of the nevus without any additional clinical symptoms.

**CONCLUSION:** Due to the risk of progression to melanoma, the lesion was removed by radical excision, and the defect was closed by tissue advancement flap.

## Introduction

Blue nevi are melanocytic tumours of dermal origin. Their prognosis is usually very good, and in most cases, they remain completely benign, asymptomatic and unchanged throughout life [1]. But there is a spectrum of "blue" lesions ranging from common benign to lesions of unknown potential as heavily pigmented epithelioid melanocytomas to aggressive malignant tumours such as the so-called "blue nevus associated melanoma" [1] [2] [3] [4]. According to blue nevi need a thorough histological work-up not to overlook a malignant transformation, in particular in adult patients where blue nevi are less common than in adolescents. Therefore, the dilemma often arises whether surgical removal of the nevus should be a priority.

## Case report

A 49-years-old man is presented in good general status. He was a smoker for 20 years (10-15 cigarettes per day). There is no evidence of family history, concomitant diseases or medication. The patient was first hospitalised at the clinic for a blue formation on the small toe of the right foot that had occurred approximately two weeks before.

During the dermatological examination, we made two independent findings: 1) cyanosis on the small toe of the right foot (Fig. 1a, and 2) nevus with blue colour on the left leg above the ankle (Fig. 1b, 1c). The patient complained of pain at palpation of the cyanosis toe, foot pain at rest and improvement of the status of the movement.

The inpatient instrumental and paraclinical examinations showed no significant abnormalities: a panoramic image of teeth – 17, 23, 27, 32 roots with granuloma; PSA (< 4.0) – negative; CEA (< 5.0) – negative; Protein C (109.7%) – normal; Protein S (119.3%) – normal; antithrombin III – normal; Factor V Leiden – normal; anti-phospholipid antibodies – 1.3 U/ml (< 10); anti-cardiolipin antibodies – 1.4 U/ml (< 10); anti beta2-glycoprotein antibodies – 3.3 U/ml (< 10); ANA-1: 100 (boundary titre), anti-streptolysine titre – normal; INR – 1.04.


*Figure 1: 1a) Cyanotic toe; 1b) Blue nevus localized on the left lower leg, above the ankle; 1c) Outlining the resection boundaries; 1d, 1e) Elliptical excision of the blue nevus with boundaries of surgical security 0.5 cm; 1f) Closure of the surgical defect by means of stretch plastic surgery*

The Doppler echography of the lower limb vascular system followed by consultation with a vascular surgeon showed no evidence of thrombosis or thrombophlebitis. Surface and deep venous system – without evidence of reflux; preserved pulsations at the levels of the foot and digital arteries of both limbs. Echocardiography showed evidence of dilated and hypertrophied right ventricle. The blue toe was found to be most likely due to cholesterol microembolism.

For microembolism, a therapy with low molecular weight heparin – Nadroparin calcium 0.4 x 1/d subcutaneously for 7 days and topical Heparoid ungentum for 7 days was introduced. After a consultation with a vascular surgeon, outpatient therapy with Cilostazol 100 mg x 2/d for 1 month and Clopidogrel 75 mg x 1/d for 1 month was assigned.

Due to the patient's current history of the observed growth of a blue formation and the likelihood of malignant transformation to melanoma, radical removal was performed under local anaesthesia. Elliptical excision with a 0.5 cm surgical field security was used (Fig. 1d, 1e), followed by gradual closure of the defect by tissue advancement flap (Fig. 1f). The histological examination confirmed the diagnosis of cellular blue nevus with free resection margins.

## Discussion

Blue nevus is a flat or slightly raised macula, papule or plaque with grey-blue to bluish-black colour [2]. It is believed that blue nevi are a collection of pigment-producing melanocytes in the dermis [1] [2]. They may be congenital or acquired [3]. They are usually presented as single lesions and are found in the area of the head, neck, and sacral region, the back of the upper and lower limbs [1]. In dermoscopy with polarised light popular exophytic lesions present with a homogenous blue-greyish globular pattern, and striking colour changes resembling the colours of a rainbow. Such a pattern is usually seen in vascular lesions only. In flat lesions, the dermoscopic pattern may resemble seborrheic keratosis. Dermoscopy alone is not a substitute for histopathology.

Although in most cases blue nevi are considered benign, there are occasions when they are likely to progress to melanomas [3] [4] [5]. Determination of the risk of occurrence of a possible melanoma based on a blue nevus, as well as which of the occurred or congenital blue nevi will have an aggressive course, may be a significant challenge for the clinician [3] [4]. In these cases, identification of certain genetic changes would help to individualise and refine their prognosis [4].

Benign and malignant lesions are characterised by genetic peculiarities. Benign blue nevi harbour mutations of the G-protein-coupled receptor subunits *GNAQ* and *GNA11*. Also, the *c-kit* (CD117) gene may be used, which is strongly positive in immunohistochemical staining for benign cellular blue nevi, in contrast to the invasive melanomas, in which this gene has a lower intensity of staining [1].

Several histological types of blue nevi are known: simple blue nevus, cellular blue nevus, atypical cellular blue nevus, combined blue nevus, blue nevus-like melanoma, and malignant blue nevus – with the worst prognosis [3] [4] [5].

The frequency of malignant transformation of blue nevi is between 5.2 and 6.3% [6]. No consensus has yet been reached on the histological features indicating malignancy in blue nevi, and this poses significant diagnostic difficulties in attempting to distinguish between benign and malignant blue nevus [7]. The presence of irregular edges with satellites (satellitosis) is considered a strong precondition for malignancy [5]. Other signs considered to be indicative of possible malignant transformation are infiltration boundaries, common mitoses, nuclear pleomorphism, hyperchromasia [7]. According to many authors, the most important histopathological sign distinguishing benign and malignant blue nevi is the widespread necrosis [7].

Malignant blue nevi are "aggressive neoplasias" that most commonly metastasises in the regional lymph nodes [3] [6] [9]. Migration or infiltration

of nevus cells to distant lymph nodes is also possible [8]. Interestingly, it is possible to have infiltration of the lymph nodes by single cells also in the event of a benign type of cellular blue nevus without any histological evidence of malignancy [1] [6] [10]. Literature data are of interest, in which axillary lymph node biopsy (performed to suspicion of breast cancer metastasis) indicates the presence of a combined blue nevus adjacent to benign non-pigmented nevus cells (within the same lymph node). This speaks in favour of the fact that blue nevus may occur as a primary finding in the lymph nodes without a skin manifestation or primary skin manifestation [11].

Virtually, the cases of enlarged lymph nodes within the congenital or acquired cellular blue nevus camouflage the clinical picture and may be misinterpreted as melanoma metastases [12].

Atypical cellular blue nevus is presented with median histology between the “malignant blue nevus” and melanoma [3]. This type of blue nevus is associated with the highest risk of proliferation to malignant melanoma and metastasis in the lymph nodes [3] [13]. There are in fact cases of “metastatic malignant melanoma”, which are often the result of incorrect diagnosis or interpretation of the available histopathological evidence indicative of a “malignant blue nevus” [13].

As on many other topics in the field of medicine, there are many questions here as well [14]. On the one hand, the underestimated blue nevi may become melanomas with metastasis in the lymph nodes, and on the other, the lymph nodes that are not always enlarged, in combination with blue nevus show progression to malignant melanoma [11] [13] [16]. According to studies evaluating the 5-years survival in cases of lymph nodes involvement from benign nevus cells, it does not differ from that in patients lacking lymph nodes involvement [17]. Currently, the recommended approach in case of lymph nodes involvement is the removal of the primary skin lesion and lymph nodes biopsy, followed by histological and immunohistochemical analysis to consider further actions [17].

Several years after lymph nodes dissection has seemed to be mandatory for the detection of a malignant blue nevus with lymph nodes metastases [18]. Complete lymph node dissection is seen today more critical especially in patients with low-risk sentinel lymph node tumour load since the prognosis is not improved by this way [19] [20].

A case of a patient with a blue nevus leading to the development of orbital-palpebral and intracranial melanoma has also been described [21].

Any case of increasing and extending blue nevus boundaries is a major indication of their surgical removal [7] [11]. The excision should be through a technique leading to complete elimination of the lesion and should ensure compliance with the boundaries of surgical security [22]. This is easily and quickly accomplished by radical excision and closing the defect using a tissue advancement flap [20]. Each patient with a resected blue nevus requires a histological examination of the lesion removed and long-term monitoring of the operative zone and the locoregional lymph nodes [6] [11] [14].

In conclusion, despite the “predominantly” benign course of the blue nevi, some of them are related to a risk of switching to malignant melanoma. This makes the early surgical removal a priority approach. The aesthetically and therapeutically acceptable surgical decision for blue nevi is the radical excision, followed by closure of the occurred skin defect using stretch plastic surgery.

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***Brief Communication***

# What Online Traditional Medicine Dictionaries Bring To English Speakers Now? Concepts or Equivalents?

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**Abstract**

Nowadays, more and more Chinese medicine practices are applied in the world and popularizing that becomes an urgent task. To meet the requiremets, an increasing number of Chinese - English traditional medicine dictionaries have been produced at home or abroad in recent decades. Nevertheless, the users are still struggling to spot the information in dictionaries. What traditional medicine dictionaries are needed for the English speakers now? To identify an entry model for online TCM dictionaries, I compared the entries in five printed traditional medicine dictionaries and two online ones. Based upon this, I tentatively put forward two samples, "阳经 (yángjīng)" and "阴经 (yīn jīng)", focusing on concepts transmitting, for online Chinese - English TCM dictionaries.

## Introduction

In 2015, the Nobel Prize in Medicine was awarded to Professor Youyou Tu, who found the active compound from a Chinese herbal medicine Artemisia to treat malaria. Middlesex University in the UK, the first ever university outside China to launch a traditional Chinese medicine degree, has now enrolled MSc students for more than ten years. Traditional Chinese medicine (TCM) is gradually attracting more and more attention in the world. To meet the requirements of TCM development, an increasing number of Chinese-English traditional medicine dictionaries have been produced at home or abroad in recent decades. But generally, they cannot meet non-Chinese users' needs due to the lack of some indispensable information. For example, some dictionaries only provide the English equivalents without an English definition for the lemmas and some do not cover all the basic TCM terms in their entries [1] [2].

To identify an entry model for Online TCM encyclopedic dictionary, I compared the microstructure of the entries in the following five printed traditional medicine dictionaries:

- A Concise Chinese-English Dictionary of Yellow Emperor's Canon of Medicine (DYECM 2011) [3];
- New Chinese-English Dictionary of Traditional Chinese Medicine (Second Edition) (NCEDTM 2013) [4];
- Classified Dictionary of Traditional Chinese Medicine (CDTCM 2002) [5];
- Terminology of Traditional Chinese Medicine (TTCM 2008) [6];
- Chinese-English Pocket Dictionary of Traditional Chinese Medicine (CEDTCM 2003) [7].

and two online dictionaries:

- Paradigm Online TCM Dictionary (POTCMD) [8];
- Translation-dictionary.net (TD) [9].

## "阳经(yángjīng)" and "阴经(yīn jīng)"in Seven Chinese-English TCM Dictionaries

In Chinese philosophy, yin and yang describe how seemingly opposite or contrary forces may actually be complementary, interconnected, and interdependent in the natural world, and how they may give rise to each other as they interrelate to one another. Many tangible dualities (such as light and dark, fire and water, expanding and contracting) are thought of as physical manifestations of the duality symbolized by yin and yang. This duality lies at the origins of many branches of classical Chinese science and philosophy, as well as being a primary guideline of TCM [10]. Accordingly, a relevant pair of terms, "阳经(yángjīng)" and "阴经(yīn jīng)" frequently emerge in TCM books.

Huangdi said, "Yin and yang serve as the law of the heavens and the earth, the fundamental principle of all things, the parents of change, the beginning of birth and death and the storehouse of spiritual being. The treatment of disease must follow this law." [11].

Huangdi said, "The meridians are not only responsible for the blood and Qi, but also are important for determining life and death, treating various diseases and regulating Deficiency and Excess. Hence it (author: should be "they") must be thoroughly understood." [12].

The meridian system is one of the basic approaches of pathological physiology in Chinese medicine, whereas yin and yang are one of the philosophical principles of TCM (Sun 2002: 8) [13]. Consequently, I selected two entries, "阳经(yángjīng)" and "阴经(yīn jīng)", from five printed Chinese-English TCM and two online TCM dictionaries to compare their microstructure treatment. I also searched for "阳经(yángjīng)" and "阴经(yīn jīng)" in different online dictionaries; they are not listed in Wiktionary, Wikipedia, or Britannica but are listed in two online dictionaries, POTCMD and TD.

### *Entry Treatment of Seven Chinese-English TCM Dictionaries*

In the dictionaries' preface, the compilers in NCEDTCM and DYECM claim that their target users are L1 users of Chinese, such as traditional medicine researchers, students and interpreters. In CEPDTCM, CDTCM and TTCM, the compilers highlight that their dictionaries are designed not only for the traditional medicine researchers, students and interpreters in China, but also for the L2 users of Chinese.

Taking "阳经(yángjīng)" and "阴经(yīn jīng)" as examples, I attempted to identify whether the seven dictionaries shared the same approach to providing lexical information, including Chinese pinyin, the Chinese tone, English equivalents, an English definition, English encyclopedic information and illustrative examples in their microstructure. Due to the limited space, I can not present all the details of the dictionaries, but provide the results of this comparison in Table 1:

**Table 1: "阳经 (yángjīng)" and "阴经 (yīn jīng)" in TCM Dictionaries**

| | Items | | | | | | |
|---|---|---|---|---|---|---|---|
| | English equivalents | | Chinese Pinyin | Chinese Tone | English definition | Encyclopedic information | Illustrative examples |
| | 阳经(yángjīng) | 阴经(yīn jīng) | | | | | |
| DYECM | Stomach meridian of Foot-Yangming; Three yang meridians | yin meridians | Y | Y | N | N | N |
| NCEDTCM | yang meridian | yin meridian | Y | Y | N | N | Y |
| TTCM | the yang channels | the yin channels | Y | Y | Y | Y | N |
| CDTCM | yang meridians | yin meridians | Y | Y | Y | Y | N |
| CEPDTCM | none | Yin vessel | Y | N | Y | Y | N |
| POTCMD[1] | Yang channel | Yin channel | Y | Y | N | N | N |
| TD[2] | the yang meridians; yang meridian; yang channels | the yin meridian; yin channels | N | N | N | N | Y |

1, accessed January 15, 2018, http://www.paradigm-pubs.com/TermList; 2, accessed January 15, 2018, http://translation-dictionary.net/chinese-english; All the dictionaries provide the English equivalents of "阳经 (yángjīng)" and "阴经 (yīn jīng)" with different treatments, including pronunciation, definitions, illustrations and encyclopedic information. Five dictionaries present Chinese pinyin and tone; one dictionary only provides pinyin without tone and neither exists in TD. In POTCMD, both of the two ways, accented pinyin (with the tone) and unaccented pinyin (without the tone) appear in the treatment. The headwords are translated into different English words or phrases in these dictionaries. Obviously, even though there is some overlap among their target users, all of the seven dictionaries have their own microstructure treatment for the entries, such as varying translations different ways of representing tone. Furthermore, not all the dictionaries provide the following items, the English equivalent, Chinese pinyin and tone, English definition, encyclopedic information and illustrative examples. It seems difficult for English speakers to fully understand what the symbols represent or embody, which would not only hinder the users' understanding the terms, but worsen the difficulties in users' accessing process, or even restrain their desire in accessing the entry

## Analysis and Suggestions for Online Chinese-English TCM Dictionaries

Chinese-English TCM Encyclopedia users could be varied in their Chinese level. For example, some TCM professionals and researchers who want to keep up with the burgeoning array of terminology found in today's medical news, some patients who hope to acquire concise and easily accessible information for descriptions of prescription medications, medical abbreviations, test procedures, medical research topics, or illnesses. Due to lack of culture background, the English speakers perhaps feel frustrated to grasp the meaning of culture-loaded headwords, or understand the tone symbols, 1st tone "¯", 2nd tone," ´", 3rd tone,"ˇ" ,4th tone "`", and 5th tone " ". Considering the fact that there are abundant of diversified English equivalents and obscure definitions for the same entry, encyclopedic information should be supplemented to provide some additional information to clarify the Chinese culture related items. Therefore, an encyclopedia, not a dictionary, should be compiled.

Henri Bejoint (2000) [14] holds the view that

the dictionary and the encyclopedia differ in the nature of their entries, their arrangement, and the nature of the information given in each entry. The entry-words of an encyclopedia are always nouns, both common nouns and proper nouns. But those nouns are not the subjects of the microstructural information contained in the entries; they are only the signs -- in the sense of road signs -- indicating the contents of the entries. They are labels attached to the entries for convenience of reference: typically, for example, an entry headed by the word garden will contain not an exploration of the concept, an explanation of the etymology of the word, etc., but a history and geography of gardening, an explanation of its techniques, etc. For many entries, a different entry-word might have been chosen, and this would not have modified noticeably the contents of the entry.

The compilers could attempt to set up a featured entry microstructure, which contains headwords, Chinese pinyin, tone, English equivalent, encyclopedic information, even some pictorial illustrations, and some hyperlinks to the reference websites.

### *Encyclopedic text*

"Users generally expect a bilingual dictionary to provide them with faithful translation. However, perfect equivalence is not always achievable ……" (Fontenelle 2015:53) [15].The English equivalents of the headwords are not identical in the seven dictionaries. I think the point is that even though there exists a range of criterion for the English equivalents, for example, the WHO International Standard Terminologies on Traditional Medicine in the Western Pacific Region (2009) [16] and International Standard Chinese-English Basic Nomenclature of Chinese Medicine (2008) [17], the compilers haven't strictly complied with that. Or, the English equivalents in the criterion can not cover all the technical terms exhaustively, which urges the compilers to find the equivalents by themselves. Consequently, different equivalents could be found out in a couple of dictionaries.

Three of the dictionaries provide the definition of the terms, for example:

阴经Including the three Yin Channels of the Hand and the three Yin Channels of the Foot, Renmai (the Anterior Midline Channel), Chongmai(The Vital Channel), Yinwei Ma(The Regulating Channel of Yin), Yinqiaomai(the Motility Channel of Yin). ----(TTCM 2005: 66) [6];

阴经a collective terms for the three yin meridians of the hand and the foot, conception vessel, thoroughfare vessel, yin link vessel and yin heel vessel -----(CDTCM 2002: 436) [5];

阴经a terminology of meridians. Refers to the yin channels including hand and foot yin channels, Ren Channels, Chong Channels,Yin springing vessel Channels and yin linking vessel -----(CEPDTCM 2003: 441) [7].

In such cases, the users would probably find there exist some beneficial information in the definition. However, all of them are made up of difficult technical terms, which still can not construct an image in users' minds. Consequently, some encyclopedic information is necessarily added to supplement the microstructure of the entries in Chinese-English TCM dictionaries. In the field of Chinese-English TCM dictionary compiling, an encyclopedia is more appropriate than a dictionary.

A majority of the TCM terms are culture-specific words or phrases, which cannot share the same denotation and connotation with western medicine terms. Consequently, diversified English equivalents could be seen in line with the same terms, and definitely, would lead to the diversified English translating treatments for the same term. Comparatively, "Encyclopedic definitions are more detailed and less vague than the meaning explanations offered in general dictionaries" (Hartman & James 2002:36) [18]. A dictionary gives a basic and short description of a (large) set of both trivial as well as non-trivial words, while an encyclopedia explains rigorously and provides background for a (small) set of non-trivial concepts and things in relatively large articles. Therefore, encyclopedic texts should be taken as an indispensable part of TCM microstructure. For users who are not familiar with Chinese culture, not a dictionary, but an encyclopedia should be provided.

### *Illustration*

Due to the cultural diversity, some TCM terms still cannot be fully understood after consulting encyclopedic texts in the microstructure. In such cases, illustrations can become another supplementary component for encyclopedic information. "There is enough space on the computer screen to enable illustrations to always be displayed at the same time as the associated word entry." (Klosa 2016: 516,517) [19]. "This is generally effective when a written description of the external form of an object reaches its limits, and the visualization of the object in an illustration contributes to a better understanding.....it is generally the case that information is retained in the memory better when it is conveyed through the use of both text and images....illustrations can also be displayed in a (smaller) window which is opened separately; this is particularly useful when a dictionary is being accessed on a smartphone. " [20]. Illustrations can be pictures, audio or video material. By clicking on the interpretation of the text content in multimedia audio and video button, the users can easily construct an image of the TCM term in their minds. Here is a sample:

灸法[jiǔ fǎ]

moxibustion: a therapy involving ignited material, usually moxa, to warm the surface of skin and meridian points with the intention of stimulating circulating through the points and harmonizing blood and qi. Practitioners claim it is especially effective in the treatment of chronic problems, "deficient conditions" (weakness), and elderly persons' diseases.

### *Hyperlink*

"Online" is not a new word. Wifi covers a large part of the world. Consequently, an online dictionary, not a printed one, is needed. Compared with the printed dictionaries, the online ones have an additional feature—hyperlinks, which could present much more usage information. Hyperlinks could play a key role in a Chinese-English TCM dictionary. For example, the entry, "灸法"can also be found in wikipedia, which would definitely provide more supplementary information. Therefore, if possible, the compiler could try to provide some hyperlinks to sources such as Wikipedia, Wiktionary, Sacred Lotus, or the Encyclopedia Britannica, which could not only help the users deeply grasp the meaning of the entry, but also help them develop a framework for traditional medicine in their minds.

Wikipedia, catering for non-experts, is very popular in the world, and can provide a brief introduction to the basic principles in TCM. Unfortunately, there are abundant terms not included in Wikipedia, Wiktionary or Encyclopedia Britannica. Furthermore, anyone can add an entry to Wiktionary and Wikipedia. That can not guarantee the correctness of the definition.

### *Chinese pinyin with tone*

Chinese Mandarin is a tonal language. In order to differentiate meaning, the same syllable is pronounced with different tones. Mandarin's tones give it a very distinctive quality, but the tones can also be a source of miscommunication if not given due attention. Mandarin is said to have four main tones and one neutral tone (Xin & Wang 2012: 53) [20]. Some dictionaries only provide Chinese pinyin without tones, or ignore both of them. It would be appropriate for the users who only need to understand the meaning of the term. However, if the users want to learn how to pronounce the word, they have to turn to other Chinese dictionaries, which is time-consuming.

Therefore, to meet the demands of different users, it would be better to add the Chinese pinyin with tone in TCM encyclopedias. For example, yángjīng, not yangjing, could be adopted for "阳经". The former is easy to understand and follow for the English speakers.

Furthermore, for the users who are not familiar with the Chinese pinyin or want to know how to read and write the Chinese characters, the compiler can provide a Chinese character stroke order, 一, 丨, 丿,丶, ,¬ , in the appendix or "help" column, in which stroke order rules could be provided. The proper stroke order is easily learned once a few basic rules are followed, which is similar to drawing on the board.

There are some popular online dictionaries in China, such as Aiciba, Youdao, Haici, and Bing. They contain a large number of Chinese characters in their microstructure, which could contribute a lot to the Chinese translations into English or TCM learning for advanced learners of Chinese. For the majority of non-Chinese users, it is frustrating to read such a block page filled with Chinese characters. Nevertheless, they still could be added as hyperlinks by advanced Chinese learners. I present the samples for"阳经yáng jīng" and "阴经yīn jīng" as follows:

阳经[yáng jīng]

yang meridians: a collective term of hand and foot yang vessels, governor vessel, yang link vessel, and yang heel vessel. Meridians are classified into yin meridians and yang meridians according to the yin or yang organs with which they are connected [21]. The line on the inside of the body of the person is yin; line on the outside of the body of the person is yang. The interior parts of limbs belong to yin meridians, while the exterior parts belong to yang meridians.

阴经[yīn jīng]

yin meridians: a collective term of hand and foot yin vessels, conception vessel, thoroughfare vessel, yin link vessel, and yin heel vessel (see also 阳经yáng jīng).

In conclusion, online TCM encyclopedias are capable of not only providing the basic information about Chinese culture and medicine, for example, Chinese pinyin, Chinese tone, English equivalents, but also providing some encyclopedic information to fill in the gap of cultural background. They feature immediate cross-reference facilities, typically activated by a click of the mouse. By consulting online Chinese-English TCM encyclopedias, some users, for example, TCM patients can not only understand the denotation and connotation of the TCM terms, but also understand the explanations for various types of illness or symptoms given by the practitioners. Consequently, with the assistance of an online TCM encyclopedia, TCM patients might be able to interact with TCM practitioners more effectively, as well as overcome the restrictions in their clinical experience. For some technical problems, compiling a comprehensive online non-Chinese users' Chinese-English TCM encyclopedia is still in a trial stage. However, we should not deny the fact that it is an appealing and challenging task. It is safe to say that Chinese-English TCM encyclopedias for non-Chinese

users will continue to be innovative in the years and decades to come, which could bring more potential benefits to TCM's dissemination in the world.

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***Brief Communication***

# The Role of *CYP2A6* Genetic Polymorphism in Nicotine Dependence and Tobacco Consumption among Bataknese Male Smokers

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**Abstract**

**AIM:** This research aimed to analyse the relationship between CYP2A6 gene polymorphism with nicotine dependence and its relation to the number of cigarette consumption among Bataknese smokers.

**METHOD:** This study was a cross-sectional study involving 140 research subjects in Medan, Indonesia.

**RESULTS:** Nicotine dependence rates were found to be significantly associated with the number of cigarette consumption expressed in the Brinkman Index.

**CONCLUSION:** The *1A wild-type alleles have a greater risk of high-very high dependence rate compared to the other variants.

## Introduction

Cigarettes are one of the causes of public health problems with an estimated mortality rate of 5 million every year [1]. Nicotine is one of the components of cigarettes which has an important role regarding physical dependence mediated through neuronal nicotinic acetylcholine receptors (nAChRs) [2]. This basic mechanism of physical dependence has been known for a long time. However, there are several other factors which have a role in the pathophysiology of physical dependence on nicotine but cannot be explained in detail as it can be influenced by several multifactorial factors. There are two factors which might influence individuals' physical dependence on cigarettes, namely environmental and genetic factors [3].

The *CYP2A6* gene is a gene encoding the P450 2A6 cytochrome which has a role in the physical dependence on nicotine. Furthermore, it is also responsible for 70-90% of nicotine metabolism in the blood into cotinine; thus, it can eliminate or decrease the effect of nicotine to stimulate the brain reward

system [4]. The nicotine dependence will be further assessed using Fagerstrom Tolerance Questionnaire (mFTQ) [5]. On the other hand, the Brinkman Index is used to identify the cumulative number of smoking habits.

In our previous report, the study about the relationship between genetic polymorphism of *CYP2A6* and nicotine metabolism in male Bataknese smokers with lung cancer was explained. Bataknese smokers, which have pure genetic inheritance, used as participants due to their tradition on smoking [6], it can give a proper model for the study related to the genetic factor and the smoking habit.

Thus, this study intends to analyse the relationship between smoking habits of male Bataknese smokers with the Brinkman Index.

## Material and Methods

The subjects of this research were 140 Bataknese male with a history of smoking, active smokers, and the age > 20 years. The participants involved were recruited from Haji Adam Malik Hospital, USU Hospital, and Elizabeth Hospital in Medan, North Sumatra, Indonesia. The nicotine dependence was measured using seven items of questionnaire modified according to Fagerstrom Tolerance Questionnaire (mFTQ) with its scoring ratings. The interpretation of this questionnaire was as follows: (1) very low nicotine dependence indicated with a score of <4; (2) low nicotine dependence indicated with a score of 5-7; (3) moderate nicotine dependence indicated with a score of 8-9; (4) high nicotine dependence indicated with a score of 10-14; and (5) very high nicotine dependence indicated with a score of 15-20. Also, the smoking status was documented through interviews. The subject can be categorised as an active smoker if he has a smoking history ≥ 100 cigarettes throughout his life [7]. The severity level of smoking can be assessed using the Brinkman Index. The Brinkman Index value was obtained from the multiplication of the average number of cigarettes smoked a day and multiplied by the duration of smoking (years). The value of Brinkman Index (IB) is mild if 0-199, moderate if 200-599, and severe if > 600 [8].

Genotyping of *CYP2A6* was conducted using the following primer: 2Aex7F (5'-GRCCAAGATGCCCTACATG-3`) and 2A6R2 (5'-AAAATGGGCATGAACGCCC-3`) [9].

The blood sample from the subject (0.5 µg), which obtained by employing Puregene DNA Isolation Kit (Promega), was added with PCR mixtures (25 µl) (It contained 1 PCR buffer, 1.5 mM $MgCl_2$, 0.4 µM of each primer, 250 µM dNTPs, and 1 U of Taq DNA polymerase). The initial denaturation was then carried out at 95°C (1 minute). After that, the application was applied with denaturation at 95°C (15 seconds), annealing at 60°C (20 seconds), and extension at 72°C (3 minutes for 35 cycles), followed by a final extension at 72°C (7 minutes). The triple-digestion with restriction enzymes, namely Eco81I, AccII, and StuI, was done on the PCR product. The analysis using electrophoresis at 2% of agarose gel was then applied to the product [10]. Data analysis was performed by using Epi Info-7 software.

## Results

Based on the data collected, which also was reported in our previous report, there were 106 subjects aged <65 (75.7%) and 34 subjects aged ≥65 (24.3%) involved in the study. The Brinkman Index obtained was 9.3% for mild, 37.9% for moderate, and 52.9% for severe. Therefore, it was discovered that the average age <65 years was the most commonly found with a severe degree of Brinkman Index value. The nicotine dependence was assessed based on the Fagerstrom score using a special questionnaire. Also, the results showed that 91 people (65%) had a very high Fagerstrom score, 31 people (22.1%) had a high Fagerstrom score, and 18 people (12.9%) had low-moderate Fagerstrom score.

Table 1 showed that individuals with the *1A wild-type alleles were 1.13 times more likely to have high-very high nicotine dependence than the variant alleles (*1B and *4A) although this relationship was not statistically significant.

**Table 1: The Relationship between *CYP2A6* Genetic Polymorphism and Nicotine Dependence**

| | | High - Very High | | Mild - Moderate | | *p-value** | OR | 95% CI |
|---|---|---|---|---|---|---|---|---|
| | | *n* | % | *n* | % | | | |
| CYP2A6 allele type | Wild type (*1A) | 109 | 44.7 | 15 | 41.7 | 0.73 | 1.13 | 0.55-2.29 |
| | Variant (*1B and *4A) | 135 | 55.3 | 21 | 58.3 | | | |
| | Total | 244 | 100 | 36 | 100 | | | |

**Logistic Regression Test.*

Table 2 showed that there was a significant relationship between the nicotine dependence level and the number of cigarettes consumed ($p = 0.015$). It can be seen that the higher the level of nicotine dependence, the more the number of cigarettes consumed.

**Table 2: The Relationship between Nicotine Dependence and Brinkman Index**

| | | Severe IB | | Moderate IB | | Mild IB | | *p-value** |
|---|---|---|---|---|---|---|---|---|
| | | *n* | % | *n* | % | *n* | % | |
| Nicotine Dependence | High – Very High | 66 | 89.2 | 48 | 90.6 | 8 | 61.5 | 0.015 |
| | Low - Moderate | 8 | 10.8 | 5 | 9.4 | 5 | 38.5 | |
| | Total | 74 | 100 | 13 | 100 | 53 | 100 | |

**Chi-Square test.*

## Discussion

The results of this study also indicated that there was a significant relationship between *CYP2A6* genotype and the Brinkman Index. However, this study could not determine which allele was associated with the degree of Brinkman Index.

Based on above results, using cigarette smoking as a paradigmatic substance-use problem, these findings suggest that the pathway to dependence is complex. Both genetic and sociocultural factors play a significant aetiological role at the stages of initiation and dependence. For example, social, environmental factors play a major role in the smoking behaviour of Bataknese because smoking becomes an important element in various cultural activities and as a treat that must be provided with food and beverages in each series of customary activities.

In conclusion, the results of this study showed that individuals with the *1A wild-type alleles had 1.13 times greater risk of severe-very severe nicotine dependence compared to the variant alleles (*1B and *4A) although this relationship was not statistically significant. Furthermore, there was a significant relationship found between *CYP2A6* genotype and the Brinkman Index. However, this study could not determine which allele was associated with the degree of the Brinkman Index.

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***Dental Science***

# Dental Number Anomalies and Their Prevalence According To Gender and Jaw in School Children 7 To 14 Years

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**Keywords:** Hypodontia; Hyperdontia; ethnics; children

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**Abstract**

**OBJECTIVES:** This study aimed to find the prevalence of Hypodontia and Hyperdontia in different ethnicities in patients from 7 to 14 years old.

**MATERIAL AND METHODS:** A group of 520 children were included aged 7 to 14 years, only the children who went to primary schools. Controls were performed by professional people to preserve the criteria of orthodontic abnormalities evaluation. The data were recorded in the individual card specially formulated for this research and all the patients suspected for hypodontia and hyperdontia the orthopantomography for confirmation was made. The data were analysed using descriptive statistical analysis using $\chi 2$ test for the significant difference for $p < 0.05$ and Fisher test for $p < 0.05$.

**RESULTS:** Hypodontia, not counting the patients with missing third molars was found in 18 patients researched or 3.46%. The most commonly missing teeth were the second lower premolars, the second upper premolars, second upper lateral incisors followed by the lower incisors. Hyperdontia not including the third molars was found in 4 cases of the participants or 0.76% from which the most frequent atypical tooth mesiodens and one case of bilateral hypodontia of a lateral upper incisor with typical shape and size. But there were no significant differences when tested between genders and jaws.

**CONCLUSION:** The prevalence we found is similar to the prevalence in the region. Our findings indicate that there is a difference between the genders in the prevalence of hypodontia, but without statistical significance, while for hyperdontia we can't see such a difference between the sexes.

## Introduction

Hypodontia and hypodontia are particular dental anomalies, they are known as number dental anomalies. Hypodontia is best defined as agenesis of one or more teeth [1] and considered to be one of the most frequently encountered oral alterations [2] [3] Agenesis of teeth can be classified as hypodontia, oligodontia or anodontia. The term hypodontia is used to describe the agenesis of one or six teeth (excluding third molars), oligodontia shows the absence of more than six teeth and anodontia represents total lack of teeth [4]. Congenital absence of teeth or hypodontia is one of the most common abnormalities in tooth development in human beings. The prevalence of hypodontia varies from 2.63% to 11.2% depending on the race [5] [6] [7].

Many authors have used similar methods of classifying the congenital absence of teeth. In general, they identify three categories of hypodontia, excluding third molars, as follows: mild with 1 or 2 missing teeth, moderate with 3-5 and severe more than 5 missing teeth [8]. Thus, hypodontia can occur either as part of a syndromic or as a non-syndromic patient, familiar form; in the latter, it occurs as an isolated trait, affects variable numbers of teeth and appears either sporadically or as an inherited condition within a family pedigree [9]. Dental anomaly reported in patients with hypodontia is a conical form of lateral incisors, defects in the enamel development and transposition of lateral incisor and canine [10] [11].

For many years the dental anthropologists have researched the evolution of human teeth. All agree that the development in food processing from

prehistory until modern times reflects in the morphological changes in human masticator space. By reducing the level of physiological teeth friction, well documented over evolution which today is completed because eating habits are stable, some authors believe in their useful effect related to dental arch; less density fewer impactions in the third molar and large stable occlusion [12] [13].

Clinicians often believe that the teeth agenesis is increasing me the recent decades. But again there is no evidence that this trend is true for the human dentition or it is purely a hypothetical observation, and it can be described under the examination and diagnostics of dental anomalies. The new genetic researchers show new fascinating horizons in this field, aiming at explaining the mystery of agenesis of the tooth, dental embryology and fetal development. The family lacks teeth were described in a research done by Arts and Vastardis [3] [4]. These anomalies are autosome dominant mutations of MSX1 gene in chromosome 4p (the short side).

Other studies indicate that hypodontia is different compared to deciduous and permanent teeth, tooth type, by gender and racial group. Early research shows that the prevalence of hypodontia is higher in patients that are relative compared to the general population. It also affects both dentitions [14]. In the deciduous dentition, it varies from 0.5-0.9% while oligodontia is rare with a prevalence of 0.25% [3]. Some authors show a higher prevalence among women hypodontia [15] [16] [17], but there are studies that found no statistically significant difference between genders [18] [19] [20]. In the deciduous dentition the anomaly is rare, and most often it appears in the upper jaw compared to the lower jaw, most often the upper lateral incisors are rarely missing the central and lateral lower incisor. Lack of central incisors, canine and deciduous molar are a rare occurrence and are most often as a symptom of ectoderm dysplasia.

In the permanent dentition, the most commonly missing teeth are the third molars, followed by the second lower premolars, upper lateral incisors [21] [22]. The following differences present the prevalence between racial groups: 1.5-3% for the white race, 6-9.2% for the oriental race and 7.7% for the African-Americans [3]. Other dental anomalies have been reported in patients with hypodontia such as defects in developing [23] and transposition of lateral with canine [9].

In the literature, there are few data for hyperdontia compared to the hypodontia in every aspect of these phenomena.

Hyperdontia is defined as an increase in the number of teeth in dental arches in the deciduous or permanent dentition. Morphological appearance of hypodontia teeth varies from those similar with the normal teeth (supplementary) to the teeth with a form that is not normal (atypical).

Although presented in both dentitions it is most often found in the permanent dentition, where the frequency ranges 0.2-0.9%. In the deciduous dentition, the hypertonic teeth are in the upper jaw with the most frequent involvement of lateral incisor. In the permanent teeth, the frequency ranges from 0.1-3.6% in the population, from the typical tooth most often the second lateral upper incisor, the third premolar and the fourth molar. Hyperdontia of the permanent canine of the upper jaw are characteristics of the orofacial digital syndrome, while the hyperdontia of the lower canine is a characteristic for dysostosis cleidocranial.

From the atypical hyperdontic teeth which in general are found in the permanent dentition, most often appear as atypical tooth localised in between or in the location of central upper incisors-mesiodens.

This study aimed to find the prevalence of Hypodontia and Hyperdontia in different ethnicities in patients from 7 to 14 years old.

## Material and Methods

### *Subjects*

In our research 520 children were included aged 7 to 14 years. We included children from town those who went to primary schools with classes in Macedonian, Albanian and Turkish language, with a total number of 364 children or 70.68% from the total number of children examined in the town of Gostivar. In rural areas, we involved primary schools from the village Cigllana and schools in the village Vrapcishte with classes in Macedonian, Albanian and Turkish language, with a total number of 156 pupils from rural areas or 29.32% of the total number of children involved. Controls were performed by professional people to preserve the criteria of orthodontic abnormalities evaluation. Detection of orthodontic anomalies was performed by the same criteria in the assessment of the existence of hypodontia or hyperdontia in all children examined. It should also be noted that all the data were recorded in the individual card specially formulated for this research, which was done by the same examiners, all to avoid possible errors that may appear during the gathering, sorting, statistical processing and interpretation of results.

### *Data Collection*

In all those cases where the data of the anamnesis has shown that there was no extraction of the appropriate tooth, those patients were separated for further research regarding verifying whether it was an extraction performed as told by the parent's anamnesis, and in all the patients the orthopantomography were made. Only then the

patients were registered as hypodontia or hyperdontia. The exams was conducted in classes, where the patients were seated and examined with dental mirrors, and dental probes and OJ and OB measurement was performed with Korkhause kit, for each pupil we took 5 pictures with a digital camera of which 2 extra oral in profile and frontal plane and 3 intraoral the left side, right and front in occlusion for verification and the complete documentation. In our study, the third molars were not included from the fact that their mineralisation and visibility in the OPT recordings is delayed even after 12-13 years of age.

### *Data Analysis*

The data obtained from systematic orthodontic controls after they were organised and systemized in the database, they were analysed using descriptive statistical analysis using $\chi 2$ test for the significant difference for $p < 0.05$ and Fisher test for $p < 0.05$.

### *Ethics*

All the protocols and data used in this research were approved by the Ethical Board.

## Results

Hypodontia, not counting the patients with missing third molars was found in 18 patients researched or 3.46% as shown in the table below. This lack of the number of the teeth was more common in the female children 65.7%, while the male children with 34.3% in the upper jaw, compared to the lower jaw were the presence was 63.5% versus 36.5% (F vs M). The most commonly missing teeth were the second lower premolars, the second upper premolars, second upper lateral incisors followed by the lower incisors.

**Table 1: Hypodontia percentage**

| | Hypodontia | Row total |
|---|---|---|
| Count | No | 502 |
| Percentage | | 96.54% |
| Count | Yes | 18 |
| Percentage | | 3.46% |
| Count | All Groups | 520 |

Pearsons Chi-square 1.99 df=1, p=16.

In table 2 it is noted that from the total number of anomalies, in the missing number of teeth there has been a difference between men and women, in the upper jaw (34.3: 65.7%) and the lower jaw (36.5: 63.5%). Using the Fisher's test we found no statistical significance for $p < 0.05$.

**Table 2: Hypodontia according to the gender**

| N | N | M/Max | F/Max | M/Mand | F/Mand |
|---|---|---|---|---|---|
| 520 | 18 | 6 | 12 | 7 | 11 |
| % | 100% | 34.30% | 65.70% | 36.50% | 63.50% |

Fisher Test, p = 0.74.

A patient with hypodontia is shown in Fig. 1 also a radiographic picture in the 2nd one where we can see all the missing teeth. While in the Fig. 2 is a patient with missing central incisor and his radiography.

*Figure 1: A) A case with hypodontia; B) A case with hypodontia of second upper and lower premolars*

Hyperdontia not including the third molars was found in 4 cases of the participants or 0.76% from which the most frequent atypical tooth mesiodens and one case of bilateral hypodontia of a lateral upper incisor with typical shape and size.

*Figure 2: A case with hypodontia of the lower central left incisor*

From the total of 520 examined, four examined (0.76%) had hyperdontia. In 516 cases (99.24%) there were no hyperdontia diagnosed which are shown in Table 3.

**Table 3: Hyperdontia percentage**

| | Hyperdontia | Row total |
|---|---|---|
| Count | No | 516 |
| Percentage | | 99.24% |
| Count | Yes | 4 |
| Percentage | | 0.76% |
| Count | All Groups | 520 |

Pearsons Chi-square 0.16, df = 1, p = 0.69.

In the statistical test we found $\chi 2 = 0.16$ and $p > 0.05$ ($p = 0.69$), so there was no significant difference. Table 4 shows that from the total number of anomalies, in the hyperdontic we found no difference between the genders and jaws regarding statistical validity for $p < 0.05$.

**Table 4: Hyperdontia according to the gender**

| N | N | M/Max | F/Max | M/Mand | F/Mand |
|---|---|---|---|---|---|
| 520 | 4 | 2 | 1 | 1 | 0 |
| % | 100% | 66.7% | 33.3% | 100% | 0 |

Fisher Test, p = 1.

In Fig. 3A we see a patient with hyperdontic teeth, atypical tooth called mesiodens and in Fig. 3B his radiography. While in Fig. 4 is a patient with hyperdontia of 2 upper lateral incisors.

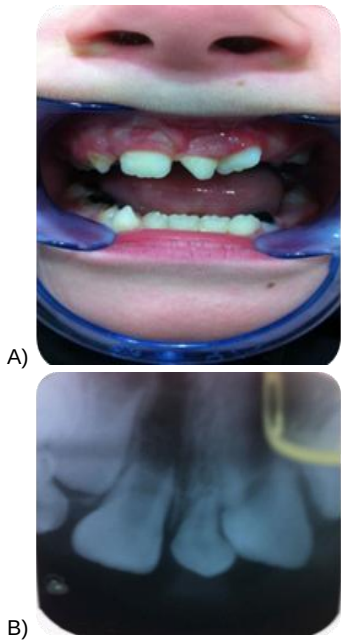

*Figure 3: A case with mesiodens*

## Discussion

These results are best compared with the results shown in the bottom table (Table 5) in which we see the prevalence of hypodontia and hyperdontia from different researchers.

*Figure 4: A case with hyperdontia of two upper lateral incisors*

In the Table 5 we can see obvious differences between authors Davis, Nik Hussein, Renkerova and Lo Muzio, where the frequency of hypodontia ranges from 2.8-6.9%, depending on the authors and countries where surveys are conducted and also a different age groups with differences from over 5 years to 12 years [24] [25] [26] [27].

**Table 5: Authors and hypodontia and hyperdontia percentage**

| Authors | Place | Years | No. investigated | Years | Hypodontia % | Common missing tooth | Hyper-don't % |
|---|---|---|---|---|---|---|---|
| Davis | China | 1987 | 1093 | 12 | 6.9 | mandibular incisors | 2.7 |
| Nik-Hussein | Malasia | 1989 | | 5.15 | 2.8 | 12,22 | x |
| Renkerova et al. | Cekoslovakia | 1989 | 4405 | 7.12 | 4 | X | x |
| Lo Muzio et al. | Italia | 1989 | 1529 | 7.14 | 5.2 | second premolar | x |
| Ignelzi et al. | USA | 1989 | 849 | 3.9 | 7.8 | X | 2.4 |
| Jangida Moris | Japan | 1990 | 4009 | 2.29 | 7.8 | second premolar | 3 |
| Dechkunakorn et al. | Tailand | 1990 | 1160 | 6.15 | 8.6 | 12,22 | x |
| Legovid et al. | Yugslavia | 1990 | 2401 | 6.18 | 6.3 | X | 1.4 |
| Amini et al. | Iran | 1999 | 3374 | x | x | X | 0.7 |
| Locht | Danmark | 1980 | 704 | 9.1 | 7.7 | X | 1.7 |
| O'Dowling | Ireland | 1990 | 3056 | X | 11.3 | X | X |
| Al-emran | Saudi Arabia | 1990 | 500 | 13.5-14.5 | 4 | 35,45 | X |
| Lynham | Australia | 1990 | 662 | 16.26 | 6.3 | 15,25 | X |
| Aaskeim Ogard | Norvegia | 1993 | 1953 | 9 | 6.5 | 35,45 | X |
| Sterzik et al. | Germany | 1994 | 3238 | X | 8.1 | X | X |

We also see that depending on the type of hypodontia teeth there is a discrepancy between the majority of the authors divided into 3 respectively 2 large groups. According to one group [25] [28] hypodontia is more common in the upper lateral incisive, while according to another group [29] [30] [31] which are also similar to our findings that show that the most missing teeth are the second lower premolars after those the upper premolars represented with 6.5% respectively 6.3%.

Hyperdontia was found in a much lower percentage 0.76% but not in all the researches done, but in the most researches, the results are similar or same. Even our results for hyperdontia are similar to different authors [32].

When it comes to hyperdontia between different authors, there is a harmony according to their results the majority of them state that the first upper lateral incisive are the most common hyperdontic (from the typical teeth), respectively mesiodens from those with atypical form.

The data from the present study and their comparison with other studies [33] [34] [35] [36] [37] [38] shows that the prevalence of Hyperdontia and Hyperdontia is found in different frequencies in many countries of the world and even within the same country among different ethnic or regional groups.

According to a study by Ghabanchi et al. in Iran, missing of wisdom teeth accounted for 7% prevalence, this was generally lower than those from other population groups [33]. The prevalence of hypodontia according to Deniz et al. in Turkish population was 5% [34] and in a study by Hunstadbraten et al. in the Norwegian population the prevalence was 10.1%. [35] The frequency of hypodontia among the Swedish subjects in the Josefsson et al. study was 5.5 percent [36].

The prevalence of the hyperdoncia was 2.4% [33] according to Ghabanchi. In Onyeaso's study in Nigeria showed a higher prevalence of hyperdontia with 14% [37]. A study from Schmuckli et al. shows a prevalence of 1.5% of hyperdontia in Swiss population [38].

Regarding the way that the creation of disorders of teeth number anomaly almost all authors put the genetic factors as the first in line. Other genetic defects and the influence of ambient factors play an important role in the distinctiveness of expressiveness of phenotype in the anomaly. Researchers show that hypodontia differs about milk and permanent teeth, tooth type, gender and racial groups. In milk dentition, the prevalence of hypodontia varies from 0.05-0.9%, whereas oligodontia is rare with a prevalence of 0.25% [3]. Some authors show the prevalence of hypodontia is higher in females [16] [17], but there are studies where no significant difference was found between genders [18] [19] [20]. In milk dentition, it is a rare anomaly, and often it appears in the upper jaw about the lower jaw, and it is often the upper lateral incisive missing and less the lateral and central lower incisors. In the permanent dentition excluding the third molars, the main tooth missing was the lower second premolars or the second upper incisor [21] [22]. Other differences were found in the prevalence between racial groups 1.5-3% for the white race, 6 to 9.2% for orientalists and 7.7% for African American [3].

When it comes to the anomaly of supper number of teeth, morphological appearance of hypodontia teeth is different. It is present in the deciduous and permanent dentition, but it is most frequent in the permanent dentition with a frequency varying from 0.2 to 1.9%. In the deciduous teeth, hypodontia is more frequent in the upper jaw, where the most common tooth is the lateral deciduous incisor. Hypodontia is a common anomaly in different populations. The highest prevalence is found in the Irish population, O'Dowlling, McNamara 1900 investigated 3056 patients where they found 11.3% of cases with hypodontia [39].

In our research female represent e high prevalence of hypodontia, these data are similar compared to other research [40]. Hyperdontia also has different prevalence in different populations, and that a lower prevalence in the Iranian population. Amini et al. in 1999 they examined 3374 participants where the hyperdontia prevalence was 0.7%, while the highest was found in Chinese children Devis 1987 in 1093 patients examined found 2.7% cases with hyperdontia and it most often affected the lower jaw incisors [41] [42].

Our research has shown a higher frequency in the absence of teeth of the second lower premolars, the second upper premolars, second upper incisor followed by the second lower incisor. In different researchers, we found different missing teeth, not including the third molars, but there is compliance between the authors. The most frequent missing dental follicles in a research done in children in Iran [43] were the lower second premolars, in a research in Chinese children the most frequent was the lower incisors missing, in Japanese, Italian, Austrian and Norwegian children the most frequent missing teeth were the upper and lower second premolars [27].

In our research, although we see differences between the sexes, there was no significant difference when we compared the means, although many authors show a difference in the prevalence of hypodontia in between the gender [44].

In conclusion, our findings for the prevalence of hypodontia are in average values within 3.46% compared with data from the literature. It is found, and it is evident that the prevalence of hypodontia compared to hyperdontia indicates a significant difference and is much higher (3.46%: 0.76%). Our findings indicate that there is a difference between the genders in the prevalence of hypodontia, but without statistical significance, while for hyperdontia we can't see such a difference between the sexes. From the total number of teeth, the most often hypodontia tooth was the second lower premolar, upper second premolar and after it the lateral upper incisor. The most hyperdontic tooth was found to be the upper lateral incisor from the typical type while from the atypical type mesiodens.

A more comprehensive study should be made including more regions to investigate these anomalies, in different families especially those in special vulnerable areas so we can achieve the connectivity of those with the etiological factor so that the anomaly can be foreseen and diagnostic and treatment can be done in time.

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# Prosthodontics Status and Treatment Needs among the Elderly in the Republic of Macedonia

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**Abstract**

**BACKGROUND:** Oral health care management among the elderly differs from the rest of the population, due to some specific physiological changes and general health status related to age. We know very little about the oral health in elderly in the Republic of Macedonia, because there are only a few articles published about dental health status and edentulism of this population.

**AIM:** The study aimed to evaluate the prosthodontic status of older adults over 65 years in the Republic of Macedonia, about their socio-economic status and individual factors.

**MATERIAL AND METHODS:** A cross-sectional study was conducted in 8 regions, in rural and urban areas of Macedonia and a representative sample of 432 people (age > 65 years) was examined. Statistical analyses of the data were made by chi-square tests and the corresponding C-coefficient.

**RESULTS:** Only 6% of all participants had not any prosthetic appliance, 9.5% had more than one bridge, 28.7% of examinees had partial dentures, both bridge(s) and partial denture(s) had 10.7% participants, and 45.1% of examinees were toothless. There was a significant difference between patients who visited the dentist more than once a year and those who did not ($\chi 2 = 14.2$; df = 4, $p < 0.01$). From all of the participants, 40.3% used public dental care organisations.

**CONCLUSIONS:** We found a high prevalence of edentulousness among older adults over 65 years in Macedonia. The study confirmed the necessity for establishing healthcare educational programs for the dental treatment of elderly in Macedonia.

## Introduction

Oral health care management among the elderly differs from the rest of the population due to some physiological and general health status changes related to age [1]. The most frequent oral diseases which affect older adults as dental caries and periodontal diseases could lead to teeth loss which is an important predictor for the oral health-related quality of life [2] [3] [4] [5]. DMFT is the most common index used for registration of dental health status in epidemiological studies, but it could not provide enough information about the functionality of remaining dentition [6]. Many epidemiological studies expressed oral functionality by a number of the remaining teeth, but it was questioned whether just the number was adequate to describe the functional status of dentition. According to Locker and Slade, the occluding pairs of natural teeth are strongly correlated with oral function [7] [8]. The number of missing teeth is increased with age, and it was recommended for future reports to include, not only the number but the additional information regarding their location, for it is very important to describe the functionality too [6]. The number of "20 natural teeth" is the generally accepted World Health Organization (WHO) operative criterion for a functional natural dentition [9].

In most worldwide countries including the developing, life expectancy is continuously increasing. It is expected that by 2030 almost one billion people will be 65 years and older, accounting for 13 percent

of the total population [10]. Population ageing is a progressive trend in the Republic of Macedonia also. The proportion of elderly at age over 65 years had increased from 11.2% in 2006 to 13.3% in 2016 [11].

There are numerous studies reporting the dental prosthetic status in edentulous elderly population and their need for prosthodontics treatment [12] [13] [14] [15]. Sometimes, there is a discrepancy between the real need for treatment and actual complaints by this group of patients [14] [16]. The need for prosthodontics appliances may be assessed by comparing the need perceived subjectively by a patient (self-perception), with that assessed by an examiner according to the (WHO) diagnostic criteria [14] [17], or through the use of the Geriatric Oral Health Assessment Index (GOHAI) [8] [14].

We have no enough information about the oral health in elderly in the Republic of Macedonia because there are only a few articles published about edentulism of this population. The last national oral health survey was conducted in 2007, but there is not any official data about oral health and prosthodontics status of the population over 65 years. To improve the oral health and quality of life among older adults, besides the prevalence of dental caries, it is necessary to know their prosthodontics status and needs for prosthodontics treatment.

This study aimed to evaluate the prosthodontics status of older adults over 65 years in the Republic of Macedonia about some socio-economic and individual factors.

## Material and Methods

A cross-sectional study was conducted in 8 regions (Skopje, Vardar, Eastern, Northeastern, Southeastern, Southwestern, Pelagonia and Polog region) in rural and urban areas of Macedonia in 2015/16 study year. A representative sample of 432 people (age > 65 years) was examined with a questionnaire, by calibrated postgraduate students following the procedures and diagnostic criteria recommended by the WHO Oral Health Assessment Form [19]. The patients excluded from the study were those without any prosthodontics appliances or those with the presence of dental crowns (without missing tooth). The patients included in the study had dental bridges, dentures, removable partial dentures or both bridges and partial dentures. There were 243 (56.3%) male and 189 (43.7%) female patients participated in the study. The average age was 74 years. According to the main demographic variables (ethnicity, sex, education and marital status), the sample represented the population well (Table 1, Table 2).

**Table 1: Demographic variable – Ethnicity**

| Nationality | Frequency | Percent |
|---|---|---|
| Macedonians | 274 | 63.7 |
| Albanians | 133 | 30.5 |
| Roma | 10 | 2.3 |
| Others | 15 | 3.5 |
| Total | 432 | 100.0 |

The participants in the study were asked to self-report their dentistry scaring experience from childhood, education, oral hygiene habits, habits in visiting a dentist, approximate last year expenditures for dental care and type of dental organisation (public/private).

The information we gathered was used to assess the relationship of the variables with the prosthodontics status. The data were analysed using the SPSS 13 statistical package. The analyses were made by chi-square tests and the corresponding C-coefficient.

**Table 2: Demographic variable - Education**

| Education | Frequency | Percent |
|---|---|---|
| Without elementary education | 56 | 13.0 |
| Elementary education | 167 | 38.7 |
| Secondary education | 114 | 26.3 |
| Higher education | 95 | 22.0 |
| Total | 432 | 100.0 |

## Results

Twenty-six of all participants (6%) had not any prosthetic appliance in mouth,so they were excluded from the study, forty-one (9.5%) had more than one bridge, 124 of examinees (28.7%) were wearer of partial dentures, both bridge(s) and partial denture(s) had 46 participants (10.7%) and 195 of examinees (45.1%) were without any tooth in the mouth (Figure 1).

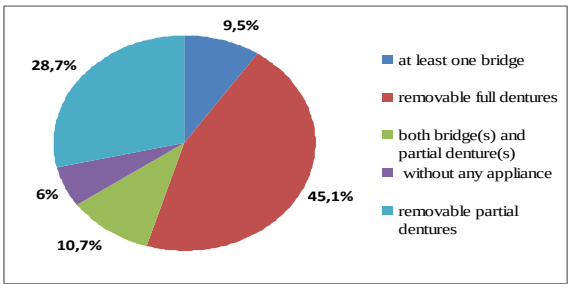


*Figure 1: Prosthodontic status*

There was a significant difference between patients who visited a dentist more than once a year and those who did not ($\chi 2 = 14.2$; df = 4, $p < 0.01$).

The correlation between the prosthodontic appliance and oral hygiene showed that the patients with removable dentures had poorest hygiene habits, while most patients with both bridge and denture,

brushed regularly, twice a day ($\chi 2 = 17.53$; df = 8, $p < 0.05$) (Table 3, Table 4).

**Table 3: Prosthodontics status and Oral Hygiene**

| Prosthetics appliance | Brushing teeth/ not every day | Brushing teeth/ once a day | Brushing teeth/ twice a day | Total |
|---|---|---|---|---|
| Without any appliance | 5 | 9 | 12 | 26 |
| At least one bridge | 15 | 15 | 11 | 41 |
| Denture | 44 | 52 | 28 | 124 |
| Both bridge and partial denture | 7 | 22 | 17 | 46 |
| Removable partial dentures | 66 | 90 | 39 | 195 |
| Total | 137 | 188 | 107 | 432 |

Last year, 317 (73.4%) of the examinees didn't visit a dentist. There was no correlation between prosthodontic status and scaring experience from childhood dentistry ($\chi 2 = 9.4$; df = 4, $p > 0.05$) (Table 5).

**Table 4: Prosthodontics status and dentist visits**

| Prosthetics appliance | Once a year or none | More than once a year | Total |
|---|---|---|---|
| Without any appliance | 12 | 14 | 26 |
| At least one bridge | 29 | 12 | 41 |
| Removable partial dentures | 98 | 26 | 124 |
| Both bridge and partial denture | 30 | 16 | 46 |
| Denture | 148 | 47 | 195 |
| Total | 317 | 115 | 432 |

There was no correlation between the cost of a dental care ($\chi 2 = 5.6$; df = 4, $p > 0.05$) (Table 6) and dental organization used ($\chi 2 = 4.9$; df = 4, $p > 0.05$). From all participants, 40.3% (174) used public dental care organizations.

**Table 5: Prosthodontics status and frightening experience(s) from childhood dentistry**

| Prosthetics appliance | Afraid | Not afraid | Total |
|---|---|---|---|
| Without any appliance | 5 | 21 | 26 |
| At least one bridge | 17 | 24 | 41 |
| Removable partial dentures | 40 | 84 | 124 |
| Both bridge and partial denture | 15 | 31 | 46 |
| Denture | 46 | 149 | 195 |
| Total | 123 | 309 | 432 |

The proportion of elderly over 65 years with 20 or more natural teeth was 24.1% (104 of examinees).

**Table 6: Prosthodontics status and cost of dental care**

| Prosthetics appliance | < 50 euro per year | >50 euro per year | Total |
|---|---|---|---|
| Without any appliance | 21 | 5 | 26 |
| At least one bridge | 30 | 11 | 41 |
| Removable partial dentures | 101 | 23 | 124 |
| Both bridge and partial denture | 40 | 6 | 46 |
| Denture | 175 | 20 | 195 |
| Total | 367 | 65 | 432 |

## Discussion

The present findings demonstrated a high prevalence of edentulousness (45.1%), removable partial dentures wearer were 27.7%, and only 6% of the patients were without any prosthetics appliance. The data about edentulous in patients over 65 years in Macedonia is almost same with Belgium old population, 45% (2007). Belgium is the country with the highest percent of edentulousness among the population over 65 years old in EU countries, and Malta has lowest 8% (2002) [20].

Differences in the prosthodontics status were associated with gender, educational background, dental attendance patterns, tooth brushing frequency, scaring experience from childhood dentistry, cost of a dental care and care organisation used. These were objective findings which could not be about the subjective patients' needs or complains.

Mac Enteeet al. reported that about two-thirds of the elderly population has poor oral health, but that only about one-third complained of a problem. In their investigation, about half (54%) of the sample identified a problem, and 83% of the subjects were either using a denture with a major fault or were missing a denture [16].

The professional criteria based on WHO guidelines also differed from the self-perceived need. The analysis is given by Colussi et al. showed that the variables age, gender, residential area and form of service, most significantly associated with a better self-perception of oral health [17]. The number of "20 natural teeth" is the generally accepted WHO operative criterion for a functional natural dentition [9] which is very important for the masticatory efficiency. Akifusa et al. reviled that85-year-old participants with > or = 20 teeth had better subjective physical health than those with < or = 19 teeth [21]. In a systematic review conducted by Zhang et al., it was reported that an average of 20 teeth was present at the age of 65 among the Chinese population [22]. The number of remaining teeth in the elderly in Japan tends to increase year by year, and an average of 14 teeth remained in the mouth even by the age of 80 [23]. The Findings in our study showed that there was a high prevalence of elderly with less than 20 teeth (75.9%) in Macedonian population, which might lead to weaker masticatory efficiency, malnutrition and other health issues.

Most of the samples examined in this study brushed their teeth once a day, but 137 (31.7%) of the examiners reviled that they did not brush their teeth or dentures every day. Kulak-Ozkanet et al. have found a statistically significant relationship between denture stomatitis, yeasts' presence and denture cleanliness in their study [24]. Sometimes teeth brushing may be in a relationship with the limitation of manual dexterity resulting from arthritis and/or stroke and special oral hygiene measures might be required for the elderly [25].

Regarding the visits to dentists, most of the examined elderly in our study reviled that they visited a dentist less than once a year (73.4%). The most common major barriers which were identified included poor general health, cost and the physical aspect of being unable to travel to a dentist. These barriers are

also most common for elderly worldwide [26]. Improving access to dental health care involves actions at individual, societal and system levels [27]. People's perception of dentists is influenced by some factors that depend on the professional, mass media and the overall health system [28]. Around 71.5% of the examined samples in our study reviled that they are not afraid of the dentist.

Dental health expenditures could be a problem for the elderly in Macedonia to visit a dentist. 85% of the patients involved in our study spent less than 50 euro per year on the dentist, and most of them were in private dental practices (69.7%). Edentulous is highly associated with socioeconomic status. Poor oral health among older adults is an important public health issue and a growing burden to countries worldwide [29]. Among elderly world population, 20-80% is edentulous, and 60-80% has immediate dental needs [30]. It is necessary to improve elders' quality of life, by minimising the risk factors and ameliorating the protective factors [31]. According to the samples that had been chosen for our study (patient's wearers of the prosthodontics devices) the treatment needs were covered with the prosthodontics status.

In conclusion, after statistical analysis of the data from our study, it can be concluded that there is a high prevalence of edentulousness in older adults (over 65 years) in Macedonia. Unfortunately, analyses showed that this population has no habit to visit a dentist regularly, but only when the dental problem appears. The anticipated connection between frequency of visiting a dentist and the prosthodontic status was also confirmed, as well as between this status and the oral hygiene habits.

This study also confirmed the necessity of establishing dental care educational programs in Macedonia which has already been done through involving the new programs and subjects at the Faculties of Dental medicine (Gerontostomatology) for doctors and oral hygienist too. Education and continuous training of dental staff towards special needs of the elderly patients should provide those particular skills and knowledge of medical, psychological and social aspects of this particular age group.

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# Evaluation of Combined Topical Ozone and Steroid Therapy in Management of Oral Lichen Planus

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**Abstract**

**AIM:** The present study aims to assess the therapeutic effect of the combination of topical ozone and steroid therapy in comparison to topical ozone alone versus topical steroid as a control in the management of atrophic - erosive oral lichen planus (OLP).

**METHODS:** Sixty-six patients are having atrophic-erosive OLP were included in the study. They were randomly divided into three equal groups to be treated with topical corticosteroids alone (steroid group) as control, topical ozone alone (ozone group) or combination of topical steroids and ozone (combined group). Assessment of pain and sign scores was done before and after each treatment modality.

**RESULTS:** The results revealed that the greatest significant percentage of change and subsequent improvement in pain and sign scores were recorded in the combined group.

**CONCLUSION:** Reported data in this study using the combination of ozone and steroid therapy could provide a new promising safe and effective adjunct therapy for management of OLP.

## Introduction

Lichen planus is a common chronic mucocutaneous disorder of uncertain cause. It has been reported that it involved about 0.5% to 2.2% of the examined populations worldwide [1].

Oral lichen planus (OLP) can be seen commonly in the fifth and sixth decades of life with a twice predominance in females as compared to males [2] [3]. The exact aetiology and pathogenesis of OLP are undetermined including a cell-mediated immune response associated with degeneration of the basal cell layer of the epithelium [4]. Six clinical forms of OLP lesions are recognised divided into two essential categories either white keratotic (plaque-like, papular, reticular) or white keratotic with red areas (bullous, erosive, atrophic) [5]. Keratotic lesions are asymptomatic with no need of treatment. Meanwhile, red sores are painful and require treatment, in addition to their potential and risk for malignant changes to squamous cell carcinoma mostly occurring in 0.4-2% of cases which should be taken into consideration [6]. OLP is generally observed bilaterally or symmetrically on the buccal mucosa, less regular on the tongue, labial mucosa and gingiva [7].

Multiple and various therapeutic approaches have been discussed in the management of OLP. As there is an alteration in disease activity, the use of a sole and definitive therapeutic modality is challenging. Existing treatment modalities are chiefly concerned with the improvement of the painful symptoms and mucosal ulcerations. The available therapies are still unable to cure the disease completely because of its refractory nature. Various available treatment options for management of OLP include topical and systemic agents. Intralesional and systemic corticosteroids are

mainly utilised yet with frequently unsatisfactory outcomes [8].

Corticosteroids still considered the main treatment for symptomatic OLP; however, its prolonged use revealed several adverse effects including atrophy of mucosal tissues with subsequent discomfort, candida overgrowth, adrenal suppression, hypertension, gastrointestinal upset and hyperglycemia. Efficient treatment option with minimal adverse effects still appears to be essential regarding the development of resistance to topical steroids with its inconveniences in some patients [9].

Ozone therapy has gained a prominent consideration in the medical and dental fields due to its strong antimicrobial activity (against bacteria, viruses, yeasts and protozoa) and as a powerful oxidising agent. It is also capable of stimulating the blood circulation and the immune system with reported analgesic effect [10].

Dental applications of ozone included prevention and management of dental caries, teeth remineralisation, control of infection, disinfection of periodontal pockets, teeth bleaching, management of pain accompanying exposed roots and tooth sensitivity, TMJ disorders, endodontic treatment, biofilm removal, enhancement of healing, tissue regeneration and control of halitosis. Ozone therapy is an alternative non-medication therapy that has also been introduced as a treatment option in the management of OLP [11].

Accordingly, this study aimed to evaluate the therapeutic effect of the combination of topical ozone and steroid in comparison to topical ozone alone and topical steroids as a control in the management of atrophic-erosive OLP.

## Patients and Methods

This randomised controlled clinical study included sixty-six patients according to the sample size calculation with an age range between 30-70 years.

Power analysis for the 3 study groups were conducted in G* power to determine the sufficient sample size using an alpha of 0.05, a power of 0.80, and large effect size (f = 0.40). Based on the assumptions above, the desired sample size was 66 patients.

Patients were recruited from the outpatient clinic of the Oral Medicine and Periodontology Department, Faculty of Dentistry, Cairo University and from the outpatient clinic of the Skin and Venereal Diseases Department, Faculty of Medicine, Cairo University.

The study protocol was approved by the Medical Ethical Committee of the National Research Centre (NRC) code no. 17 115. After the study procedures were explained before starting the treatment to the patients, they all signed an informed consent form stating their approval. This contemplates conducted on atrophic-erosive OLP patients affecting the tongue or buccal mucosa. Diagnosis of OLP Patients was based on the diagnostic steps criteria approved by the World Health Organization (WHO) [12].

Medical data were collected from the patients according to the Modified Cornel Medical Index questionnaire [13]. Smokers, pregnant or lactating ladies and patients under topical or systemic steroids during the last two months were excluded from the study. Patients using lichenoid reaction-inducing drugs, patients with positive hepatitis C virus (HCV) antibodies, those having systemic diseases that may contribute in the occurrence of OLP such as uncontrolled diabetes and hypertension were not allowed to participate in this study. Patients having amalgam filling adjacent lesions are also not included. All participants in the study groups underwent adequate oral hygiene performance measures with complete removal of plaque and calculus as they implement intraoral inflammation and intensify both extension and symptoms of OLP lesions. Patients were advised to evade accidental trauma on soft tissues using soft bristles toothbrush. Acidic, spicy, hard, hot food and beverages were avoided.

The included 66 patients were randomly assigned, by preoperative envelope drawing, to be treated in the different study groups. The patients were divided into three equal groups. The steroid group (n = 22) as a control in which patients were treated by topical steroid alone. The ozone group (n = 22) in which patients were treated with topical ozone alone. The combined group (n = 22) in which patients were treated with a combination of topical ozone and topical steroid therapy.

Topical steroid therapy involved use of commercially available ointment (triamcinolone acetonide 0.1%, Kenacort-A Orabase®, Turkey) repeated four times per day for four weeks. Topical ozone therapy was done by using an ozone generator. Ozone generator type N 1888A, China was used in the application procedures of gaseous ozone with an ozone rate of 500 mg/hour.

An ozone measuring device was used to confirm the ppm of ozone delivered and a flow meter was used to confirm the flow rate immediately before the start of the treatment. Ozone was applied on the lesions through special disposable glass cups that permitted adequate seal to avoid gas escape which ensured the safety of the machine for human use. No ozone could escape and therefore no ozone smell could be detected which allowed blinding. Ozone was applied intraoral with an intensity of 60% for 1 minute

according to the manufacturer instructions in each session twice a week for four weeks. Combined topical ozone and steroids therapy involved both topical ozone application (twice weekly) followed by topical steroid use (four times daily) for four weeks with at least 2 hours interval between topical ozone and steroid application in the day of ozone session as previously mentioned. All the patients in the three groups were followed up weekly during the four weeks.

All cases in the three groups were assessed using the sign scoring scale of Thongprasom et al., 1992 [14] as follows: 5 (white striae with an erosive area > 1 $cm^2$), 4 (white striae with an erosive area < 1 $cm^2$), 3 (white striae with an atrophic area > 1 $cm^2$), 2 (white striae with an atrophic area < 1 $cm^2$), 1 (mild white striae only), and 0 (no lesions, normal mucosa).

Pain assessment for all cases of the study groups was done using grade of pain scale before, during and after different treatments according to Garnick et al., 1998 [15], as follows: grade 0 (no symptoms), grade 1 (mild discomfort and capable of eating), grade 2 (moderate discomfort but still capable of eating), grade 3 (severe discomfort and unable to eat), grade 4 (tolerated pain and unable to eat).

Data were analysed by using SPSS ver. 18.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics included mean, median, and standard deviation (SD) values. The paired t-test was used to compare sign scores at baseline and after the end of the treatment within the same group. Friedman test was used to evaluate the difference in the sign and pain scores of lesions throughout the study. The percentage of change was also calculated. Comparison of the percentage of change in the study groups was performed using one-way analysis of variance (ANOVA) test, followed by Tukey's post hoc test for multiple comparison. Values of $p < 0.05$ was considered statistically significant.

## Results

A total of 66 patients (39 female and 27 males) having atrophic-erosive OLP having an age range from 30-70 years (mean 54.67 ± 4.63 years) were included in the study.

In this contemplate topical ozone application alone and the combination of topical steroid and ozone did not cause any unwanted tissue reactions or complications. It was found that 3 cases in the steroid-treated group complained from oral candidiasis.

No statistically significant difference was shown between mean age values ($p = 0.14$) and gender distributions ($p = 1$) in the three groups as presented in Table 1).

**Table 1: Mean age values and gender distributions in the three study groups**

| | Age | | | | Gender | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Steroid | Ozone | Combined | | Steroid | | Ozone | | Combined | |
| | | | | | Male | Female | Male | Female | Male | Female |
| Mean ± SD | 56.2 ± 5.5 | 53.4 ± 4.2 | 54.4 ± 4.2 | Number (%) | 9 (40.9%) | 13 (60.1%) | 9 (40.9%) | 13 (60.1%) | 9 (40.9%) | 13 (60.1%) |
| F value | | 2.028 | | $X^2$ | | | 0 | | | |
| P (ANOVA) | | 0.140 ns | | P value (chi square) | | | 1 ns | | | |

Significance level p < 0.05; * = significant; ns = non-significant.

Within each group, sign scores decreased after treatment. Paired t test revealed that this difference was statistically significant in steroid (p = 0.016), ozone (p = 0.0038) and combined group (p = 0.0004), (Table 2, Figure 1).

**Table 2: Comparison of sign scores expressed as mean ± SD at baseline and after the end of treatment within the same group (paired t-test)**

| Groups | Before treatment | | After treatment | | t value | | P value | |
|---|---|---|---|---|---|---|---|---|
| | Sign score | Pain score | Sign score | Pain score | Sign score | Pain score | Sign score | Pain score |
| Steroid | 4.6±0.55 | 3.6±0.55 | 3.6±0.55 | 0.8±0.45 | -0.4 | -0.14 | 0.016* | 0.0001* |
| Ozone | 4.2±1.1 | 3.4±0.89 | 3±1 | 2.8±0.45 | -0.6 | -2.45 | 0.0038* | 0.07 ns |
| Combined | 4.2±0.84 | 3.2±0.84 | 1.6±0.44 | 0.6±0.15 | -10.6 | -10.6 | 0.0004* | 0.0004* |

Significance level p<0.05; *=significant; ns=non-significant.

Within each group, pain scores decreased after treatment. Paired t-test revealed that this difference was statistically significant in steroid (p = 0.0001), and combined group (p = 0.0004). However, the difference between baseline and after treatment values of pain score in ozone group was not statistically significant (p = 0.07), (Table 2, Figure 1).

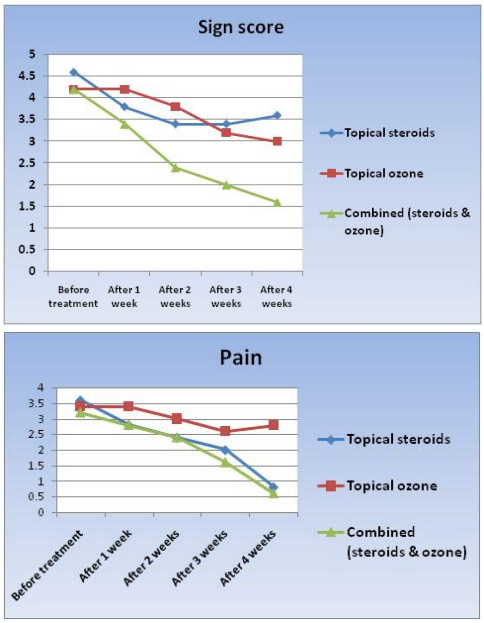


*Figure 1: Sign score and pain during the treatment*

**Table 3: Comparison of sign and pain scores t baseline and after 1, 2, 3 and 4 weeks within the same group (Friedman test)**

| | | Sign score | | | | | Pain score | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Baseline | After 1 w | After 2 w | After 3 w | After 4 w | Baseline | After 1 w | After 2 w | After 3 w | After 4 w |
| Steroid | Mean ± SD | 4.6 ± 0.55 | 3.8 ± 0.84 | 3.4 ± 0.89 | 3.4 ± 0.89 | 3.6 ± 0.55 | 3.6 ± 0.55 | 2.8 ± 0.45 | 2.4 ± 0.89 | 2 ± 0.71 | 0.8 ± 0.45 |
| | Median | 5 | 4 | 4 | 4 | 4 | 4 | 3 | 3 | 2 | 1 |
| | Min | 4 | 3 | 2 | 2 | 3 | 3 | 2 | 1 | 1 | 0 |
| | Max | 5 | 5 | 4 | 4 | 4 | 4 | 3 | 3 | 3 | 1 |
| | Mean rank | 4.9 | 3.1 | 2.2 | 2.2 | 2.6 | 4.7 | 3.7 | 3.1 | 2.5 | 1 |
| | P value | Chi square = 96.38, P < 0.0001* | | | | | Chi square = 106.6, P < 0.0001* | | | | |
| Ozone | Mean ± SD | 4.2 ± 1.1 | 4.2 ± 1.1 | 3.8 ± 0.84 | 3.2 ± 1.1 | 3 ± 1.0 | 3.4 ± 0.89 | 3.4 ± 0.89 | 3 ± 0.71 | 2.6 ± 0.55 | 2.8 ± 0.45 |
| | Median | 5 | 5 | 4 | 4 | 3 | 4 | 4 | 3 | 3 | 3 |
| | Min | 3 | 3 | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| | Max | 5 | 5 | 5 | 4 | 4 | 4 | 4 | 4 | 3 | 3 |
| | Mean rank | 4.20 | 4.20 | 3.20 | 1.80 | 1.60 | 3.8 | 3.8 | 2.9 | 2.1 | 2.4 |
| | P value | Chi square = 99.79, *p* < 0.0001* | | | | | Chi square = 61.5, *p* < 0.0001* | | | | |
| Combine | Mean ± SD | 4.2 ± 0.84 | 3.4 ± 0.55 | 2.4 ± 0.55 | 2 ± 1.0 | 1.6 ± 0.44 | 3.2 ± 0.84 | 2.8 ± 0.45 | 2.4 ± 0.89 | 1.6 ± 0.55 | 0.6 ± 0.15 |
| | Median | 4 | 3 | 2 | 2 | 2 | 3 | 3 | 3 | 2 | 1 |
| | Min | 3 | 3 | 2 | 1 | 0 | 2 | 2 | 1 | 1 | 0 |
| | Max | 5 | 4 | 3 | 3 | 3 | 4 | 3 | 3 | 2 | 1 |
| | Mean rank | 4.9 | 4.1 | 2.5 | 2 | 1.5 | 4.6 | 4 | 3.3 | 2 | 1.1 |
| | P value | Chi square = 112.18, *p* < 0.0001* | | | | | Chi square = 110.13, *p* < 0.0001* | | | | |

Significance level p<0.05; *=significant; ns=non-significant.

Comparing the sign and pain scores at baseline and throughout the study at 1, 2, 3 and 4 weeks within the same group using Friedman test it was revealed that a significant difference in each of the 3 groups was obtained (Table 3).

Regarding the sign scores, the greatest percentage of change was noted in the combined group, whereas the least percent of change was recorded in the steroid group. ANOVA test revealed that the difference was statistically significant (p<0.0001). Tukey's post hoc test revealed a significant difference between every 2 groups (Table 4).

Regarding the pain scores, the greatest percentage of change was also noted in the combined group, whereas the least percentage of change was recorded in the ozone group. ANOVA test revealed that the difference was statistically significant (p<0.0001). Tukey's post hoc test revealed no significant difference between combined, ozone and steroid groups (Table 4).

**Table 4: Comparison between groups regarding sign and pain scores percentage of change after treatment (ANOVA test)**

| Groups | % of the change in Sign score | % of the change in Pain score |
|---|---|---|
| | Mean ± SD | Mean ± SD |
| Steroid | $-22.00^{c}$ ± 2.74 | $-78.33^{a}$ ± 12.64 |
| Ozone | $-29.33^{b}$ ± 8.94 | $-15.00^{b}$ ± 4.69 |
| Combined | $-65.00^{a}$ ± 21.45 | $-83.33^{a}$ ± 15.59 |
| F value | 92.78 | 327.536 |
| *p*-value | <0.0001* | <0.0001* |

Tukey's post hoc test: means sharing the same superscript letter are not significantly differe

Significance level p<0.05; *=significant; ns=non-significant.

Improvement of OLP lesion following combined ozone and steroid therapy reported healing in the area treated as shown in Figure 2.

## Discussion

Complete curative management of OLP has not yet accomplished because of the chronic and refractory nature of the disease [16].

The inflammatory and immunologically mediated characters of OLP recommended the use of corticosteroids; thus topical, intralesional and systemic steroids are utilised. Corticosteroids are accepted as a palliative and relieving therapy rather than a therapeutic agent in the management of OLP [17] [18].

*Figure 2: Improvement of OLP lesion following combined ozone and steroid therapy*

In addition to corticosteroids, various interventions have been presented for management of OLP including immunosuppressants (e.g., cyclosporine and tacrolimus), topical or systemic retinoids, and oral metronidazole. Also, various herbal extracts and laser therapy are among the different modalities that have been introduced in the management of OLP. All remedies have been applied in an attempt to improve OLP lesion and associated symptoms such as pain and burning sensation [19] [20].

Topical steroid is considered the first-line effective treatment option for erosive-atrophic OLP with promising outcomes as regarding pain and soreness relief. Several patients encountered various adverse effects with this treatment modality including candidal overgrowth and mucosal atrophy as previously documented [21] [22] [23].

Thus, various randomised clinical trials suggested diverse treatment options in addition to

topical steroids as a combination or as a substitute for steroids [24].

New non-medication treatment modalities are suggested including ozone. Ozone has been utilised effectively for the treatment of different disorders for over 100 years. Its special properties incorporate immuno-stimulant, pain relieving, antihypnotic, detoxicating, antimicrobial, bio-energetic and biosynthetic activities with powerful wound healing properties. Ozone is capable of interacting with blood constituents (erythrocytes, platelets, leukocytes, and endothelial cells) and induces oxygen metabolism, cell energy and immuno-modulatory changes. Ozone can enhance the antioxidant defence system and stimulate the microcirculation in tissues [23].

The findings of this study revealed that the sign scores decreased after treatment within the 3 groups where this decrease was statistically significant. This is by the results demonstrated by Kazancioglu and Erisen, 2015 [21].

The outcomes of this study also showed that pain scores decreased after treatment within each group. This difference was statistically significant both in the steroid and combined groups. However, this difference was not statistically significant in the ozone group. This is against the findings previously conducted. This may be due to the difference in the ozone generator used and follow up periods between the studies [21].

Also, the results of this contemplate showed that topical ozone application prevented the candidal overgrowth in ozone and combined groups. This was in line with a study conducted by Arita et al., 2015 who concluded that the use of ozonated water might be useful in oral candidal treatment due to the strong and effective antifungal properties of ozone [24].

The bactericidal, fungicidal, and virucidal properties of ozone may be explained on the basis of its powerful oxidising ability with the creation of free radicals and direct destruction of almost all pathogenic microorganisms. Adding to that, ozone favours tissue healing and increases blood perfusion. It can improve blood flow and immunological reaction. Ozone affects both the cellular and humoral immune responses, oxidises poisons making their discharge simpler, empowers the creation of immunocompetent cells and immunoglobulins, enhances phagocytosis capacity of macrophages, which closures inflammation and fasten tissue healing. Besides, ozone improves the oxygen conveying limit of blood causing better metabolism of inflamed tissues cells and more usage of energy using actuation of aerobic pathways of metabolism. What's more, oxidant action of ozone helps protein production and upgrades cell ribosomes and mitochondria. Thus, cell action and recovery possibilities will be enhanced with the improvement of the tissue healing process. This might explains the study outcomes reporting the greatest percentage of change which was noted in the combined group in both sign and pain scores [24].

Considering the various beneficial effects obtained from the reported data in this study using the combined ozone and steroid therapy a new promising adjunct therapy might be presented for management of OLP. However, with the limitations of this study many more long-term studies are needed to substantiate the use of this combination.

Accordingly, it could be concluded that topical ozone can be combined with topical steroid therapy as a new more effective and safe treatment modality for symptomatic OLP.

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# How Is Developing the Sense of Belonging in Iranian Adolescent Girls? A Qualitative Study

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**Abstract**

**BACKGROUND:** Communicating with adolescents is associated with many challenges for parents and healthcare providers.

**AIM:** This qualitative study was conducted for exploring the sense of belonging in Iranian adolescent girls.

**METHODS:** In this study, deep semi-structured interviews were carried out with 27 adolescent girls, 10 experts, and 10 parents. Purposeful sampling was used and continued until data saturation. The data were coded and categorised through a conventional content analysis method by MAXQDA 10.

**RESULTS:** Three main categories were obtained from the analysis of the participants' descriptions: "family; a haven of tranquillity", "dominated by peers", and "concerns about differences in gender socialization". According to our results, most of the girls achieved calmness through being emotionally accepted by their families. But this sense of belonging and tranquillity was shaken by their peers' showing off, in a way that adolescent girls were always struggling to gain acceptance among their peers. Also for fear of being rejected by their peers' group, they sometimes began to make friends with the opposite sex. Meanwhile, traditional attitudes towards gender roles and adolescent girls' feelings about their lower social participation as compared to that of boys had also led to their concern about differences in gender socialization and a lower sense of community belonging among some adolescent girls.

**CONCLUSION:** Adequate parental education and the proper management of girls' interactions with the family and society can play an important role in the development of a sense of belonging among adolescent girls.

## Introduction

The transition from childhood to adolescence is associated with social, behavioural, and environmental pressures and challenges for the adolescent [1]. Adolescence is a stage of human growth, during which adolescents experience puberty, and in addition to biological changes, they undergo cognitive behavioural changes [2] [3] [4]. Adolescence is one of the longest developmental stages, which lasts at least 10 years, and typically occurs between the ages of 11 and 22 [5]. One of the most important and effective meanings, which has always been considered in the relationship between the adolescent and the world around them, is the sense of belonging. Belonging is defined as "deep mutual understanding and identification" between individuals or groups. It is one of the essential components of successful interaction and behaviour among individuals. Some of the important aspects of belonging include family solidarity, practical and emotional support, family culture, common norms, values, and aspirations, which in fact lead to individual and social self-confidence in the adolescent [6]. The results of a

qualitative study in the United States suggested the need for the conceptualisation of belonging at schools, based on school-centred interventions [7]. In a study on the immigrant population of Canada, the disintegration of ethnic identity and the absence of citizenship belonging had led to adolescents' tendency toward high-risk groups [8].

Healthcare providers need to know the effects of the family and peers on the development of adolescents through the stages of early (11-14 years of age), mid (15-17 years of age), and late adolescence (18-21 years of age) [5]. Since understanding the identity and social roles begins from adolescence, scrutiny on the challenge of belonging can be important in this group. Also, the need for further studies to explain the sense of belonging is felt more than ever before [9].

Whereas the Qualitative studies are appropriate for a deep understanding [10], this study was designed for exploring the sense of belonging with a qualitative approach.

## Methods

This study was conducted in 2017 through deep semi-structured interviews with the participation of 27 Iranian adolescent girls, 10 experts, and 10 parents in the urban population of Isfahan. Purposeful sampling with maximum variation was used and continued until data saturation. The participants in this study were apparently healthy adolescent girls ranging in age from 11 to 21 years, who were invited to participate in the study and asked to sign an informed consent. About girls aged 11 to 18 years, their family's consent was also obtained to participate in the study. The time and place of interviews were determined according to the participants' opinions. And the participants were asked for permission to digitally record their voices. The duration of each interview was 30 to 60 minutes long. The interview began by asking the following questions: How do you feel about being a girl? How and when did this feeling begin? What things and which people were effective in your thoughts? To facilitate the communication of the participants in the study with the researcher, the researcher's phone number and email address were put at their disposal. They were also asked at the end of the interview, to complete a personal data form containing questions about age, birth order, and family education.

Each interview was recorded and transcribed on the paper word for word. Before transcribing the audio file, the researcher carefully listened to the interview several times, so that she could choose the main ideas out of the participant's words. Then the interview was converted into a text file. After initial analysis of the text of each interview, the next interview was planned and scheduled.

Data analysis was carried out through the Graneheim and Lundman content analysis using the MAXQDA software Version 10 [11]. Transcripts were reviewed, and the main concepts were extracted in the form of initial codes. The codes were grouped into six subcategories and the subcategories, together, formed three main categories. By maximum variation in the selection of adolescent participants, continuous and prolonged involvement of the researcher for 11 months, peer review, and the participants' review, we tried to enhance the credibility of data. To strengthen confirmability, several codes and categories were assessed through member check, and external check and a good deal of agreement were obtained.

To assess the transferability of the data, the results were presented to some adolescent girls and specialists who had not participated in the study, which was requested to compare the results with their own experiences of the matter. The ethical principles of the study including voluntary participation, obtaining written informed consent from the subjects to involve them in the research and to record their conversations, explaining the research objectives, inviting the subjects to attend the interviews, voluntary withdrawal of the study at any time and ensuring confidentiality were observed.

## Results

Deep semi-structured interviews were carried out with 27 adolescent girls. The age range of the Iranian female adolescents was from 11 to 21 years old (Table1). According to adolescents' statements, we interviewed with 10 parents including 5 fathers and 5 mothers, and 10 experts in psychiatry and sociology who could help us to clarify our data. Three main categories were obtained from the analysis of the participants' descriptions: "family; haven of tranquility", "dominated by peers", and "concerns about differences in gender socialization" (Table 2).

**Table 1: Characteristics of participating adolescent girls**

| Age | Number | Percent |
|---|---|---|
| 11-14 | 7 | 26 |
| 15-17 | 14 | 52 |
| 18-21 | 6 | 22 |
| Birth Rate in the family | | |
| 1 | 10 | 37 |
| 2 | 10 | 37 |
| 3 | 5 | 18.5 |
| >3 | 2 | 7 |
| Fathers' education level | | |
| Under Diploma | 8 | 30 |
| Diploma | 10 | 37 |
| Academic Degree | 9 | 33 |
| Mothers' education level | | |
| Under Diploma | 5 | 18.5 |
| Diploma | 7 | 26 |
| Academic Degree | 15 | 55.5 |

Analyzing the viewpoint of the adolescent girls showed that "the adolescent girl's assurance about parents' satisfaction with her sex" and "the adolescent girl's tranquility in case of her emotional acceptance in the family" were the two main dimensions of the family's role in the acceptance of female children, especially their sex, which is known as the main category: "Family; haven of tranquility".

**Table 2: Main Categories and Sub-categories for a sense of belonging in Iranian adolescent girls**

| Sub-categories | Main categories |
|---|---|
| - The adolescent girl's assurance about parents' satisfaction with her sex<br>- The adolescent girl's tranquillity in case of her emotional acceptance in the family | Family; a haven of tranquillity |
| - The instability of the foundations of belonging to the family due to the peers' showing off<br>- Friendship with the opposite sex for fear of being rejected by their peers | Dominated by peers |
| - The adolescent girls' feelings about their limited social participation in society<br>- Traditional attitudes towards gender roles | Concerns about differences in gender socialization |

The interviews carried out with adolescent girls and their parents showed that one of the adolescent girls' serious needs was their need for their parents to express their satisfaction with their sex. Most participating girls wanted to make sure that their parents were satisfied with having a girl in the family. And to them, this assurance alone was not sufficient. They wanted their parents to express this satisfaction repeatedly. The adolescent girls would feel relaxed if their sex was accepted by their parents. And this feeling needed to be repeatedly strengthened by their parents' verbal expression.

*"My mom was very happy in her pregnancy when she knew I was a girl. My parents always say this to me. That's why, since the very beginning, I've been very happy with my being a girl. And I know that they love me a lot."* (An 11-year-old girl; the first child of the family)

Participating parents also stated that adolescent girls were in doubt as to whether their parents love them or not and frequently needed parental confirmation about their sex.

*"Many times my daughter asked me if I liked my first child to be a girl. I told her I always liked my first child to be a girl, and as for the second, if the child were a girl, she would be a sister for the first one, and if the child were a boy, I would have both a girl and a boy. And now they constantly ask me if I am satisfied with their being girls or not."* (A mother of two adolescent girls who are 11 and 14 years old)

In addition to their need to be assured about their parents' satisfaction with their sex, the adolescent girls repeated many times in their interviews that they needed their parents to express their love to them.

*"I love my father a lot. See what short messages he sent to me. He wrote: «My dear, how much God loved me that granted me a daughter, and I love you very much. » I'm glad and feel relaxed to see my father adores me."* (A 13-year-old girl; the only child of the family)

The instability of the foundations of belonging to the family due to the peers' showing off, and friendship with the opposite sex for fear of being rejected by their peers was known as the main category: "Dominated by peers ".

By entering adolescence, tendencies toward peers were more outstanding, and adolescent girls thought that the family no longer met their emotional needs like in previous years.

*"When I compare myself today with two or three years ago, I realize that my family don't understand me as they did in the past. Now, I would only like to chat with one of my intimate friends, to share with her what I cannot tell my family."* (A 17-year-old girl; the first child of a family)

One of the participating mothers said about her daughter's relationship with her same-sex peers:

*"My daughter is constantly trying to find friends from among her peers. Whatever I tell her these relationships are not worthy of our family, she doesn't listen. I have told my daughter many times that if she wants love, her father and I are here. But she says that we do not understand her. I don't know how to manage her relationships with friends.*" (The mother of a 16-year-old girl)

One of the areas of belonging to the peer group was about making friends with the opposite sex. By modelling their same-sex peers, most of the participants attempted to make friends with the opposite sex. They also stated that by having a friend of the opposite sex, in addition to not being labelled among their peers, they also increased their acceptance among their same-sex friends. Whereas according to their families and the governing sociocultural context, it is not accepted for adolescents of this age to make friends with the opposite sex.

*"If I don't have a boyfriend, I will be labelled as ugly by my friends. In our intimate chats, we have a general rule: If a girl does not have a boyfriend, she is not beautiful, and if a boy does not have a girlfriend, he is clumsy."* (A 17-year-old girl; the second child of a family)

It also seemed that a sense of belonging to the same-sex peer group showed up more than anything else in obtaining sexual information. Also, most of the adolescent girls stated that they mainly received their sexual information from their same-sex peers than anybody else.

*"The school staff and consultants try to provide us with a lot of information about adolescence and issues related to puberty. But we mainly obtain our sexual information from our friends during break*

*time at school."* (An 18-year-old girl; the first child of a family)

The peers' temptation of girls uninterested in sexual issues could also be effective in choosing friends of the opposite sex. They stated that even in girls who don't like to find friends, peers' temptation makes them change their behaviour.

*"I had no interest in finding boyfriend until last year. But since I changed my school and found new friends, they have always been talking to me about themselves going out and enjoying with their boyfriends."* (A 15-year-old girl; the only child of the family)

The adolescent girls' feelings about their limited social participation in society and traditional attitudes towards gender roles were the main dimensions of the category: "Concerns about differences in gender socialization".

Most of the participating girls were happy with their gender and considered being a girl as an advantage to them. But they stated that their participation in society was not as great as boys', and felt a difference between themselves and boys as they passed through puberty. In fact, in their words, they expressed their belief in the existence of gender differences as follows:

*"As you know, it's not important to be a boy or a girl. Because a girl also can play a role in society to the extent that a boy can. But the balance of advantages is in favour of boys. When I compare myself with a boy at my age, I see that he has more freedoms than I do. Of course, I'm free too, but not as much as he is. They can become independent of the family very soon. They can travel and have fun with their friends. In my opinion, we rarely have enough recreational sporting places in a way that is suitable for girls."* (A 15-year-old girl; the second child of the family)

According to experts participating in the study, the belief in gender differences is, in fact, different from gender discrimination.

*"Believing in the existence of gender differences has many reasons, most of which primarily refers to the family and how the family acts toward the institutionalisation of gender roles. Different parental strategies to differ between girls and boys transmit this belief into society."* (Specialist in psychiatry)

Also, according to another expert, the way the family and society look at gender roles for girls and boys can play an important role in believing in the existence of gender differences for girls, and make it a concern for them.

*"Traditional attitudes to gender roles among families are another factor for rooting this belief in society. By demarcating expectations and attitudes in girls and boys, families change girls' and boys' attitudes toward gender roles. And from the very beginning, they bring up girls and boys for their gender-related roles, which can play a role in women's feeling in their inability in society and sense of community belonging."* (Specialist in sociology)

## Discussion

The present study was designed with the aim of exploring the sense of belonging in Iranian adolescent girls. The findings of this study showed that the family had an important role for adolescent girls to feel a sense of belonging to, as well as their haven of tranquillity. The adolescent girl's need for parental expression of satisfaction with her sex and the emphasis on her parents' verbal satisfaction were among the needs expressed by the adolescent girls. Responding to this category of needs can increase the adolescent's attachment to their parents and family, and assure them about their emotional acceptance in the family.

In a study in Iran with the aim of "The family's role in increasing crimes among children", the lack of love from parents was introduced as one of the factors of children and adolescents' tendency toward crimes [12]. In another study in Canada, the disintegration of family relationships among immigrant adolescents had resulted in the challenge of identity and sense of belonging, as well as increasing their tendency to high-risk criminal groups [8].

In the present study, adolescent girls tried to maintain their acceptability among their peers, with a kind of sense of belonging to them. One of the ways of obtaining and maintaining this acceptability is to make friends with the opposite sex, to which they sometimes resort for fear of being labelled and notoriety among their peers, and sometimes for obtaining sexual information. The family's negligence and inability on the one hand, and the adolescent's intense desire for independence and breaking the family's taboos on the other hand, cause the adolescent to unpreparedly enter complex and sometimes sexual relationships with opposite-sex peers, and resign themselves to any relationships for fear of being rejected by their peers. In this study Fear of the label of ugliness, as a common stigma among peers can easily lead girls towards peers' acceptable identity. Being accepted by peers is one of the adolescents' primary goals, especially at high school ages, which is sometimes more important for adolescents even than their academic goals [7][13]. In the study in Iran the need for belonging attracted adolescents to peer groups [14]. Although the primary focus of peer groups in different studies is on the risks of these relationships, socialization by peers is not necessarily harmful. Peers can help the adolescent in altruistic behaviours, cooperation and participation,

and achieving academic goals [15]. In a study titled " Peer group membership and a sense of belonging", girls had a greater sense of belonging to the peer group than boys did [16]. Also, friends' role is effective both in choosing the goals and in influencing the adolescent to achieve them [17]. The results of a review study showed that peer education plays an important role in improving adolescents' socio-mental health [18].

The findings of this study also showed that part of the adolescent's sense of belonging was defined as their social acceptability in society, which had led adolescent girls to feel the existence of gender differences among them. In fact, different parental strategies to differ between girls and boys transmit this feeling into society. Most studies refer to the key role of parents in gender socialization. Also, intensification occurs in gender socialization during adolescence, when parents have traditional attitudes to gender. In a study on Spanish-speaking families living in the United States, it was found out that most parents brought up their daughters by traditional roles which were expected by society and mothers encouraged their daughters to gain girls' gender roles more than their fathers did [19]. The socialization process begins at birth. Families behave differently towards their infants depending on their gender [20]. In a study titled "Differences between men and women in gender socialization among Indian youth and its relationship with mental health", it was found that in families where men were preferred to women, the women of those families had more psychological problems than men did. Also, in that study, women expressed that they participated less in decision-making in everyday life, and had more limitations to their independence than men did [21]. In another study, the stress due to gender stereotypes had led to anxiety and depression symptoms among girls [22].

In conclusion, correct parental strategies for the institutionalization of gender roles and Proper management of girls' interactions by the family, school, and society can play an important role in the development of a sense of belonging among adolescent girls in a way that concerns about differences in gender socialization turn into the opportunity to benefit from girls and women's abilities in society.

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# Epidemiology of Cesarean Delivery in Qassim, Saudi Arabia

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**Keywords:** Cesarean delivery; KSA; Obesity; Age; Parity

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**Abstract**

**BACKGROUND:** There is a global increase in the rate of cesarean deliveries, with the higher morbidity and mortality. Few published data on cesarean delivery exist in Qassim, Kingdom Saudi Arabia (KSA).

**OBJECTIVES:** To investigate the incidence, type, indications, maternal and perinatal outcomes of cesarean delivery.

**METHODS:** A retrospective study was conducted during three months (August-October 2016) at Maternity and Children's Hospital (MCH), Buraidah, Qassim, KSA. The medical files of parturient women during the period were revised and the data extracted through questionnaires.

**RESULTS:** There were 936 deliveries during the study period. The mean (SD) of their age, parity and gestational age were 28.6 (6.3) years, 3.0 (2.1) and 38.8 (1.6) weeks, respectively. Out of these 936 deliveries, 396 (42.3%), 21 (2.2%), 114 (12.2%), 405 (43.3%) were vaginal, instrumental, elective and emergency cesarean deliveries, respectively. The indications of the cesarean delivery were; repeated cesarean deliveries (201, 21.5%), failure to progress (87, 9.3%), fetal distress (72, 7.7%); breach (60, 6.4%), antepartum hemorrhage (54, 5.8%), hypertension (36, 3.8%) and diabetes mellitus (9, 1.0%) and more than one indication (6; 0.6%). In binary regression, while age, parity, birth weight and newborn gender were not associated with cesarean delivery, education ≤ secondary level (OR = 2.40, 95% CI = 1.59-3.61, P < 0.001), obesity (OR = 2.30, 95% CI = 1.51-3.48, P < 0.001 and morbid obesity (OR = 3.48, 95% CI = 2.16-5.60, P < 0.001) were associated with cesarean delivery. Nine (2.2%) vs three (0.6%), P = 0.03 women in the group of the cesarean and vaginal delivery respectively developed endometritis. Apgar score at one minute was significantly lower in newborn delivered by cesarean. There were three stillbirths (all of them were delivered by emergency cesarean), P = 0.120. Fifty-four of the newborn was admitted to the nursery; 39 (7.5%) vs.15 (3.6%) were delivery by cesareans vs vaginal delivery; P = 0.010.

**CONCLUSION:** There is a high incidence of cesarean delivery in this hospital; most of them were due to repeated cesarean delivery. Obese women were at higher risk of cesarean delivery.

## Introduction

There is an alarming increase in the rate of cesarean delivery worldwide [1]. World Health Organization (WHO) recommends a cesarean delivery rate of 10-15% [2] [3]. There is a great variation in the prevalence of cesarean delivery where a low rate of a cesarean delivery observed in some circumstances in countries with low resources indicate an unmet need for this procedure while the high rate in others countries might reflect unnecessary intervention. The rise in cesarean delivery rates is a major public health problem worldwide because of an increase in the maternal and the perinatal adverse effects [2] [4] [5] [6].

In spite of the safety of cesarean delivery that has dramatically improved over time with recent advances in medicine, there are still risks associated with this operation, e.g. hemorrhage, visceral injury, thromboembolism, infections, and risks to subsequent pregnancies, including miscarriage, antepartum hemorrhage, uterine rupture, preterm labor and neonatal mortality [7] [8] [9]. Furthermore, a growing based-evidence documenting recent complications that were not included in the previous traditional reports, e.g. cesarean delivery is associated with offspring obesity, allergy, metabolic disturbance and

even cerebral palsy for which cesarean was previously advocated was not reduced but even increased by cesareans [10] [11] [12].

Previous reports from the different regions of the kingdom of Saudi Arabia (KSA) showed an increase in the overall cesarean delivery rate by 80.2% from 10.6% in 1997 to 19.1% in 2006 [13]. Likewise, Bondok et al., [14] observed that the cesareans delivery rate exceeded the acceptable (15%) rate recommended by the WHO at King Fahd Armed Forces Hospital, Jeddah, KSA [3].

Research on the incidence, indications, maternal and perinatal outcomes of cesareans delivery is of paramount for practising clinicians and health planners. The current study was conducted to investigate the epidemiology of cesarean delivery (incidence, types, indications, predictors, maternal and perinatal complications of Cesarean delivery) at Maternity and Children's Hospital (MCH), Buraidah, Qassim, KSA.

## Methods

A retrospective study was conducted during three months (August-October 2016) at MCH, Buraidah, Qassim, KSA. The MCH is the main tertiary hospital in the region. The hospital accepts the referred cases from the other hospitals in the area as well as the women who have antenatal visits in the hospital. The medical files of parturient women during the period were revised and the data extracted through questionnaires. Twins deliveries were excluded. The data were; age, parity, gestational age, educational levels, Job and body mass index (BMI) was computed from the weight and height and expressed as weight/ square height in meter, Table 1. Then the mode of the delivery was recorded as well as gender, birth weight and Apgar score.

**Table 1: Definition and categories of variables used in the analysis of the caesarean delivery**

| Variable | Definition and categories |
|---|---|
| Maternal age in years | Original variable presents the mother's age as continues variable. However, in the analysis mother's age was entered as it is and it was not significant. If it was significant, we planned to a categorical variable. |
| Parity | The original variable was continuous, and it was not significant in the analyses. If it was so we planned to group it into three categories; primiparae, porous (2 - 5 children) and multiparae > 5. |
| Maternal education | Variable was constructed from two variables; 1st is ever attended school. Women who reported that they attended secondary or university levels were grouped. Therefore categories were; illiterate/intermediate or less (≤8 years) and secondary or above (>8 years. |
| Body mass index | Was calculated from the weight/ (height, m) $^2$ and entered as a continuous variable which was significant. Then it was categorised as underweight (<18.5 kg/m$^2$, normal weight, (18.5-24.9 kg/m2), overweight (25-29.9 kg/m2) obese (≥30 kg/m2) and morbidly obese. |
| Child sex | Male /female |
| Gestational age | It is the pregnancy duration in weeks. |
| Birth weight | It the birth weight in g. |
| Stillbirth | It the delivery of a dead infant after 24 weeks of gestation. |

A sample size of 936 women was calculated guided by the previous rate of cesarean delivery in the area [13]. This sample size has over 80% power to detect a difference of 5% at $\alpha = 0.05$. We assumed that 10% of the women might not respond or have incomplete data.

### *Statistics*

The data were entered in the computer using SPSS software for Windows version 20.0(SPSS, Inc, Chicago, IL). The mean (SD) and the proportions of the maternal and perinatal characteristics were compared between the cases with cesarean and vaginal delivery using Student t- test, $X^2$(and Fisher exact tests), respectively. The binary regression analysis was performed where cesarean delivery was the dependent variable, and independent variables were age, parity, education, antenatal care, residence, body mass index, haemoglobin and fetal gender. The corrected Odd ratios and 95% confidence interval were calculated. P value < 0.05 was considered statistically significant.

The work received ethical clearance from the Research Board at Ministry of Health, Qassim, KSA.

## Results

There were 936 deliveries during the study period. The mean (SD) of their age, parity and gestational age were 28.6(6.3) years, 3.0 (2.1) and 38.8 (1.6) weeks, respectively.

Around three-quarter (777; 83.0%) of these women had secondary education and three-quarter (711; 76.0%) were housewives.

There were 9 (1.0%), 159 (17.0%), 345 (36.9%), 216 (23.1%) and 207 (22.1%) women who were underweight, normal weight, overweight, obese and morbidly obese, respectively.

Out of these 936 deliveries, 396 (42.3%), 21 (2.2%), 114 (12.2%), 405 (43.3%) were vaginal, instrumental, elective and emergency cesarean deliveries, respectively. The incidence of the cesarean delivery (both elective and emergency) was 55.4%.

The indications of the cesarean delivery were; repeated cesarean deliveries (201, 21.5%), failure to progress (87, 9.3%), fetal distress (72, 7.7%); breach (60, 6.4%), antepartum hemorrhage (54, 5.8%), hypertension (36, 3.8%) and diabetes mellitus (9, 1.0%) and more than one indication (6; 0.6%), Figure 1.

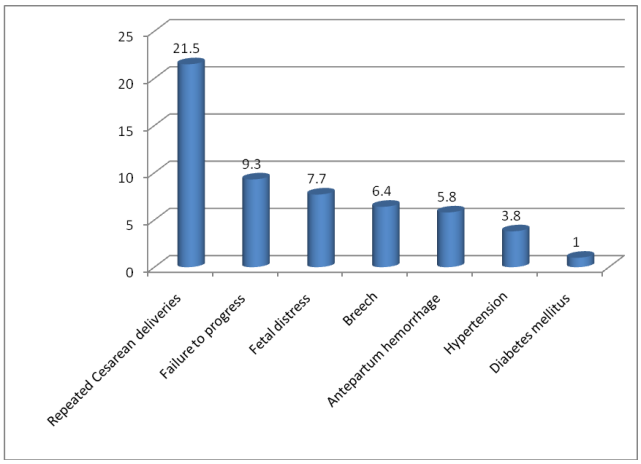


*Figure 1: Indications of Cesarean delivery at Maternity and Children Hospital, Qassim, Kingdom of Saudi Arabia*

While there was no significant difference in the mean (SD) of the parity and haemoglobin level in the women who delivered vaginally and women who delivered by cesarean, women who delivered by cesarean were elder and had significantly higher BMI, Table 2.

There was no significant difference in the job and gender of the newborn between women who delivered vaginally and those who delivered by cesareans. Significantly a higher number of women with education≤secondary level delivered by cesarean, Table 2.

**Table 2: The mean (SD) of the maternal variables in women who delivered vaginally and women who delivered Cesarean at Qassim, Kingdom of Saudi Arabia**

| Variable | Vaginal delivery (n = 417) | Cesarean delivery (n = 519) | P |
|---|---|---|---|
| Mean (SD) of | | | |
| Age, years | 28.1(5.7) | 29.1(6.7) | 0.016 |
| Parity | 2.9(1.8) | 3.1 (2.3) | 0. 159 |
| Gestational age, weeks | 38.9 (1.7) | 38.8(1.5) | 0. 641 |
| Body mass index, kg/cm$^2$ | 29.0 (5.4) | 31.0 (6.1) | < 0.001 |
| Hemoglobin, g/d | 10.7(1.3) | 10.7(1.2) | 0.869 |
| Frequency (%) of | | | |
| Housewives | 309 (74.1) | 402 (77.5) | 0.249 |
| Education≤secondary level | 324 (77.7) | 453 (87.3) | < 0.001 |
| Male gender | 202 (48.4) | 265(51.0) | 0.379 |

There was no maternal death. Nine (2.2%) vs three (0.6%), P = 0.03 women in the group of the cesarean and vaginal delivery respectively developed endometritis. While Apgar scores at one minute were significantly lower in newborn delivered by cesarean, the mean (SD) of the birth weight and Apgar scores at five minutes were not different between the two groups, Table 3.

**Table 3: Comparing birth weight and APGAR scores between neonates delivered vaginally and by Cesarean at Qassim, Kingdom of Saudi Arabia**

| Variable | Vaginal delivery (n = 417) | Cesarean delivery (n = 519) | P |
|---|---|---|---|
| Birth weight, g | 3176.7 (487.1) | 3189.2 (521.4) | 0.706 |
| APGAR score at one minute | 7.5 (1.0) | 7.2 (1.3) | < 0. 001 |
| APGAR score at five minutes | 8.0 (0.9) | 7.9 (0.9) | 0. 503 |

In binary regression, while age, parity, birth weight and newborn gender were no associated with cesarean delivery, education ≤ secondary level (OR = 2.40, 95% CI = 1.59-3.61, P < 0.001), obesity (OR = 2.30, 95% CI = 1.51-3.48, P < 0.001 and morbid obesity (OR = 3.48, 95% CI = 2.16-5.60, P < 0.001were associated with cesarean delivery, Table 4.

**Table 4: Binary regression analyses for factors associated with cesarean delivery in Qassim, KSA**

| Variable | OR | 95% CI | P |
|---|---|---|---|
| Age, years | 1.02 | 0.99 –1.05 | 0.149 |
| Parity | 1.03 | 0.94–1.13 | 0. 493 |
| Housewives | 1.01 | 0.71–1.41 | 0.964 |
| Education≤ secondary level | 2.40 | 1.59–3.61 | <0.001 |
| Body mass index, kg/cm$^2$ | | | |
| Normal weight | Reference | Reference | |
| Overweight | 1.38 | 0.87–2.19 | 0.163 |
| Obese | 2.30 | 1.51–3.48 | <0.001 |
| Morbidly obese | 3.48 | 2.16–5.60 | <0.001 |
| Hemoglobin, g/d | 1.01 | 0.91–1.13 | 0.763 |
| Male gender | 0.99 | 0.73–1.33 | 0.961 |
| Birth weight | 1.00 | 0.99–1.00 | 0.041 |

There were three stillbirths (all of them were delivered by emergency Cesarean), P = 0.120. Fifty-four of the newborn was admitted to the nursery; 39 (7.5%) versus 15 (3.6%) were delivery by cesareans vs vaginal delivery; P = 0.010.

## Discussion

The main findings of the current study were; there was a high rate of cesarean delivery (55.4 %.), and obese women were at higher risk to deliver by cesarean. This goes with the previous report which showed an increase of in the rate of cesarean delivery in the different region of KSA. Both vaginal breech and operative vaginal deliveries showed a significant decrease of 38% and 29%, respectively [13]. In a retrospective analysis, the cesareans delivery rate exceeded the acceptable 15% rate suggested by the World Health Organization (WHO) [3] at King Fahd Armed Forces Hospital, Jeddah, KSA [14]. In contrast, a lower rate (12%) cesarean delivery has been estimated in developing countries based on nationally representative data from 82 countries [6]. Recent reports from sub-Saharan Africa showed that out of 1276 women underwent a cesarean delivery, the most common indications were obstructed labor (399, 31%), poor presentation (233, 18%), previous cesarean delivery (184, 14%), and fetal distress (128, 10%), uterine rupture (117, 9%) and antepartum hemorrhage (101, 8%) [15]. Likewise, it has been shown that previous cesarean, a referral from another facility and suspected cephalopelvic-disproportion, vaginal bleeding near full term, hypertensive disorders, previous cesarean delivery and premature rupture of membranes were the main indications of cesarean delivery [16]. It has recently been shown that repeated previous cesarean, failure-to-progress,

breech presentation and hypertensive disorder, were the main indications of cesarean delivery in Khartoum Sudan [17]. On the other hand, many African couturiers have a low rate of cesarean delivery, e.g. the rate of cesarean section was 4.5% in Congo, and it was between 0.1% and 1% in Kenya, Rwanda, Southern Sudan, and Uganda [18] [19]. Many explanations could behind the low rate of cesarean deliveries in African countries such as hospital/institution distance where the lowest cesarean delivery rates were found in the more remote part of the hospital catchment area in Rwanda [20]. However recent reports showed that one third and two-fifths of the deliveries in Yemen and Sudan respectively were cesareans [17] [21].

The high rate of cesarean delivery in this hospital could be explained by the low rate of instrumental delivery as well as because the hospital is tertiary referral hospital receiving the high-risk patients. It has been mentioned that in Jordan there was rising rate of cesarean sections and a significant decrease in the use of both the vacuum extractor and the forceps after the new millennium during the 15-year period [22].

Repeated cesarean deliveries, failure to progress, fetal distress and breech were the main indication of cesarean delivery in the current study. Previous reports showed that fetal distress, previous cesareans and breech presentation were the most common indications for cesarean delivery in a tertiary level hospital in Jeddah, KSA [14]. Kamil et al. observed that fetal distress was the commonest indication for cesareans at Women Specialized Hospital Riyadh, King Fahd Medical City, KSA [23].

Maternal age and parity were the most associated factors for cesareans, and fetal distress was the commonest indication for cesareans at Women Specialized Hospital Riyadh, King Fahd Medical City, Kingdom of Saudi Arabia [23]. In the current study; repeated cesarean delivery, failure to progress, fetal distress; breech, antepartum haemorrhage, hypertension and diabetes mellitus were the main indications of cesarean delivery. It has recently been shown that 44.2% of cesarean deliveries were emergency ones and repeated previous cesarean, failure-to-progress, breech presentation and hypertensive disorder, were the main indications of cesarean delivery in Khartoum Sudan [17].

Likewise in, the vast majority (three-quarters) of the Cesareans were emergencies, and maternal indications accounted for two-third of the cesareans [24]. The current study showed that body mass index was significantly higher in women delivered by cesareans. Previous recent reports showed that obese women were at four times at higher risk to deliver by cesarean delivery [17]. The previous study showed that maternal age and parity were the most associated factors for cesareans and fetal distress was the commonest indication for Cesareans at Women Specialized Hospital Riyadh, King Fahd Medical City, Kingdom of Saudi Arabia [23].

In the current study, there was no difference in the gestational age, Apgar scores and there was no maternal death. This goes with the previous reports where Gasim et al., 2013 reported no maternal death in their recent study of complicated cesareans. They reported that the number of preterm birth and low Apgar scores<7 at 5 minutes were significantly higher in complicated Cesareans [25].

In conclusion, there is a high incidence of cesarean delivery in this hospital; most of them were due to repeated cesarean delivery. Obese women were at higher risk of cesarean delivery.

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# Evaluation of the Directly Observed Treatment's Acceptance by Tuberculosis Patients in the Republic of Macedonia

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**Keywords:** directly observed therapy; home visits; treatment outcome

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**Abstract**

**BACKGROUND:** Directly observed therapy (DOT) is a specific strategy endorsed by the World Health Organization in 1994 to improve adherence by requiring health workers, community volunteers or family members to observe and record tuberculosis (TB) patients for taking each dose. The implementation of DOT strategy in the National Tuberculosis Programme (NTP) in Macedonia was introduced in 2006 and was realised by 6 nurses engaged through the Project of Global Fund against HIV/AID and TB.

**AIM:** This study aimed to evaluate how these visits conducted by the DOT nurses engaged through the GF were accepted by TB patients and to evaluate the impact on the treatment outcomes.

**PATIENTS AND METHODS:** In this prospective study 105 TB patients who started treatment in 2016 and who were supposed to be visited by DOT nurses at their homes were included. All of these patients filled out a questionnaire compiled of 6 questions related to their opinion about the efficacy and usefulness of the visits managed by the nurses when they would come for check-ups at the Institute for Lung Diseases and Tuberculosis. The assessment of the efficacy of the work of DOT nurses was performed by analysis of the answers received by TB patients. The data were analysed with the method of description and was statistically prepared in the program SPSS for Windows, 17.0.

**RESULTS:** The results from the analyses showed that the number of visited patients were not satisfied, because 29.52%% were never visited by a DOT nurse at their homes. A lot of patients (61.91%) were not willing to take medicines under the observed control by nurses. Those TB patients who were visited by DOT nurses thought that this type of visit is useful for them: they were satisfied by the attitude of the nurses during the visits, and they received enough explanations regarding TB.

**CONCLUSION:** We can conclude that the DOT visits to TB patients are useful, contribute patients to complete the therapeutic regime without interruption, take care for every individual patient effectively, and protect the rest of society by preventing the development and spread of TB, including drug-resistant strains.

## Introduction

Despite the widespread availability of cheap, effective treatment, tuberculosis (TB) remains a major cause of severe illness and death, with an estimated nine million new cases and two million deaths occurring annually [1]. One barrier to global TB control is the long duration of TB treatment- a minimum of six months- which frequently results in patients taking their medications erratically or not at all [2]. Interruption of the treatment can lead to relapse and even death in individuals, and also has important public health consequences, such as increased TB transmission and development of drug resistance [3]. Directly observing TB patients taking their anti-TB therapy, either daily or several times per week, was first piloted in the 1950s as a way to ensure adherence and treatment completion [2] In 1994, based on the reported success of directly observed therapy (DOT) in increasing treatment completion rates and preventing drug resistance, the World Health Organization (WHO) adopted DOT as a principal component of its global TB control strategy named as DOTS - Directly Observed Treatment Short-course strategy [4].

DOT is a specific strategy endorsed to improve adherence by requiring health workers, community volunteers or family members to observe and record patients taking each dose. Cure rates in DOTS programs have been reported to be as high as

95%, and the World Bank considers the DOTS strategy to be one of today's most cost-efficient health interventions [5].

Although WHO and other international agencies strongly advocate DOT, controversy remains whether its benefits have been proven. Randomized controlled trials (RCT) have shown either modest or no benefit of DOT in improving TB treatment success rates, and a meta-analysis of 10 RCTs concluded that the evidence base for WHO's DOT policy is insufficient. A recent Cochrane review reports no benefit from DOT on adherence and cure rates when compared with self-administered treatment (SAT) regimens [6]. The effectiveness of DOT, it has been argued, should be judged by how well it prevents drug resistance, specifically to rifampicin, and not by improvements in treatment success rates [7].

The implementation of DOT strategy in Macedonia started in 2004, but all principles and activities in the National Tuberculosis Programme (NTP) were introduced in 2006. In the period from 2006-2016 when the Project of Global Fund against HIV/AID and TB (GF) was presented in Macedonia with significant financial support to our NTP, DOT activities were realised in a big proportion from 6 nurses engaged through the GF. A small part of DOT activities was controlled by the nurses from public health institutions. The role of the DOT nurses was to visit TB patients at their homes especially during the continuous phase of treatment regime few times per week with the aim to observe the intake of medicines, to educate TB patients about diseases and to provide social or some other kind of support where it was needed. DOT nurses from the GF covered TB patients from the four biggest cities in the country.

The objective of this study was to evaluate how these visits conducted by DOT nurses engaged through the GF were accepted by TB patients and to evaluate the impact over treatment outcomes.

## Patients and Methods

In this prospective study, 105 patients were included who were considered to be eligible due to confirmed TB disease and who started treatment in 2016. Out of the total number of TB patients who were supposed to be visited by DOT nurses at their homes, this group of 105 TB patients was randomly selected. The general information regarding TB patients was provided from the evidence of DOT nurses engaged through the GF. The data regarding the disease of the patients like treatment outcomes were obtained from the electronic base of the Central Register for TB patients from the Institute for Lung Diseases and Tuberculosis.

All of these patients received a questionnaire comprised of 6 questions prepared by the doctors from the Institute for Lung Diseases and Tuberculosis. The questions were related to the opinion of TB patients for the satisfaction and usefulness of the visits managed by the nurses.

The patients filled out the questionnaires when they would come for check-ups at the Institute for Lung Diseases and Tuberculosis. This activity was conducted and controlled by 3 nurses from the Institute, who was not part of the DOT activity organised by the GF. So we conducted the assessment of the nurses' engagement in DOT through the questionnaire filled out by TB patients.

### *Statistical analyses*

The data were analysed with the method of description and was statistically prepared in the program SPSS for Windows, 17.0.

## Results

In the next text, we present the analyses of the patient' s answers to all 6 questions from the questionnaire separately:

### *Question No1. How many times per month were you visited by DOT nurses?*

According to the results, one third 31(29.52%) was never visited by DOT nurses. 74 patients (70.47%) were visited during their TB treatment: 32 (30.48%) were visited less than 3 times per month, the same were visited between 3 and 9 times, and 10 patients were visited more than 10 times per month (9.52%) (Table 1).

**Table 1: Number of DOT visits per month to TB patients**

| How many times per month were you visited by DOT nurses? | n (%) |
|---|---|
| Never | 31 (29.52) |
| <3 times | 32 (30.48) |
| 3 – 9 times | 32 (30.48) |
| >10 times | 10 (9.52) |
| Total | 105 (100.0) |

### *Question No 2. What was the attitude like of the DOT nurses towards you during the visits?*

Nearly all TB patients (73-69.52 %) who were visited by a DOT nurse answered that the DOT nurse had a good positive attitude towards them. Only one patient was dissatisfied from the nurse' s attitude (Table 2).

**Table 2: The attitude of DOT nurses towards TB patients during the visits**

| What was the attitude like of the DOT nurses towards you during the visits? | n (%) |
|---|---|
| Not visited | 31 (29.52) |
| Good | 73 (69.52) |
| Bad | 1 (0.95) |
| Total | 105 (100.0) |

### *Question No 3. Do you find the DOT nurses' visits useful for you?*

Regarding the usefulness of DOT nurses' visits except for 2 patients, the others thought that that kind of visits was very useful for them and their disease (Table 3).

**Table 3: Assessment of the usefulness of DOT nurses' visits**

| Do you find the DOT nurses' visits useful for you? | n (%) |
|---|---|
| Not visited | 31 (29.52) |
| Yes | 72 (68.57) |
| No | 2 (1.9) |
| Total | 105 (100.0) |

### *Question No 4. Were you satisfied with the DOT nurses' answers to your questions regarding your* **disease?**

As for the question about the TB patients' satisfaction and the answers received by DOT nurses, 73(69.52%) answered positively with "yes (Table 4). Only 1 patient was dissatisfied with the answer to the questions regarding the disease.

**Table 4: Satisfaction of TB patients to the answers regarding their disease received by a DOT nurse**

| Were you satisfied with the DOT nurses' answers to your questions regarding your disease? | n (%) |
|---|---|
| Not visited | 31 (29.52) |
| Yes | 73 (69.52) |
| No | 1 (0.95) |
| Total | 105 (100.0) |

### *Question No 5: Did you take the medicines under the nurses' control?*

The results showed that most of the visited patients (65 - 61.91%) did not take the medicines under the nurses' control. It was odd that only 9 patients (8.57%) took medicines during the visits of DOT nurses (Table 5).

**Table 5: Were the TB patients observed when they took the medicines?**

| Did you take the medicines under the nurses' control? | n (%) |
|---|---|
| Not visited | 31 (29.52) |
| Yes | 9 (8.57) |
| No | 65 (61.91) |
| Total | 105 (100.0) |

### *Question 6: Treatment outcomes of TB patients*

At the time when we finished this study, all of the TB patients who started treatment in 2016 and were included in the study, did not finish their therapeutic regime. So, the treatment outcomes of these 105 TB patients were: 36 or 34.29% still on treatment, treatment completed 64 or 60.95% and those who died 5 or 4.76% (Table 6). If we exclude those 36 TB patients who were still on treatment, the number of patients that remained for assessment regarding the known treatment outcomes was 69.

**Table 6: Treatment outcomes of TB patients**

| **Treatment outcomes** | **n (%)** |
|---|---|
| Treatment completed | 64 (60.95) |
| Died | 5 (4.76) |
| Still on treatment | 36 (34.29) |
| Total | 105 (100.0) |

We performed statistical analyses by applying the Fisher exact test to see if there is a correlation between the number of visits to the patient's home by the DOT nurses and the treatment outcomes. The results showed that there were statistically significant differences between the number of visits and treatment outcomes ($p < 0.008$). Most of TB patients who completed the treatment (24-37.5%) were visited 3-9 times per month, followed by the group of 21 patients (32.81%) that were visited less than 3 times per month. We have to note that among patients never visited at their homes; there were 12 (18.75%) who completed treatment. In regard to the patients who have died, we have information from the medical documentation that those were older patients with comorbidities: these types of patients should be visited more times at their homes, not less than 3 according to the results.

What is characteristic among the active TB patients who are still in treatment, half of them (50%) have not been visited by DOT nurses not even once.

**Table 7: Correlation between the number of visits by the DOT nurses and treatment outcomes**

| The number of visits by the DOT | Treatment outcomes | | |
|---|---|---|---|
| | Completed | Died | Still on treatment |
| Never | 12 (18.75) | 1 (20) | 18 (50) |
| <3 times | 21 (32.81) | 4 (80) | 7 (19.44) |
| 3 – 9 times | 24 (37.5) | 0 | 8 (22.22) |
| >10 times | 7 (10.94) | 0 | 3 (8.33) |
| | Fisher exact $p < 0.008$ | | |

## Discussion

TB incidence in the Republic of Macedonia has declined during the last ten years and from 27.8/100 000 inhabitants in 2007, dropped to 12.9/100 000 inhabitants in 2016 (absolute number of 267 new TB cases in 2016). In this period the treatment success rate was between 86.3% and 91.3% of all registered cases. Regarding multidrug-resistance TB (MDR-TB), there are only 2-4 new cases per year (2 cases in 2016).

These positive results are due to all activities performed through our NTP in which DOT presents an important portion (part). DOT is designed to promote

proper adherence to the full course of drug therapy to improve patient outcomes and prevent the development of drug resistance.

The results from the analyses showed that the number of visited patients was not satisfactory, because 29.52%% were never visited by a DOT nurse at their home at all. The explanation for the reason why DOT nurses did not visit these TB patients was that some of these patients had support from other family member or some patients refused to be visited without reason or because of stigma. It is correct that DOT nurses should assess that of the patients should be visited less than it was recommended, especially in the case when the patients are aware and educated about their disease well enough. We know that stigma in our country is a big problem among TB patients and sometimes can be a barrier for diagnosing TB in time, starting with treatment and curing the disease.

But in those TB patients who were visited by DOT nurses, nearly all cases thought that this type of visit is useful for them: they are satisfied by the attitude of the nurses during the visits, and they received enough explanations regarding TB.

A lot of patients (61.91%) were not willing to take medicines under the observed control by nurses. Only 8.57% of patients answered that they accepted to take medicine when the nurses had visited their homes.

Regarding the treatment outcomes, we found statistically significant differences between the number of visits and treatment outcomes: nearly half of the patients who completed treatment (48.44%) were visited more than 3 times per month. TB patients who died during the treatment were visited less than 3 times per month because the time of death had happened at the beginning of the treatment regime. However, the analyses showed that there was good compliance by the patients, good adherence to the treatment, good cooperation between patients and nurses which led to the completion of the treatment and cure without any patients who interrupted the treatment. Also, to improve patients' adherence to the treatment, packages with food and hygiene products were provided as an incentive by the GF in the RM. In this study, we did not conduct the analysis how DOT strategy influenced to the country economic burden.

The rates of success of different DOT programs reported in the literature shows great variability.

Six trials from South Africa, Thailand, Taiwan, Pakistan and Australia compared DOT with self-administered therapy for treatment. Trials included DOT at home by family members, community health workers (who were usually supervised); DOT at home by health staff; and DOT at health facilities. TB cure was low with self-administration across all studies (range 41% to 67%), and direct observation did not substantially improve this (RR 1.08, 95% CI 0.91 to 1.27; five trials, 1645 participants). Treatment completion showed a similar pattern, ranging from 59% to 78% in the self-treatment groups, and direct observation did not improve this (RR 1.07, 95% CI 0.96 to 1.19; six trials, 1839 participants [8].

A meta-analysis on the efficacy of DOT, which included 32 studies, reported that only 11 of those were prospective, and of these only two were randomised clinical trials. The majority of the TB programs included in that meta-analysis incorporated other components besides the five key elements of the WHO-recommended DOTS strategy, with great heterogeneity within programs [9]. These other components included incentives, facilitators, follow-up of defaulters, legal sanctions, and patient centred-care and health personnel motivation programs [10]. The potential confounding effect of such co-interventions was evident. In general, DOT completion rates are significantly higher for those receiving DOT plus an incentive than for those receiving DOT without incentives [11]. To focus exclusively on supervised swallowing of the pills must be considered as an extremely simplistic approach to a very complex problem. Patient adherence is multifaceted and is affected by several factors that range from the individual's characteristics to the qualities of the social environment [12]. To attain acceptable rates of treatment adherence might require additional social services and economic incentives [9] [13].

The DOTS strategy imposes an additional economic burden on the health system of a country, and also on the patients treated under this regimen due to the need for the system to provide the necessary infrastructure and personnel for the supervision of treatment and the time and costs involved in the requirement for the patient to travel to the health center every day or three times a week. Moreover, the DOT component can potentially intensify the stigma associated with TB and diminish a patient's ability to maintain privacy about their health [14].

Although some TB treatment programs have health workers who conduct DOT in patients' homes rather than health facilities, no official numbers exist on how many programs administer TB treatment through this method [15].

Nonetheless, between 1995 and 2012, an estimated 56 million individuals were successfully treated for tuberculosis under the DOTS/Stop TB strategy in 184 countries [15]. Worldwide, approximately 86 % of TB patients complete treatment; however, treatment success and cure rates vary widely by geographic region and per capita income [16]. DOT is only one part of the comprehensive case management of each patient with TB. Rigorous monitoring of all patients who have started treatment and rapid response to ensure that patients who interrupt their treatment are returned to care are also essential components of effective case

management and community-wide TB control.

On the basis of the results from our study we can conclude that DOT visits to TB patients are useful, contribute patients to complete therapeutic regime without interruption, take care for every individual patient effectively, and protect the rest of society by preventing the development and spread of TB, including drug-resistant strains. NTP in Macedonia and public health policy-makers should find the best way how to strengthen and continue with DOT in the future and assess which are the most important ingredients for success in our particular program. The prime responsibility of a TB control programme for patients and the community is to ensure cure while preventing drug resistance. DOT is the only current documented means to meet this commitment.

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# Antenatal Deworming and Materno-Perinatal Outcomes in Calabar, Nigeria

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**Abstract**

**BACKGROUND:** Studies have shown that administration of anthelmintic drugs in pregnancy can reduce the incidence of maternal anaemia; however, data on other maternal and perinatal outcomes are limited.

**AIM:** This study was therefore conducted to evaluate the direct impact of mass deworming on delivery and perinatal outcome.

**MATERIAL AND METHODS:** A total of 560 healthy pregnant women in their second trimester were randomised to receive a single dose of oral mebendazole (500 mg) and placebo. Each participant received the standard dose of iron supplement and malaria prophylaxis. They were followed up to delivery and immediate postpartum period to document the possible impact on maternal and perinatal outcomes.

**RESULTS:** The prevalence of anaemia at term, 37 weeks gestation and above, among the treatment arm was 12.6% compared with 29.9% in the placebo arm ($p < 0.001$). Caesarean section rates was higher in the treated group and the placebo ($p = 0.047$).There were no statistically significant differences in incidences of postpartum haemorrhage ($p = 0.119$), Puerperal, pyrexia ($p = 0.943$), low birth weight ($p = 0.556$) asphyxia ($p = 0.706$) and perinatal death ($p = 0.621$).

**CONCLUSION:** Presumptive deworming during the antenatal period can significantly reduce the incidence of peripartum anaemia. However, more studies may be needed to prove any positive perinatal outcome.

## Introduction

Intestinal parasitic infections are of public health importance because of likely morbidity related to iron deficiency anaemia due to chronic blood loss suffered by the hosts [1]. The burden is much for children and pregnant women because of the increased requirement for iron and other nutrients [2]. About two decades ago, a study in the general population in Calabar by Ejezie GC and Akpan IF [3] showed a high prevalence of intestinal nematodes of 28.5% and recently, 23.6% prevalence has been reported by Ozumba UC et al., [4] among pregnant women at a University Teaching Hospital, in Southern Nigeria. However, these studies did not assess the correlation with anaemia among the subjects which necessitates more prospective studies to determine the impact of this high helminths prevalence on the haematological status and pregnancy outcome in the pregnant populace.

It has been reported that majority of the pregnant women in the tropics have depleted or borderline iron stores due to menstrual blood loss and the demands of previous pregnancies, and only a few women in low-income countries begin pregnancy with sufficient iron stores [5] [6] [7] [8]. Combined with the increased iron demands in pregnancy due to the expansion in red cell mass and the requirement of the developing foetus, many women become iron deficient during childbearing [4]. The prevalence of iron deficiency anaemia in pregnancy is quoted at 56% in developing countries [7]. Hence, parasitic diseases particularly soil-transmitted intestinal helminthic infections are important public health problems confronting many women in the developing countries [6] [8].

Furthermore, it is estimated that anaemia may be responsible for as much as 20% of all maternal deaths in sub-Saharan Africa through three main mechanisms [9]: Firstly, anaemia makes women more susceptible to deaths from haemorrhage by lowering their haemoglobin reserves for blood loss especially at birth. Severe anaemia is associated with increased susceptibility to infection due to lowered resistance to disease, and haemoglobin level of less than 4 g/dl is associated with high-risk cardiac failure, particularly during delivery or in the immediate post-partum period, making the women likely to die if not properly managed [9].

Although it is widely believed that deworming can improve anaemic status in pregnant women [10] [11] little information is available about the degree to which anaemia improves after deworming in Calabar and improvement in the pregnancy outcome. Hence, it is necessary to conduct this randomised controlled study to suggest effective evidence-based methods of controlling anaemia and improving maternal health. And despite WHO recommendation of a single dose of Albendazole or Mebendazole treatment after the first trimester for infected pregnant women and mass policy (presumptive treatment) in areas where the infection is endemic (prevalence>20-30%) and where anaemia is highly prevalent, [12] it is yet to be included in routine antenatal care package in the University of Calabar Teaching Hospital, Calabar and many other tertiary health institutions in Nigeria. Low birth weight due to maternal chronic anaemia is a major factor influencing neonatal and infant survival. So treatment of helminthiasis in pregnancy may be beneficial to maintain appropriate fetal weight [12] [13]. Anthelminthics alone may halt the iron loss, or reduce the rate of loss, and the addition of iron supplements is likely to improve the haemoglobin or packed cell volume (PCV) levels.

This study was therefore conducted to evaluate the direct impact of mass deworming on delivery and perinatal outcome.

## Material and Methods

The research was conducted at the University of Calabar Teaching Hospital Antenatal Clinic from 1st January to 31st December 2015. The hospital is the only tertiary health facility in Calabar Metropolis, the capital of Cross River State which is located in South-South Zone of Nigeria. A review of antenatal records revealed that the hospital provides obstetrics services to between 1500 and 2200 pregnant women annually. The hospital practices routine antenatal care schedules, i.e. four weekly visits for the 1st 28 weeks after that 2 weekly till 36 weeks, then weekly till delivery. The study population comprised all consenting women attending antenatal care at the University of Calabar teaching hospital who meet the criteria during the study period.

Of the 560 participants in this study, 300 received mebendazole while 260 made up the placebo arm. A sample size of 300 was adopted for the treatment arm using Leslie Kish formulae. The actual calculated sample size was 280 but to cater for attrition or “drop out” 300 women were recruited into the treatment arm. The sample size was determined using 23.6% prevalence from a recent study among pregnant women with precision at 0.05 and 95% confidence interval.

Women who presented for antenatal care at the University of Calabar teaching hospital were recruited in their second trimester during the study period. The research and its purpose and expected benefits to the patients and the community were explained, and consent was obtained from the willing participants. To facilitate easy completion of questionnaires, semi-structured and closed-ended questions were used. Consenting women were assured of confidentiality. They were informed that no money would be charged for the test performed or for the anthelminthic/placebo drugs.

This was a randomised placebo-controlled study. The study consisted of two groups of pregnant women, the treatment and the placebo group. The study design was suitable for this particular research because the placebo group constituted the baseline of what can be assumed to be expected the outcome in normal circumstances without treatment. The participants were assessed for eligibility using the inclusion and exclusion criteria below. The women were then randomised to receive either a single 500 mg oral dose of Mebendazole plus a daily iron supplement, (60 mg elemental iron) and folic acid or a single dose placebo plus a daily iron supplement (60 mg elemental iron) and folic acid. The administration of the anthelmintics was done by directly observed therapy (DOT) to ensure 100% compliance. Computer-generated random numbers were used for sampling. Single tablet (500 mg) of mebendazole was wrapped in a paper and labelled with a number for identification from the placebo which was also wrapped with same paper to blind the patients and the dispenser. Presumptive treatment was adopted in this study due to ethical issues in leaving some women with proven infections from stool microscopy untreated. All the participants received intermittent preventive treatment for malaria (IPT) according to the National protocol. The baseline packed cell volume (PCV) was noted at recruitment. All the participants in both the treatment and the placebo groups were then followed up to term and delivery. The maternal and perinatal outcomes were documented. Outcomes of interest included the prevalence of peripartum anaemia (PCV < 33%), mode of delivery post-partum haemorrhage, and other maternal morbidity like puerperal pyrexia; perinatal outcome included the

proportion of low birth weight, birth asphyxia, congenital abnormally and perinatal mortality.

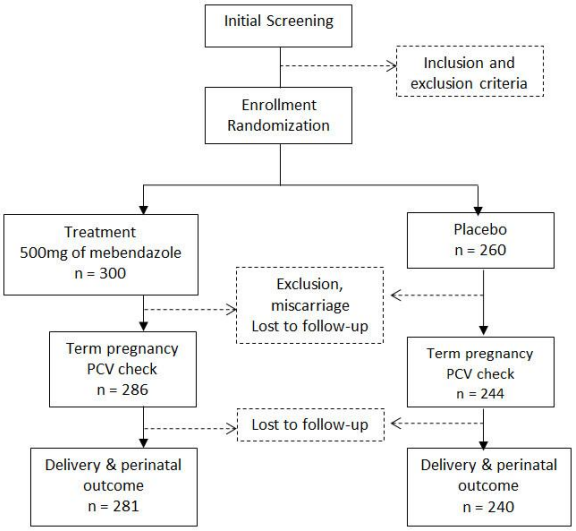


*Figure 1: Summary of the study design (Flow diagram)*

Data were collected using pre-tested interviewer-administered questionnaire which contains socio-demographic characteristics (age, educational qualification, occupation, the occupation of spouse marital status, place of resident) obstetric and gynaecological history; drug history (haematinics, antimalarial, anthelminthics) and previous drug reaction or allergy; past and present medical and surgical history.

Data were analysed using SPSS version 19. Description of means, frequency, proportions and rates of given data for each variable was calculated. Bivariate analysis was done to see the association of each independent variable with the outcome variable. A multivariate logistic regression model was used to identify the effect of each independent variable with the outcome variable. A p value of less than 0.05 was considered statistically significant.

***Inclusion criteria:*** 1) consenting women; 2) gestational age from 14 weeks to 28 weeks by LMP or ultrasound, and 3) singleton pregnancy.

***Exclusion criteria:*** 1) history of vaginal bleeding in current pregnancy; 2) medical, surgical or obstetric complication; 3) diagnosed or suspected multiple pregnancies; 4) patients with haemoglobinopathy; 5) women with moderate to severe anaemia; and 6) allergy to Mebendazole or sulphadoxine.

Formal approval was obtained from the research ethics committee of the University of Calabar Teaching Hospital before the research was commenced. Written informed consent was obtained from each study participant after they were introduced to the purpose of the study and informed about their right to interrupt the researcher or withdraw at any time. Women who were detected to be anaemic were given appropriate treatment. Confidentiality was maintained at all levels of the research.

The research assistants were trained in history taking, physical examination and data recording. They were trained on filling the questionnaire in a standard way. Haematological tests were done in the same laboratory. The laboratory internal and external quality control measures were adhered to. A haematologist reviewed the haemograms. There was a meticulous recording of laboratory investigation results. The same haematinic was given to all the participants. To avoid contamination of placebo group with the treatment arm, each participant's folder was identified with a number on a sticker which helped the investigator to differentiate them. This was also used to follow-up the women to term and delivery. The sticker was removed at the time of discharge from the hospital before the folder was sent to health record department for filing.

## Results

Out of the 300 women recruited into the treatment arm, 281 (93.7%) of them continued till delivery giving a drop-out rate of 6.33%. The mean age of respondents in both groups was similar; 29.3 ± 4.4 years for the study group and 29.7 ± 4.6 years for the control group (p = 0.062). Table 1 summarises the socio-demographic characteristics of respondents.

**Table 1: Socio-demographic characteristics of respondents by group**

| Variable | Study N = 286 Freq (%) | Control N = 244 Freq (%) | Total N = 530 Freq (%) | X2 | P-value |
|---|---|---|---|---|---|
| Age (years) | | | | | |
| < 20 | 4 (1.4) | 1 (0.9) | 5(0.9) | Fisher's Exact | 0.062 |
| 20-29 | 136 (47.6) | 118 (48.4) | 254 (47.9) | | |
| 30-39 | 146 (51.0) | 120 (49.2) | 266 (50.2) | | |
| ≥ 40 | 0 (0.0) | 5 (2.0) | 5 (0.9) | | |
| Mean ±SD | 29.3 ± 4.4 | 29.7 ± 4.6 | 29.5 ± 4.5 | | |
| Residence | | | | | |
| Urban | 276 (96.5) | 234 (95.9) | 510 (96.2) | | |
| Rural | 10 (3.4) | 20 (3.8) | 10 (4.1) | Fisher's Exact | 0.825 |
| Parity | | | | | |
| 0 | 96 (33.6) | 49 (20.1) | 145 (27.4) | | |
| 1-4 | 187 (65.4) | 187 (76.6) | 374 (70.6) | 14.269 | 0.001* |
| > 4 | 3 (1.0) | 8 (3.3) | 11 (2.1) | | |

A higher proportion of participants in the control group (29.9%) compared with the study group (12.6%) had anaemia at term. The PCV difference of respondents in the study and control groups was assessed. Participants with a positive difference were more (57.0%) in the Mebendazole group compared with (29.9%) in the placebo group. Those with either no difference or negative difference were more (22% and 61.1% respectively) in the control group compared with the study group (6.3% and 36.7%

respectively). These differences were statistically significant (p < 0.001). In the mebendazole group, 64.3% of the anaemic women had mild anaemia (Hb = 10 to 10.9 g/dl) while the remaining (35%) had moderate anaemia (7 to 9.9 g/dl). There was no incidence of severe anaemia in the treatment arm (Hb < 7.0 g/dl). One case of severe anaemia was noted in the placebo group while 79.2% and 19.4% of the reported anaemia in this group was mild and moderate respectively.

Table 2 shows multivariate logistic regression analysis of factors associated with anaemia among study respondents. Only parity (OR = 5.063, 95% CI = 1.531-16.743) was found to be an independent predictor of anaemia among the study group. Those with parity above 3 were significantly more likely to have anaemia at term.

**Table 2: Determinants of anaemia among respondents in the study group**

| Characteristic | Odds Ratio | 95% Confidence interval | P-value |
|---|---|---|---|
| Age | | | |
| ≤29 | 1.815 | 0.816-4.039 | 0.144 |
| >29 | 1 | | |
| Parity >3 | 5.063 | 1.531-16.743 | 0.008 |
| Yes | 1 | | |
| No | 5.063 | | |
| Social class | | | |
| 1 to 2 | | 0.324-1.485 | 0.346 |
| 3 to 5 | 1 | | |
| Gestational age at term | | | |
| <24 | 0.829 | 0.396-1.736 | 0.618 |
| >24 | 1 | | |
| Residence | | | |
| Urban | 1.091 | 0.128-9.291 | 0.937 |
| Rural | 1 | | |

Table 3 below shows the birth weight group of respondents' babies. Majority of respondents had babies with normal birth weight 455 (85.8%). This was slightly higher among study group 248 (86.7%) compared with control group 207 (84.4%). Overall, 40 (7.5%) had babies with higher than normal birth weight, 4 kg and above, slightly higher in the study group 23 (8.0%) compared with control group 17 (7.0%) while 20 (3.8%) had low birth weight babies, slightly lower in study group 9 (3.1%) compared with control group 11 (4.5%). These differences were not statistically significant (p = 0.556). The overall mean birth weight of babies was 3.23 ± 0.629 kg; 3.26 ± 0.582 kg in the study group and 3.19 ± 0.679

**Table 3: Birth weight group of respondents' babies by study arm**

| Variable<br>Birth weight group/Kg (%) | Study group | Control group | Total | $\chi 2$ | P-value |
|---|---|---|---|---|---|
| ELBW (<1.0) | 6 (2.1) | 9 (3.7) | 15 (2.8) | 2.079 | 0.556 |
| VLBW (1-1.4) | 0 (0.0) | 0 (0.0) | 0 (0.0) | | |
| LBW (1.5-2.4) | 9 (3.1) | 11 (4.5) | 0 (3.8) | | |
| Normal (2.5-3.9) | 248 (86.7) | 207 (84.8) | 455 (85.8) | | |
| Macrosomia (≥4) | 23 (8.0) | 17 (7.0) | 40 (7.5) | | |
| Mean birth weight ± SD | 3.23 ± 0.629 | 3.19 ± 0.679 | 3.26 ± 0.582 | | |

Most of the participants, 94.7% in the study group and 95.1% in the controlled group, did not experience postpartum haemorrhage (PPH) while 4.2% in the study group and 4.9% in the control group had PPH. The slight differences were not statistically significant (p = 0.119). Also, incidences of puerperal pyrexia were compared. Almost equal percentage in both study (94.8%) and control (94.3%) had no incidence of puerperal pyrexia, and almost equal proportion had pyrexia; 3.5% in the study group and 3.7% in control group. There was no statistically significant difference in pyrexia by study arm (p = 0.943).

The perinatal deaths were: 2.9% in (29 per 1000) study group and 3.3% (33 per 1000) in control group (Fig. 2).

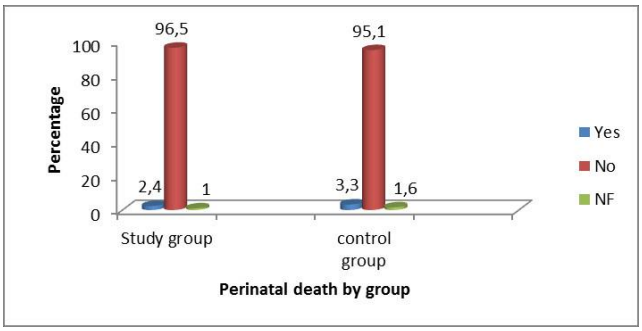


*Figure 2: Perinatal death by a group*

Only two babies, one from each group, were born with a gross congenital anomaly. Furthermore, the incidences of birth asphyxia were comparable. The study shows the percentage of participants whose babies had asphyxia (5.6% in the study group and 7.05% in control group) and had no asphyxia (93.4% in the study group and 95.1% in control group). The slight difference in this experience was not significant statistically (p = 0.706). Table 3 summarises proportion of normal birth weight (2.5-3.9 kg), low birth weight (LBW), very low birth weight (VLBW) and extremely low birth weight (ELBW).

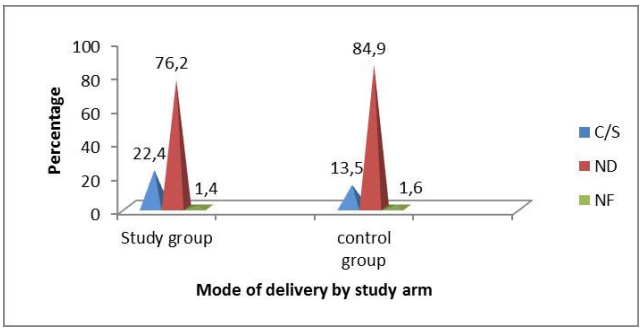


*Figure 3: Mode of delivery by study arm (p = 0.047); C/s = caesarean section; ND = Normal delivery; NF = Lost to follow up*

The study shows that more women who received mebendazole were delivered by caesarean section. Fig. 3 below shows the mode of delivery of participants by study arm. The majority of both study and control had normal delivery; 76.7% in the study group and 84.9% in control group. In the study group, 22.4% delivered by C/S compared with 13.5% in the control group.

## Discussion

The drop-out rate of 6.33% in this study was similar to 7% obtained in a previous randomised trial in Uganda [14]. As every participant was dully counselled on the need for continued care at the centre, the drop-out could have been due to miscarriages, change of place of residents/relocation or other factors. The prevalence of anaemia at term (after treatment) in the study group was 12.6% and was significantly lower than the prevalence of 29.9% among the control/ placebo arm (p=0.000l). This statistically significant difference is the evidence of the negative effect of helminth infection on maternal haemoglobin concentration with associated impact on overall maternal health. Perhaps the elimination of intestinal parasites has significantly contributed to the reduction in the incidence of anaemia at term among the treated women. Several studies have found a strong association between anaemia and intestinal parasitic infestations [4] [15] [16] [17] [18].

In controlling for the co-founders or other related risk factors for anaemia using multiple logistic regression, the study shows that high parity was an independent risk factor for anaemia at term among the participants. The women with more than three previous pregnancies had significantly higher rates of anaemia. This is in keeping with findings from other studies especially in Africa and Latin America [19]. Also, a study in south India [20] reported a higher incidence of anaemia for the parity index more than four. This might be due to the increase in women's nutritional needs during pregnancy in the setting of low iron store especially due to short inter-pregnancy intervals. This further highlights the role of contraceptives in improving maternal health.

The observed higher caesarean section rate among the treatment group might have been contributed by the relative increase in the incidence of macrosomic births (birth weight > 4.0 kg) among other indications. However, the study failed to show any significant statistical difference in the incidence of postpartum haemorrhage (p = 0.119), and puerperal pyrexia (p = 0.943) between the treatment and the placebo arms. Improved maternal care in the facility may have significantly reduced the risk of these morbidities since the study was conducted in a tertiary health centre with the availability of specialised care. The association between maternal anaemia and increased risk of postpartum haemorrhage has been reported. Significant anaemia may cause postpartum haemorrhage by impairing myometrial contraction (atony) following delivery [21]. Similarly, puerperal sepsis has been linked to peripartum anaemia [22]. In this study, most of the anaemic women had mild anaemia probably because of iron supplementation they received during pregnancy, and this might have influenced the low incidence of postpartum haemorrhage among the participants.

In this study, although there was a remarkable improvement in the haematologic status of women treated with the anthelminthic drug in pregnancy, these did not significantly translate to improvement in fetal or neonatal well-being. The research showed a slightly higher mean birth weight in the study (3.26 ± 0.582 kg) than the placebo group (3.19 ± 0.679). There was no statistically significant difference in low birth weight (p = 0.556). The incidence of very low birth weight and extremely low birth weight were very rare in this study. Also, there was no significant difference in perinatal death (p = 0.621) and birth asphyxia (p = 0.706). Two congenital abnormal infants were delivered, one in each group. Similar findings were documented in previous studies [28] [29]. Furthermore, several other studies on helminths infection and anaemia on pregnancy outcomes have yielded inconsistent results [30] [31] [32] [33] [34] [35] [36] [37] [38] [39] [40] [41] [42]. For instance, these findings in this study contrast with 2 previous observational studies [41] [42] in which women who received mebendazole or albendazole were compared with those who did not. One possible mechanism for a beneficial effect on birth weight would be through increased maternal haemoglobin level, and an effect of anthelminthics on perinatal mortality might be mediated by improved birth weight. It is possible that women in those other studies cited who missed one or both doses of albendazole may also have missed their haematinics doses. Similarly, the findings of a cross-sectional study in Sierra Leone [42] in which a lower rate of perinatal deaths occurred among women who took mebendazole may have been affected by selection bias and unmeasured confounders due to lack of randomisation.

In our study since every participant received the standard dose of iron supplement in addition to malaria control strategy (IPT and LLIN) which possibly resulted in the overall decline in the prevalence of anaemia in both arms with very few of them having moderate to severe anaemia, the incidence of low birth weight was low. This must have impacted positively on the perinatal outcome in both groups. Low birth weight has been associated with other perinatal complications including birth asphyxia and perinatal death [34] [42]. Furthermore, deworming is important to prevent some direct pathological effects of worms on the newborn. This is because helminths infection has been linked to the long-term effect on the development of the foetal immune system and risks for disease susceptibility in later life [34].

In conclusion, mass deworming in pregnancy is effective in reducing the incidence of anaemia in advanced gestation. However mild to moderate anaemia may not significantly impact negatively on the perinatal outcomes. The same may not, however, apply to women who are not on routine iron and folic acid supplement during pregnancy as severe anaemia may worsen life-threatening obstetric complications and infant health. There is need to consider 2nd-

trimester presumptive treatment for inclusion in our routine antenatal care practice.

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# Food Safety Knowledge and Practices of Male Adolescents in West of Iran

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**Abstract**

**BACKGROUND:** Every year many people around the world become infected with food-borne infections. Insufficient knowledge and skills related to food safety and hygiene are among the factors affecting the incidence of food-borne diseases, especially in adolescents.

**AIM:** The purpose of this study was to determine the knowledge and practices associated with food safety and hygiene in Ilam city male adolescents.

**MATERIAL AND METHODS:** Three hundred and eighty of male adolescents aged 13 to 19 were selected randomly and entered the cross-sectional study. Data were collected using a researcher-made questionnaire From December 2016 to February 2017. Descriptive statistics, Pearson correlation, independent t-test and one-way ANOVA were used to analyse the data in SPSS software (version 19.0).

**RESULTS:** The findings of the study showed a positive and significant relationship between knowledge and practices related to food safety and hygiene ($r = 0.122$; $p = 0.018$). Also, the findings showed that food safety knowledge and practice of adolescents were significantly affected by the level of their education, parental education level, parental employment status and household economic conditions, ($p < 0.005$). Also, the results showed that the participants generally obtained 57.74% of the knowledge score and 57.63% of practices score. The subjects had the most knowledge about food supply and storage (60%), and the highest practice was related to personal and environmental hygiene, (61.73%).

**CONCLUSION:** The inadequacy of knowledge and performance of adolescents about food safety and hygiene shows the need for implementation of health education interventions in this area.

## Introduction

Food-borne diseases are among the most important public health challenges and one of the major barriers to socioeconomic development worldwide [1]. The results of various studies show that a wide range of infectious diseases spread to humans through unhealthy foods and cause food poisoning, diarrhoea and death [2]. According to the World Health Organization (WHO), in 2010 there were a total of 600 million cases of illness and 420,000 deaths from food-borne diseases worldwide which most of them were related to diarrheal disease agents. Also, according to the report, 31 risk factors for food-borne diseases have been identified, which are responsible for 32 food-borne diseases [1]. Also, food-borne diseases impose high costs on the health system of countries. WHO has estimated the global burden of foodborne diseases to be around 33 million DALYs in 2010 [1]. Pathogenic agents are transmitted to food from all stages of production to distribution and consumption of food through contaminated equipment, food handlers and also final consumers [3]. Several factors affect the incidence of food-borne diseases; the most important of them are contaminated food supplies, inappropriate food storage, providing food from unhygienic sources, and poor personal hygiene [4]. In addition to the factors

mentioned above, inadequate monitoring of food production, distribution and supply, and inadequate health education programs are other reasons for the growing incidence of food-borne diseases, especially in developing countries [5] [6] [7]. On the other hand, demand for cheap and ready-to-eat foods that often are produced in unhealthy conditions is being increased [6] [7]. The results of various studies show that if the food health and safety principles are maintained from the production stages to the consumption, many of the water and food-borne diseases are prevented and controlled and food security of consumers is provided [8] [9]. Food safety is defined as the degree of confidence that food will not cause illness or harm to consumers [7]. Consumers are the last link in the chain of food production to consumption, and they are the most important step in combating food-borne diseases through proper selection, purchase, storage and preparation of food products [8] [11]. However, most studies on food hygiene have focused on food handlers but, food consumers have been less targeted by various studies. Previous studies have shown that people especially in less developed countries have poor knowledge and skills in relation to food safety and hygiene [12]. Meanwhile, adolescences need a more educational program in the field of food safety [7] [8] [13]. Adolescences have a significant role in buying, storing and even preparing food, and in the future, their role will be greater [7] [14]. On the other hand, adolescents' exposure to puberty leads to an increase in the spirit of independence and tendency toward peer groups. So, they are more likely to consume fast food/snack away from home rather than at home meal. Due to this reason, adolescents are more at risk to food-borne diseases [14] [15] [16]. Previous studies have reported that knowledge is the most important predictor of safe nutritional behaviors in adolescents [17]. Therefore, assessing the level of adolescents' knowledge and practices in relation to food safety is a fundamental step towards planning future educational interventions. The purpose of this study was to assess food safety-related knowledge and practices of male adolescents in the west of Iran.

## Material and Methods

A cross-sectional study was conducted from December 2016 to May 2017 on food safety knowledge and practice of adolescent consumers in the Ilam city (west of Iran). A total of 380 voluntary male adolescents were selected by sampling and included in the study. Research Ethics Committee of Ilam University of Medical Sciences approved the present study.

The data collection was carried out through a 56-items researcher-made questionnaire as self-report. The questionnaire consists of three sections:

1. *A demographic section* includes 10 items (Age, educational status, parents' educational level, parents' employment status and family economic status).

2. *Food safety knowledge* scale includes 25 items (For example consumption of food and hot drinks in disposable plastic cups and dishes is dangerous to health). The knowledge section answers scoring was as Yes = 2, I don't know = 1 and No = 0.

3. *Food safety practices* scale includes 21 items (For instance: how often do you pay attention to the date of production and expiration when you purchase food?). The practice section answers were scored with a five-point Likert scale from never = 1 to always = 5.

Reliability of the questionnaire was estimated using Cronbach's alpha coefficient of internal consistency. The Cronbach's alpha for food safety knowledge and practice was estimated to be 0.81 and 0.86, respectively. Time needed to complete the questionnaire was approximately 25 minutes.

The data analysis was conducted with SPSS software (version 19.0). Descriptive statistics, Pearson correlation, independent t-test and one-way ANOVA were used to analyse the data. *P*-values less than 0.05 were considered as statistically significant.

## Results

Three hundred and eighty boys aged 13 to 19 years old in Ilam city, with an average age of 16.38 ± 1.65 years, participated in this study. Most participants are being educated in high school (74.07%) and had a moderate economic status (54.07%).

**Table 1: Absolute frequency, relative frequency, mean and standard deviation of knowledge and practice scores regarding demographic variables**

| Variables | N (%) | Knowledge | | Practices | |
|---|---|---|---|---|---|
| | | *M ± SD* | *p-value* | *M±SD* | *p-value* |
| Educational Status | | | | | |
| Student | 284 (74.7) | 28.27 ± 7.96 | *< 0.010* | 59.91 ± 15.44 | *< 0.090* |
| Graduated | 96 (25.3) | 30.61 ± 7.43 | | 62.34 ± 11.01 | |
| Father Educational Status | | | | | |
| Under Diploma | 158 (41.6) | 27.19 ± 7.14 | | 59.24 ± 13.97 | |
| Diploma | 88 (23.2) | 28.48 ± 8.23 | *< 0.001* | 61.59 ± 14.84 | *< 0.346* |
| University education | 134 (35.3) | 31.08 ± 8.02 | | 61.33 ± 14.80 | |
| Mother Educational Status | | | | | |
| Under Diploma | 211 (55.5) | 28.73 ± 8.23 | | 60.00 ± 14.65 | |
| Diploma | 82 (21.6) | 27.01 ± 6.33 | *< 0.005* | 63.35 ± 12.69 | *< 0.122* |
| University education | 87 (22.9) | 30.94 ± 7.94 | | 59.12 ± 15.40 | |
| Father Job Status | | | | | |
| Unemployed | 19 (5.0) | 24.36 ± 5.76 | | 55.42 ± 15.06 | |
| Employee | 166 (43.7) | 30.50 ± 8.05 | *< 0.001* | 61.10 ± 16.19 | *< 0.270* |
| Self-employed | 195 (51.3) | 27.91 ± 7.61 | | 60.53 ± 12.74 | |
| Mother Job Status | | | | | |
| Housekeeper | 294 (77.4) | 28.81 ± 7.71 | | 61.48 ± 14.22 | |
| Employee | 67 (17.6) | 30.41 ± 8.58 | *< 0.010* | 58.46 ± 14.72 | *< 0.018* |
| Self-employed | 19 (5.0) | 24.21 ± 6.30 | | 52.89 ± 15.19 | |
| Family Economic Status | | | | | |
| Weak | 64 (16.8) | 26.06 ± 7.02 | | 57.45 ± 15.40 | |
| Moderate | 208 (54.7) | 28.92 ± 7.06 | *< 0.002* | 59.75 ± 13.69 | *< 0.010* |
| Good | 108 (28.4) | 30.42 ± 8.50 | | 63.82 ± 14.91 | |

Absolute and relative frequencies, mean and standard deviation of knowledge and practice scores regarding demographic variables are shown in Table 1.

The results showed that adolescents graduated from high school ($p < 0.01$), adolescents with more educated parents ($p < 0.005$), adolescents with employed parents ($p < 0.01$), as well as adolescents with a good economic situation ($p < 0.02$) had a higher level of knowledge about food hygiene. However, the results of food safety related to practice showed that the higher levels were only found in the adolescents with householder mothers ($p < 0.01$) and in the adolescents with a good family economic status ($p < 0.05$). Also, Pearson correlation test showed a positive and significant relationship between knowledge and practices related to food safety and hygiene ($r = 0.122$; $p = 0.018$).

**Table 2: Mean and standard deviation of the total knowledge and practices scores related to food hygiene and three sub-scales**

| Variables | *Mean* | *SD* | *Obtainable Scores Range* |
|---|---|---|---|
| Total Food Hygiene Knowledge | 28.87 | 7.88 | 0 – 50 |
| Personal and Environmental Food Hygiene | 7.18 | 2.72 | 0 – 12 |
| Food Preparation and Preservation | 13.20 | 3.85 | 0 – 22 |
| Food Consumption | 8.46 | 3.04 | 0 – 16 |
| Total Food Hygiene Practices | 60.52 | 14.47 | 21 – 105 |
| Personal and Environmental Food Hygiene | 18.52 | 5.42 | 6 – 30 |
| Preparation and Preservation of Food | 20.46 | 5.74 | 7 – 35 |
| Food Consumption | 21.53 | 5.42 | 8 – 40 |

Table 2 shows the mean and standard deviation of the total knowledge and practices scores related to food hygiene and three sub-scales including personal and environmental hygiene, food supply and storage, and food consumption. As seen, the participants obtained 57.74% of the knowledge score and 57.63% of practices score. The highest and lowest obtained scores of knowledge were related to the food supply and storage (60%) and food consumption (52.87%), respectively. Also, the highest and lowest obtained scores of practices were related to personal and environmental hygiene (61.73%) and food consumption (53.82%), respectively. Moreover, 33.4% of the adolescents reported that they had not received any education regarding food hygiene. Figure 1 illustrates the contribution of different educational resources to increase the adolescents' information regarding food hygiene and safety. As it can be seen, the Internet had the highest roll with 22.3%, and health staff (8.4%) had the lowest role in adolescent food safety education.

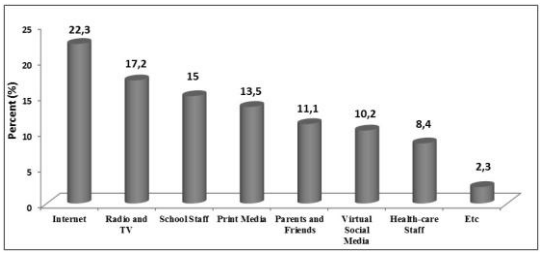


*Figure 1: Sources of information of study subjects on food safety and hygiene (%)*

## Discussion

This study aimed to determine the status of knowledge and practices of adolescent boys about food safety and hygiene. The subjects reported moderate levels of food safety knowledge and practice. These findings indicated inadequate knowledge and practice of the subjects about food safety and health. In this study, the knowledge and practice of the adolescents were studied both in total and regarding three areas include 1) Personal and Environmental Food Hygiene (PEH), 2) Food Preparation and Preservation (FPP), and 3) Food Consumption (FC). Accordingly, the results of the study showed that the level of adolescents' knowledge in two areas of FPP and PEH was better than of FC. The food consumption practice was also weaker in two other areas. Findings of other studies indicated that food consumers had poor knowledge and practice in relation to food safety [2] [7] [18]. Socioeconomic factors seem to play a crucial role in the knowledge and practices of male adolescents in the field of food safety and hygiene. The results of present study showed that adolescents' knowledge regarding food safety was significantly affected by their educational status (Studying or have graduated), parents' educational level, parents' job status and household economic conditions. So that, the results indicated the higher knowledge of adolescents with higher educated parents and graduated adolescents. Additionally, adolescents with employed parents and also adolescents with good household economic status significantly had more knowledge about food safety and hygiene. However, the role of socioeconomic factors was more limited in adolescents practice in relation to food safety and hygiene. So that, only the mother's job status and family economic status had a significant effect on the practice. Accordingly, the well-being of the family and stay-at-home mothers had a positive and significant influence on the practice of the adolescents in relation to food safety and hygiene. Therefore, in several studies, the effect of socioeconomic factors on the knowledge and practice of adolescents in relation to food safety has been reported [8] [18] [19] [20] [21]. Generally, it can be said that the higher education level of adolescents and their parents has a positive effect on food safety-related knowledge [19] [20] [21], although it does not guarantee a proper practice [22]. However, the job status of the parents and the household's economic situation have a positive impact on the knowledge as well as improving the practice of adolescents in relation to food safety [20].

In the present study, adolescents with employed mothers were more knowledgeable about food safety and hygiene, while the children of the housewife's mothers reported better practice. These findings can be explained by the fact that employed mothers have more access to knowledge sources related to food safety due to higher levels of literacy

and more presence in the society. Therefore, much of their knowledge can be passed to their children. Of course, resources that generate knowledge in adolescents do not necessarily enhance their behaviour, and vice versa, resources that affect behaviour may not be a good source of knowledge. Hence, housewife mothers can be good role models for learning skills related to the prevention of food-borne diseases in adolescents [23] [24]. Adolescences, especially adolescent boys, tend to model from out-of-home patterns including peers and friends, and for this reason, they spend more time outdoors than their childhood [14] [25] [26]. As a result, they eat more out-of-home foods that often include ready-to-eat meals, junk foods and street foods that increase the risk of food-borne diseases [25] [26]. Health education seems to be the most important need for adolescents to prevent food-borne diseases [7] [13]. With the passing of the childhood, the social responsibility of adolescents is increased. So, they play a more active role in the selection, purchase, preparation and preservation of foods [8]. Accordingly, teenage is a great opportunity to implement health education interventions and develop knowledge and skills related to food safety and hygiene [14]. The fact is that as long as the adolescents are not adequately aware of the potential dangers of food-borne contamination, they will not have enough motivation to adopt the preventive behaviours [8] [27]. According to the results of present study, about one-third of the participants reported that so far they have not received any education about food safety and hygiene. Of the adolescents who were educated, only 15% and 4.3% of them were educated by school staff and healthcare system, respectively. These findings indicated that only small proportion of food safety and hygiene education of the adolescents provided by healthcare staff or school health care providers. On the other hand, the role of mass media in educating adolescents about food safety more reported [8] [28]. In this study, the Internet and virtual communication social networks were provided 32.5% of the food safety education to the adolescents. Also, radio and television (17.2%) and printed media (13.2%) had a significant contribution to the education of food safety to the adolescents. Similar studies in other developing countries also indicated that there were not adequate educating programs in the field of food safety and hygiene, especially for adolescents [29].

In today's world, Web-based educational resources play an important role in promoting health-related knowledge and practices of adolescents [30, 31]. However, the educational content provided by the Internet may not have sufficient scientific credibility and always cannot be trusted. Therefore, due to the high interest of adolescents to Web-based educational resources, it is suggested that these methods be used in further educational interventions in the field of food safety and hygiene. In this way, the interest and selection of adolescents are respected and can also be acted as a credible scientific resource for educating health topics in the Internet environment.

In summary, the results of this study indicated the inadequate knowledge and practices of male adolescents in relation to food safety and hygiene. Since adolescents are vulnerable and at risk for food-borne diseases. Consequently, they should be more targeted at food safety educational interventions.

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# Knowledge and Awareness of Osteoporosis among Saudi Physicians and Nurses: A Cross-Sectional Study

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**Abstract**

**AIM:** We aimed to evaluate the awareness and knowledge of osteoporosis in a sample of 141 health professionals.

**MATERIALS AND METHODS:** A group of 141 health professionals (medical, surgical, primary health care and nursing departments) were enrolled in this cross-sectional study in the period from August 2017 to November 2017. The participants completed a questionnaire composed of 19 questions about osteoporosis which covering the main domains of knowledge on osteoporosis. Each correct answer carried 1 point whereas incorrect or 'don't know' carried 0 points. This gave a total score range of 0-19. A cut-off level of < 10 points was considered as poor knowledge while ≥ 10 was regarded as good knowledge.

**RESULTS:** Out of 141 respondents, 78 were females, 63 males, mean age of the participants was 38.17 ± 9.6 years, less than 40 years (n = 89), more than 40 years (n = 52). Nurses (n = 54), medical (n = 29), surgical (n = 35), primary health care (n = 23). Most of the study group with experience of fewer than 10 years (n = 66), 127 (90.1%) had good knowledge and 14 (9.9%) had poor knowledge p-value < 0.01.

**CONCLUSION:** Our study revealed that Saudi health professionals have a good of knowledge about osteoporosis and no significant difference in osteoporosis knowledge between the health professional subgroups.

## Introduction

Osteoporosis is defined as a systemic bone disease characterised by decreased bone mass and deterioration of micro-architecture of bone tissue leading to increased risk of fracture [1]. Osteoporosis is a major health problem in both developed and developing countries [2], and it has been considered as the second most important health concern after heart diseases in developed countries [3].

Several studies from different countries have pointed out the lack of awareness of osteoporosis, its risk factors and measures of prevention not only among the general population but also among physicians [4] [5]. Thus expanding the knowledge and awareness of osteoporosis are important measures warranting better care of osteoporosis with subsequent improvement of life quality and decrease disease burden.

A recent study demonstrated that physician knowledge and education improve patient care and outcome [6]. It worth mentioning that there is a deficiency in the number of Saudi health professionals [7]. Therefore, adequate and updated knowledge of osteoporosis by health professionals is of great importance towards improving patient care.

This study aims to evaluate the knowledge and awareness of osteoporosis in 141 Saudi health professionals.

## Methods

This a cross-sectional study conducted at King Abdullah General Hospital in Bisha from August 2017 to November 2017. A total of 141 health professionals (medical, surgical, primary health care and nursing departments) were enrolled in the study. The participants were selected randomly. Ethical approval was sought from Human Research Ethics Committee at the University of Bisha. All participants were given oral informed consent. The data was collected using a structured questionnaire.

The following data were taken from each participant: age, gender, type of health profession and years of experience. The questionnaire also included 19 questions on osteoporosis knowledge, which cover the definition, risk factors, management, prevention and complications of osteoporosis. Each item has Yes, No, and "Do not know" options. The latter option was designed to avoid guessing. Each correct answer carried 1 point whereas incorrect or 'don't know' carried 0 points. This gave a total score range of 0-19. A cut-off level of < 10 points were considered as poor knowledge while ≥ 10 was regarded as good knowledge.

Statistical analysis was conducted using Statistica (version13.2). Descriptive statistics were used to demonstrate the characteristics of the study population. Continuous variables were expressed as mean ± standard deviation whereas categorical variables were measured as frequency and percentages. Kolmogorov-Smirnov and Shapiro-Wilk W test were used to determine nature of the distribution. Kruskal Wallis test was applied to assess the degree of knowledge among the different subgroups where the health profession, age group, gender, and experience were entered as dependent variable while the degree of knowledge was entered as an independent variable, statistical significance is considered as $P < 0.05$.

## Results

The characteristics of respondents are shown in Table 1.

**Table 1: Respondents characteristics**

| Variable | Category | No | Percentage |
|---|---|---|---|
| Age group | Less than 40 | 89 | 63.1 |
| | More than 40 | 52 | 36.8 |
| Gender | Male | 63 | 44.6 |
| | Female | 78 | 55.3 |
| Speciality | Medical | 29 | 20.5 |
| | Surgical | 35 | 24.8 |
| | Primary care | 23 | 16.3 |
| | Nurse | 54 | 38.2 |
| Experience | Less than 10 | 66 | 46.8 |
| | 10-20 | 57 | 40.4 |
| | More than 20 | 18 | 12.7 |

The mean age of the participants was 38.17 ± 9.6 years. Concerning their age group, the majority were less than 40 years (n = 89), more than 40 years (n = 52). In terms of gender, the majority were females (n = 78), males (n = 63). A large number of the respondents were nurses (n = 54), medical (n = 29), surgical (n = 35), primary health care (n = 23). Respondents with experience of fewer than 10 years were greater than other groups (n = 66) (see Table 1). Responses to osteoporosis questions are shown in Table 2.

**Table 2: Responses to the questionnaire questions**

| Question | Correct No/%) | Incorrect (No/%) | Do not know (No/%) |
|---|---|---|---|
| Pain is common in individual with osteoporosis | 56 (39.7%) | 80 (56.7%) | 5 3.5%) |
| Osteoporosis is a condition characterised by fragile bones | 130 (92.1%) | 9 (6.3%) | 2 (1.4%) |
| Osteoporosis and osteomalcia are different conditions | 131 (92.9%) | 7 (4.9%) | 3 (2.1%) |
| Osteoporosis is common in women than men | 136 (96.4%) | 4 (2.8%) | 1 (0.7%) |
| Bones are strongest between the ages of 20 to 50 years | 125 (88.7%) | 11 (7.8%) | 5 (3.5%) |
| Early menopause is a risk factor for osteoporosis | 136 (96.5%) | 3 (2.1%) | 2 (1.4%) |
| Excessive alcohol intake is a risk factor for osteoporosis | 124 (87.9%) | 4 (2.8%) | 13 (9.2%) |
| Sun light reduces the risk of getting osteoporosis | 128 (90.8%) | 12 (8.5%) | 1 (0.7%) |
| Lack of exercise is a risk factor for osteoporosis | 123 (87.2%) | 11 (7.8%) | 7 (4.96%) |
| Awareness of HRT in prevention of osteoporosis | 131 (92.9%) | 4 (2.8%) | 6 (4.2%) |
| High chance of sustaining a fracture if the previous history of fragility fractures | 131 (92.9%) | 3 (2.1%) | 7 (4.96%) |
| Family history predisposes to osteoporosis | 101 (71.6%) | 25 (17.7%) | 15 (10.6%) |
| low BMI is risk factor for osteoporosis | 90 (63.8%) | 41 (29.1%) | 10 (7.1%) |
| Osteoporosis increases risk of fractures | 131 (92.9%) | 4 (4.2%) | 6 (2.8%) |
| Physical activity is beneficial for osteoporosis | 121 (85.8%) | 16 (11.3%) | 4 (2.8%) |
| If you have osteoporosis, you become shorter due to bent spine | 106 (75.1%) | 21 (14.89%) | 14 (9.9%) |
| Osteoporosis is treatable disease | 107 (75.9%) | 31 (21.98%) | 3 (2.1%) |
| There are no effective treatments for osteoporosis available | 73 (51.8%) | 52 (36.9%) | 16 (11.3%) |
| Calcium supplements and Vitamin D can prevent osteoporosis | 129 (91.5%) | 8 (5.7%) | 4 (2.8%) |

Out of 141 respondents, 127 (90.1%) had good knowledge and 14 (9.9%) had poor knowledge - p-value < 0.01. There was no significant difference in the level of knowledge on osteoporosis among the health professionals' subgroups (age groups, sex, experience, and speciality) as shown in Table 3.

**Table 3: Level of knowledge on osteoporosis among the health professionals' subgroups**

| | Category | Degree of knowledge | | No | P value |
|---|---|---|---|---|---|
| | | Good | Poor | | |
| All respondents | All respondents | 127 (90.1%) | 14 (9.9%) | 141 | < 0.01 |
| Age groups | less than 40 | 79 (89%) | 10 (11%) | 89 | |
| | more than 40 | 48 (92%) | 4 (8%) | 52 | |
| Specialties | All Groups | 127 (90%) | 14 (10%) | 141 | 0.4897 |
| | medical | 29 (21%) | 0 (0%) | 29 | |
| | surgical | 31 (22%) | 4 (3%) | 35 | |
| | primary care | 21 (15%) | 2 (1%) | 23 | 0.0591 |
| | nurse | 46 (33%) | 8 (6%) | 54 | |
| Gender | All Groups | 127 (90%) | 14 (10%) | 141 | |
| | male | 59 (42%) | 4 (3%) | 63 | 0.2030 |
| | female | 68 (48%) | 10 (7%) | 78 | |
| Experience | All Groups | 127 (90%) | 14 (10%) | 141 | |
| | less than 10 | 57 (40%) | 9 (6%) | 66 | |
| | 10-20 | 53 (38%) | 4 (3%) | 57 | |
| | more than 20 | 17 (12%) | 1 (1%) | 18 | 0.1730 |
| | All Groups | 127 (90%) | 14 (10%) | 141 | |

## Discussion

Osteoporosis is a substantial health problem, particularly with an increase in ageing population. The cornerstone of prevention of osteoporosis is to improve knowledge and awareness among health professionals. Physicians in practice have only limited information about the proper and best treatment strategies for osteoporosis [8]. Although there were many studies described osteoporosis knowledge in populations, only scarce data addressed osteoporosis knowledge among health professionals [9].

This study explored the knowledge of health professional on osteoporosis in Saudi Arabia. In our study, 90% of the study group expressed good knowledge on osteoporosis which is significantly high. These findings are almost in accordance with the results of another study concluding that 88% of physicians had a good knowledge of osteoporosis [10]. Furthermore, Saeedi et al. described a range of osteoporosis knowledge between 36.5% and 92.2% among physicians [11]. Moreover, German study reported good knowledge of osteoporosis in 83% of primary care physicians [12]. On the other hand, some studies reported a bit low level of osteoporosis knowledge. In 2013 Al-Musa et al. described an average level of knowledge of 67% [13]. Another study conducted in 490 primary care physicians, 50% demonstrated adequate knowledge of calcium and vitamin D supplementation, 51% were aware of the main therapeutic purposes of osteoporosis [14].

Concerning the questions about osteoporosis, the majority of the respondents demonstrated a good level of knowledge; however, a large gap in knowledge of osteoporosis was detected in two questions assessing symptoms and treatment of osteoporosis with correct answers only of 56% and 73% respectively. Beshyah et al. described poor knowledge about osteoporosis treatment among practising physicians [15]. These results lower than what we observed and it could be explained by several reasons including; the difference in study samples, methods of evaluation of knowledge level and the type of the questions in the assessment questionnaires.

About age groups and length of experience, no statistically significant differences in the degree of osteoporosis knowledge between the various groups, although several studies demonstrated a significant inverse relation between physicians' age and knowledge about osteoporosis [16] [17] [18]. This discrepancy is likely attributed to our small sample, but could also be linked to the heterogeneity of our study population.

In this study, health professional gender did not affect the level of knowledge about osteoporosis, while other studies described that females are more knowledgeable than men [12] [18] [19].

In the present study, the degree of knowledge among the different health professionals' subgroups (medical, surgical, primary health care and nursing) showed no significant differences. However, one previous report showed an increased degree of knowledge in family physicians than did general practitioners [16].

Of note, our study has several limitations; the cross-sectional design decreased the power of the study and outcome bias cannot be excluded due to the small sample. A large sample would have led to a better conclusion.

In conclusion, this present study showed that health professionals have a good knowledge about osteoporosis, but there is no significant difference in osteoporosis knowledge between the health professional subgroups (age groups, sex, experience, and speciality). However, there are a few gaps in knowledge that need to be addressed by adopting educational programs for health professionals to robust patient care and decrease disease burden.

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# The Concept of Withdrawal of Divorce Petition Based on the Theory of Planned Behavior: A Qualitative Study

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**Abstract**

**AIM:** The present study sought to explore the experiences of participants in divorce process according to the theory of planned behaviour.

**MATERIAL AND METHODS:** This qualitative study was conducted using content analysis method. In this research, 27 participants involved in the divorce process were selected. The data were coded, and the qualitative content analysis was performed.

**RESULTS:** Based on four constructs of the theory of planned behaviour, the subcategories of instrumental attitude were "Divorce as the last solution" and "Divorce as damage for individuals and society". From the perceived behavioural control theme, two subcategories of behavioural control and self-efficacy were drawn; the first subtheme included "Others' meddling in the married life", "Social problems reducing behavioural control power" and "Personality characteristics affecting the behavioural control power"; and the second one included: "Education as a means for developing self-efficacy" and "barriers to self-efficacy". The injunctive norms theme included three subcategories of "Others help to reconcile", "Others meddling and lack of reconciliation", and "Families support to reconcile". The descriptive norms theme was "High divorce rate and misuse of satellite channels and social networks as factors making reconciliation difficult".

**CONCLUSION:** It seems that education and counselling, within a predefined framework, such as applied theories, can be useful.

## Introduction

In recent decades, divorce has increased all over the world [1]. In Iran, like other developing countries, the divorce rate has significantly increased [2]. Although in some cases divorce and even its increasing rate cannot be considered as negative [3], in Iran, due to the importance of the family, any harm to this institution is seen as an unpleasant and investigable social issue [4]. Globally, divorce has been accompanied by many changes in economic social and cultural terms. For example today in the western societies we face the grey divorce phenomenon which refers to the increasing divorce rate for older couples in long-lasting marriages. Roughly 1 in 4 divorces in 2010 occurred to persons aged 50 and older [5].

Increasing divorce rate is considered as a social problem that has various consequences and puts mental health of the family members and therefore society at risk. There are several ways to reduce divorce rate such as counselling and Couple therapy before marriage, during marital life and before divorce [6]. The theory of planned behaviour is one of the patterns used to change behaviours such as addiction, nutritional behaviours, pregnancy prevention, and Internet and so on [7] [8] [9] [10] [11]. It seems that the theory of planned behaviour and its components are also suitable for changing the behaviour of couples (withdrawal of divorce petition) because the withdrawal of divorce can be considered as a behaviour affected by different behavioural models.

Attitude is the first determinant of behavioural intention and one of the components of the planned

behaviour theory. It refers to the degree to which a person has a favourable or unfavourable evaluation of the behaviour of interest and entails negative or positive beliefs about performing the behavior [12]. The individual's attitude toward divorce is one of the most influential factors in his decision making. Attitude can be divided into two categories: instrumental and Experiential. Instrumental attitude is determined by beliefs about outcomes of behaviour, and experiential attitude refers to a judgment about the pleasantness or unpleasantness of performing a certain behaviour [13] [14].

Another important and effective factor in the decision about divorce is subjective norms. Subjective norms or perceived norms reflect the social pressure that a person perceives about doing or not doing a certain behaviour. The norms are classified into two categories: Injunctive norm, the Descriptive norm. Injunctive norm refers to normative beliefs about what others think one should do and motivation to comply. Descriptive norm refers to perceptions about what others in one's social or personal networks are doing. Meant to capture situations where there is strong social identity [12].

The other important factor in performing or changing behaviour is evaluation (perception) of a person's own capability to accomplish a certain level of performance. The perceived behavioural control includes behavioural control and self-efficacy. When there is no limitation, individual may have complete control over the behaviour. But at the end of this continuum, there may be no control over the behaviour. Control over some behaviours requires resources, facilities and skills that people may not have. Control factors are either internal or external. Internal factors are personal such as skills, abilities, knowledge, feelings of stress, and external factors include environmental or occupational factors. The other construct of this theory is the behavioural intention. Intention refers to the motivational factors that influence a given behaviour where the stronger the intention to perform the behaviour, the more likely the behaviour will be performed [15].

In the past few years, joint efforts were undertaken to increase reconciliation between couples and withdrawal of divorce, by the Judicature and the State Welfare Organization. Divorce applicants were introduced to the Family Counseling Center by the judicature. After different education and counselling programs, if they refuse to reconcile, they will be referred to the Judicature to pursue legal separation procedures. This research attempted to examine the concept of divorce and its withdrawal, by carrying out qualitative work and interviewing people, who are involved in the issue of divorce in the Family Counseling Center in Yazd, based on the four constructs of the theory of planned behaviour within the framework of personal experiences. Yazd is a central and a relatively industrial city of Iran, and regarding climate, is located in a warm and dry region of the country. The population of the city is 1 040 286, of whom 87.4% live in urban areas, and 12.6% live in rural areas. Men comprise 51.3% of the city's population, and female population is 48.7%. The language spoken in the city is Persian, and the religion of its residents is Islam. In Yazd, the life expectancy rate is 75.7 years, the unemployment rate is 11.2%, and the average family size is 3.54 persons. In recent years, there have been many cultural changes in the city due to migrant workers and students, including an increase in divorce rates for various reasons.

The present study sought to explore the experiences of participants in divorce process according to the theory of planned behaviour.

## Methods

This qualitative study was conducted by directed content analysis. Data were collected by in-depth interviews to evaluate participants' experiences about the concept of withdrawal of divorce. Participants included various groups such as divorce applicants, their families, social workers and counsellors of the Family Counseling Center and Family Court Judges. One of the authors was the interviewer, and the interviews were conducted from May 1 to September 1, 2016, at the Family Counseling Center. The only condition for the participation of the subjects was their satisfaction with interviewing and collaborating with the researchers. The condition for leaving the study was also the lack of consent of the participants to continue the interview. Table 1 revealed demographic characteristics of participants.

**Table 1: Demographic characteristics of participants**

| Participants | N (%) | Gender N (%) | | Age N (%) | | Occupation N (%) | | Education N (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Female | Male | 31≤ | = 30 | Unemployed | Employed | Master and higher | Diploma to bachelor | Primary |
| Couples | 10(37) | 4(40) | 6(60) | 7 (70) | 3(30) | 2(20) | 8(80) | 3(30) | 1(10) | 6(60) |
| Parents | 7(25.9) | 6(85.7) | 1(14.3) | 7(100) | 0 | 6(85.7) | 1(14.3) | 0 | 0 | 7(100) |
| Counselors and social workers | 7(25.9) | 5(71.4) | 2(28.6) | 7(100) | 0 | 0 | 7(100) | 5(71.4) | 2(28.6) | 0 |
| Family Court Judges | 3(11.1) | 1(33.3) | 2(66.7) | 3(100) | 0 | 0 | 3(100) | 2(66.7) | 1(33.3) | 0 |
| Total | 27 | 16 | 11 | 24 | 3 | 8 | 19 | 10 | 4 | 13 |

To get a full understanding of the concepts of divorce and withdrawal of divorce, we tried to conduct in-depth interviews with different people in each group. The interview was initially conducted with a social worker and two divorce applicants to obtain an appropriate framework for the interviews. In this research the key questions based on the four constructs of TPB included. Table 2 revealed the key questions based on the four constructs of TPB.

**Table 2: Interview questions TPB constructs**

| Constructs of TPB | questions |
|---|---|
| Attitude | What is your experience of divorce? |
| | Please describe your experience of reconciliation or withdrawal of divorce. |
| | How will your life be after withdrawal of divorce? |
| Subjective norms | As you experienced, what roles do family members have in the withdrawal of divorce? |
| | Based on your experience, who do play roles in the withdrawal of divorce? |
| | Who in your personal life was involved in creating or escalating your married life problems? |
| Perceived behavioural control | What are barriers to the withdrawal of divorce petition, according to your personal experience? |
| | What does your experience show you about conditions leading to the withdrawal of divorce petition? |
| | Based on your experience, are all the conditions for [couples'] living together in one's control? |
| Intention | How can you give up your intention to divorce? |
| | Do you have any experience with someone who wants to continue his/her married life despite filing a petition for divorce? |

The questions were completely flexible so that if a question was needed to be asked for participants'' better understanding, they were asked. It was important because Participants' level of education ranged from the academic education of counsellors and social workers to primary school education of couples and their families. Therefore, the researcher should make the question simpler, if necessary, or raise follow-up questions like: Can you explain more about this? Exactly how?

The interviews were recorded except two interviews with the Family Court Judges who did not allow the recording. As a result, the researcher wrote down the essential points of the interview so that after the interviews, other points were also added before forgetting. Except these two, all interviews took place at the Family Counseling Center. Interviews lasted 40 to 65 minutes. Interviews were conducted in each group to reach data saturation so that in the last three interviews no new code was obtained. Interviews were conducted with 27 participants in several groups.

The recorded interview was immediately typed in full, and then the data was encoded. Then the qualitative content analysis based on Graneheim and Lundman 5-step method was used which includes: gaining overall insight by reading interviews for several times, dividing the text into semantic units, abstraction of semantic units and tagging them by codes, dividing codes into subthemes by comparing their similarities and differences, setting themes as indicators of hidden content and main themes including attitudes, subjective norms, perceived behavioral control, and intention of participants. To analyse data MAXQDA 10 was operated.

To check the trustworthiness of data, Lincoln and Guba 's method was used [16] [17]. The diversity of the participants in both the general characteristics and the various roles they had in the process of divorce, e.g. couples, their parents, judges and counsellors, increased the credibility of the data. While the interviews were reviewed and encoded and data were analysed, two supervisors assisted and controlled the process, therefore encoding and classification were compared by the researchers (Dependability). In the case of discrepancies, consensus over final themes was reached through discussion between the researchers and an external observer. To reach the confirmability of data, interviews were repeatedly studied and also colleagues comments on them. By a full and exact description of methodology, participants' interviews and data analysis, possibility of using our methodology in similar cases (transferability) was reached.

## Results

Using the theory of planned behaviour, the present study provides appropriate information which makes the behavioural change, i.e. withdrawal of divorce more possible. Four constructs of the theory of planned behaviour include attitudes, perceived behavioural control, subjective norms and intentions, each based on the encoding of interviews consisted of categories and subtheme. Reviewing 27 interviews, the total number of primary codes was 853. After omitting duplicate codes, 396 codes remained, among which 57 as the main codes were by the four constructs of the theory of planned behaviour. Other codes were also categorised into 40 categories, out of the constructs, which can be used in future studies. The attitudes consisted of two categories of instrumental and empirical attitude, each of which had two subcategories. Within the theme of perceived behavioural control, two categories of behavioural control and self-efficacy were obtained, having 3 and 2 subcategories, respectively (To be published) [18]. The theme of subjective norms had two categories of injunctive and descriptive norms, having 1 and 3 subcategories, respectively. Intention also had two categories of definitive intention, with one subtheme or non-definitive intention, with three subcategories. Review of all construct along with related categories, subcategories and codes are as follows:

A: Instrumental attitude (the degree to which a behaviour is thought to be enjoyable)

- Divorce as a solution to insoluble problems

In some cases, those who refer to the counselling centre believed that to continue marital life would damage their children's upbringing. One of the men referring to the Center said: "I don't like that my children grow like their mother and to behave like her. Even if divorce harms my life, it is good for my children". (Referral No. 3)

B: Experiential attitude (a judgment about the pleasantness or unpleasantness of performing a certain behaviour)

- An annoying concept

"No one loves to lose what he owns. Man is

accustomed to and get interested in what belongs to him and will suffer while losing it (Judge No. 2)

Fear of being alone was seen among participants' attitudes toward divorce, one of them said: divorce is not a good event. For me, it means loneliness. It is as if you wonder. (Referral No. 4)

- Divorce; not very bad and the easiest solution

Counsellors believed that in some cases, people thought that filing divorce petition is the easiest way to solve their marital problems and they often regret when emotions subside. "a man was a pigeon racer who accused his wife of escaping a pigeon. They argued. In the morning they refer to the Family Court, after living together for 19 years, and fill the divorce petition. After taking legal measures and getting a divorce, they reconciled. They had have chosen the easiest way" (Counselor No. 5). Table 3 revealed the description of codes and categories of the main theme of attitude.

**Table 3: Description of codes and categories of Attitude related to the concept of withdrawal of divorce petition**

| Main Theme | Theme | Subtheme | Extracted codes |
|---|---|---|---|
| Attitude | Instru-mental Attitude | Divorce as the last solution | 1) to continue stressful life or a forced compulsory disrupt healthy upbringing children |
| | | | 2) divorce a solution to prevent physical harm in unsuccessful lives |
| | | | 3) divorce as the last solution to marital problems |
| | | | 4) a divorce means a wrong choice that needs to be erased and no more |
| | | Divorce as damage for individuals and society | 1) divorce is stressful |
| | | | 2) divorce is an obstacle to progress. |
| | | | 3) divorce as a harmful social phenomenon |
| | Empirical attitude | Divorce as an annoying concept | 1) to accept divorce is annoying |
| | | | 2) divorce is not a beautiful word |
| | | | 3) negative judgment of society toward divorced people |
| | | | 4) divorce means loneliness and confusion. |
| | | | 5) unacceptability of the concept of divorce |
| | | Divorce, to escape the painful situation | 1) divorce as freedom |
| | | | 2) divorce is not so bad |

Perceived behavioural control (includes two categories of behavioural control and self-efficacy)

A: Behavioral control power

The initial codes showed that the relevant codes could be classified into several subcategories including meddling of others, social problems affecting behavioural control and personality characteristics which were put under behavioural control. The obtained codes under subcategories of education as a means for developing self-efficacy and barriers to self-efficacy constitute the categories of self-efficacy. All codes mentioned above, categories and subcategories form the theme of perceived behavioural control. (This construct is independently written in an article in print.)

- Meddling of others

Occasionally conflicts occur between couples' families and make them more difficult to reconcile. One of the counsellors said: "Families are involved. They want to synthesise and do something but engage themselves and make things worse." (Counselor No. 3)

- Social problems

In many cases, social problems initiate or intensify the couples' conflicts. Chronic and incurable addiction and betrayal are the most important factors arising conflicts. The importance of addiction was repeatedly stated by divorce applicants, their families, counsellors and judges. Mother of a divorcee said:" we wait for 5 years. We prayed and sacrificed a sheep. We encouraged him .he quitted three times. But again get addicted besides his father. Our efforts had no result."(Mother of Referral No. 2)

*B: Self-efficiency*

- Education

A Social worker believed that counselling is very useful for couples: "they come to the Center, learn solutions, their minds open, they recognise problems; therefore, they will learn those things which were not taught in the family, such as skills of marital affairs and marital relationship. Consequently, their problems are solved" (social worker No. 3). Table 4 revealed the description of codes and categories of the main theme of Perceived behavioural control.

**Table 4: Description of codes and categories of perceived behavioural control related to the concept of withdrawal of divorce petition**

| Main Theme | Theme | Subtheme | Extracted codes |
|---|---|---|---|
| Perceived behavi-oural control | Beha-vioural control | Others' meddling in the married life | 1) impossibility of reconciliation due to conflicts between parents and the couples |
| | | | 2) inability to reconcile due to the conflicts between families |
| | | | 3) inability to reconcile due to having one child and families meddling |
| | | | 4) ability to reconcile without families meddling |
| | | | 5) difficulty of reconciliation without couples families help for solving married life problems |
| | | Social problems reducing behavioural control power | 1) difficulty of reconciliation due to easy access to adultery in the society |
| | | | 2) difficulty of reconciliation due to woman's assurance about the dowry |
| | | | 3) inability to reconcile due to imprisonment of the spouse |
| | | Personality characteristics affecting the behavioural control power | 1) inability to reconcile due to his/her distrust and suspicion |
| | | | 2) difficulty of reconciliation due to lack of independent decision-making by the spouse |
| | | | 3) difficulty of reconciliation due to lack of responsibility |
| | | | 4) difficulty of reconciliation due to the violence of spouse |
| | | | 5) inability to reconcile due to sexual reluctance and coldness |
| | | | 6) inability to reconcile since the spouse is on trial or imprisoned |
| | | | 7) ability to reconcile in the case of spouse's moral consistency |
| | Self-efficacy | Education as a means for developing self-efficacy, | 1) ability to reconcile by receiving appropriate counselling and education at the centre |
| | | | 2) ability to reconcile by receiving appropriate counselling and education at organisations like NGOs, drug rehab centres, etc. |
| | | | 3) ability to reconcile because of an opportunity to think during the divorce process |
| | | Barriers to self-efficacy (the ability to reconcile) | 1) reconciliation is impossible due to the failure of the previous one |
| | | | 2) inability to compromise due to low tolerance in a new generation |
| | | | 3) difficulty of reconciliation due to forced marriages |

*Subjective norms*

A: Injunctive norms as preventive norms refer to normative beliefs about what others think one should do and motivation to comply. A man referring to the Center said: “at first we live peacefully, but little by little her mother interfered. My mother-in-law was good to me. But she began to interfere. She wishes I divorce my wife. She said I would pay her living expenses. My wife wants to divorce under her influence. (Referral No. 6)

B: Descriptive norms: Includes perceptions of the individual's behaviour about what some people in the community are doing. One of the counsellors said: “Divorce is no longer a bad thing, it is very effective. When a man faces some behaviours within the society, he becomes one step closer to doing it. E.g. the destructive effect of watching pornographic videos is to bring someone closer to doing the same. (Counselor No. 3). Table 5 revealed the description of codes and categories of the main theme of Subjective norms.

**Table 5: Description of codes and categories of subjective norms related to the concept of withdrawal of divorce petition**

| Main Theme | Theme | Subtheme | Extracted codes |
|---|---|---|---|
| Subjec-tive norms | Descrip-tive norms | High divorce rate and misuse of satellite channels and social networks as factors making reconciliation difficult | 1) high divorce rate and less negative view of it<br>2) difficulty of reconciliation due to misuse of satellite channels and social networks |
| | | Others help to reconcile | 1) religious comments encouraging marital life and solving problems<br>2) counsellors and social workers encourage to reconcile |
| | Injunctive norms | Others meddling and lack of reconciliation | 1) girl/boyfriends or concubines as supporters of divorce<br>2) parents help to divorce<br>3) mother as the main supporter of her daughter’s divorce<br>4) couples families encourage to divorce |
| | | Families support to reconcile | 1) couples families encourage to reconcile<br>2) couples families support to divorce |

*Intention*

Some of the divorce applicants fill the petition as the last solution: a woman said: “I tolerated for 18 years because I wanted to continue my marital life. He gets me to his concubines’ house, I tolerated. I tolerated many things”. (Referral No. 5).

In some cases, they decided to divorce: “she granted her dowry, I don’t think there is a possibility for reconciliation. This situation lasted for 4 years. Her parents say she is young, tolerate her, but it was not useful. If was, we should have seen it during these 4 years (Referral No.1)

In some cases wives don’t want to get divorced, they only try to punish their husbands, but this is not a solution. One of the counsellors said: “They said by going to the court, their husbands will be punished, but it resulted in something different. Men usually don’t accept to continue living with those who take them to court. Referring to the court and ask for dowry make reconciliation more difficult. (Counselor No. 4). Table 6 revealed the description of codes and categories of the main theme of intention.

**Table 6: Description of codes and categories of Intentions related to the concept of withdrawal of divorce petition**

| Main Theme | Theme | Subtheme | Extracted codes |
|---|---|---|---|
| Intention | Non-definitive intention | Withdrawal due to emotional decision | 1) decide to the withdrawal of divorce while hurry to file for divorce<br>2) intention to reconcile when emotions subside |
| | | Decide to continue marital life by tolerating a difficult situation | 1) tendency to reconcile and to file divorce petition to frighten the spouse<br>2) tolerate marital problems in order to reconcile |
| | | Withdrawal of divorce after referring to the centre | 1) tendency to reconcile in spite of families disagreement<br>2) intend to reconcile despite filling agreed divorce petition |
| | Definitive intention | Decide to divorce | 1) intend to reconcile despite chronic addiction of the spouse<br>2) intend to divorce due to the unchanged ability of abnormal personality<br>3) unwillingness to continue marital life due to betrayal<br>4) lack of intention to continue marital life after leaving the spouse for years |

## Discussion

Considering the importance of family preservation and the efforts of various institutions and organisations to reduce divorce rate especially in cases of preventable or contested divorce, the present study is planned to examine this social problem in depth and qualitatively through interviews, using the theory of planned behaviour and its constructs. The results of this study showed that most people had a negative attitude toward the concept of divorce and considered it an annoying and bad event, even if it is accepted as the last resort. It is noteworthy that, compare to the past, many changes had taken place in people’s attitudes toward divorce, it is accepted despite being annoying. It was not seen before in Yazd [19]. Nowadays, due to the changes in technology and its effects on the lives and cultures of the people, especially the youth, divorce is more readily accepted and there is no longer a negative attitude toward it. The change in the attitude toward divorce is confirmed in other studies. According to another studies, this change in attitudes and the less negative views about divorce, especially in metropolises or among educated people, was clear [19] [21] [22]. Considering the experiences of counselors and social workers and even the couples , it seems possible to correct or complete these attitudes by using appropriate education and counselling [23] [24].

The present study revealed that subjective norms of divorce applicants are affected by different factors. The frequency and increasing rate of divorce lessen the negative views about it. As some

counsellors believed divorce became one of the options some choose as the first and easiest solution for their problems. It might be said that programs of Satellite TVs and misuse of online social networks in traditional societies like Yazd made divorce easier. In the other side, it is noteworthy that increase in the divorce rate in a traditional society shows changes in the attitudes of females and decrease of pressures to conform to wrong traditions [25] [26] [27] [28]. However, the increasing rate of divorce might be seen as harmful and severe. Subjective norms are determined by the perceived social pressure from significant others for an individual to behave in a certain manner and their motivation to comply with those people's views. Therefore parents' role become greater here. This is seen more among women because of their greater dependency to their parents. Other studies also revealed the effects of families' interference on couples' decision making. Family Intervention Divorce Girl's Dependency Family Intervention Divorce Girl's Dependency [29] [30]. According to data drawn from our interviews, the effects of parents are mainly negative. Most of the counselors believed that it is difficult to involve parents in the process of solving couples' problems. Sometimes due to their low literacy, age, and ignoring others' advice, it seems impossible to change parents decisions. Therefore, counselors focus on strengthening the independence of decision making by couples because dependent decision making may result in worsening the problems. Some studies showed the positive effects of education in decision making [31] [32], in steps to establish and stabilize the family, the importance of independent thinking is necessary [32].

Perceived behavioural control is one of the important variables effects on the ability of individuals to control their decision to divorce, as a behaviour. In this study, it included categories of social problems, others meddling, personality characteristics, and sexual problems of couples. The effects of these factors were also confirmed in other studies [33] [34]. Although social, economic and cultural problems are very influential on solidity and stability of the families, both directly and indirectly; people are not able to solve these problems and at most they, especially by learning problem-solving and decision-making at the primary school, can only reduce the negative effects of these problems on their family structure. Anyway, about those couples referring to the Family Counseling Center, education and counseling may improve their self-efficacy [35], since some characteristics or lack of knowledge reduce the couples' self-efficacy and self-esteem on solving problems and controlling the situation [36] (results of this section is independently studied in an article in print) [18].

According to the interviews, most of the couples, referred to the Center, fully intended to end their married life. However, a few of the divorce applicants only hoped to punish their spouses and force them to behave better. As the counsellors reported, bringing the spouse to the court will worsen the situation and lead to retaliatory actions by the other side.

Due to the sensitivity and difficulty of addressing the issue, it was difficult to obtain permission from the Judicature and the State Welfare Organization to conduct the research and interview the people involved in the divorce process. In many cases, couples and their parents reject our request for being interviewed. Moreover, the authors could not find similar studies in which the theory of planned behaviour was used to explain divorce. The family members' meddling in the process of divorce and their role in solving couples' problems are probably one aspect of Iranian culture reflected in the laws since in the studies on family conflicts in other countries, this is seen only before or after the divorce process.

In conclusion, the findings revealed that individuals' attitudes towards divorce were considered annoying and inevitable. This view was shaped under the influence of the family, particularly parents, and general social atmosphere. However, most of the codes drawn belonged to the perceived behavioural control which was affected by different personal and social problems. Participants felt that they had lost control over their lives; in other words, their self-efficacy had decreased.

According to the findings, it can be argued that a qualitative study on divorce is the best choice for researchers to investigate this issue. Conducting a qualitative study, using a finely determined protocol, designing proper questions based on the constructs of the theory, and also enrolling various groups involved in divorce process were the strengths of the present study. It seems that findings of this study can provide a suitable context to conduct similar studies, particularly by encouraging the researchers to use mixed and interdisciplinary, e.g., sociology and health education, methods and researchers.

## Ethical Approval

Participants' satisfaction and observance of their rights were confirmed at the ethical committee of Shahid Sadoughi University of Medical Sciences in Yazd with the code of ethics IR.SSU.SPH.REC.1395.43. Participants were informed of the research and were told that they had the right to withdraw at any time during the interview process or the study and that their names and interviews would be fully trusted.

## Authors' contributions

This was a part of PhD thesis of the first investigator (NA). (MAMS, SARA) supervised the development of work. (AV, SSMM and SAAR) Were the study advisors. All authors read and approved the final version.

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# Linguistic Validation and Cultural Adaptation of Bulgarian Version of Hospital Survey on Patient Safety Culture (HSOPSC)

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**Keywords:** Patient safety; HSOPSC; Linguistic validation; Cultural adaptation

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**Abstract**

**BACKGROUND:** Patient safety (PS) is one of the essential elements of health care quality and a priority of healthcare systems in most countries. Thus the creation of validated instruments and the implementation of systems that measure patient safety are considered to be of great importance worldwide.

**AIM:** The present paper aims to illustrate the process of linguistic validation, cross-cultural verification and adaptation of the Bulgarian version of the Hospital Survey on Patient Safety Culture (B-HSOPSC) and its test-retest reliability.

**METHODS:** The study design is cross-sectional. The HSOPSC questionnaire consists of 42 questions, grouped in 12 different subscales that measure patient safety culture. Internal con-sistency was assessed using Cronbach's alpha. The Wilcoxon signed-rank test and the split-half method were used; the Spear-man-Brown coefficient was calculated.

**RESULTS:** The overall Cronbach's alpha for B-HSOPSC is 0.918. Subscales 7 Staffing and 12 Overall perceptions of safety had the lowest coefficients. The high reliability of the instrument was confirmed by the Split-half method (0.97) and ICC-coefficient (0.95). The lowest values of Spearmen-Broun coefficients were found in items A13 and A14.

**CONCLUSION:** The study offers an analysis of the results of the linguistic validation of the B-HSOPSC and its test-retest reliability. The psychometric characteristics of the questions revealed good validity and reliability, except two questions. In the future, the instrument will be administered to the target population in the main study so that the psychometric properties of the instrument can be verified.

## Introduction

Patient safety (PS) is a key determinant of healthcare quality in medical facilities and is considered a priority in most countries [1] [2] [3]. The World Health Organization views PS as a global issue and has established a World Alliance for Patient Safety to promote international cooperation and facilitate the process of improving PS worldwide [4].

Studies from all over the world have documented that between 4% and 16% of all hospitalised patients are victims of medical errors, which are preventable to a great extent [5] [6]. Medical services are complex, specific, and not always predictable. Even if doctors and other care providers have proven medical expertise, and even if all applicable rules and procedures have been adhered to, undesirable event or complications could occur [7] [8].

To ensure that PS standards are followed and to establish breaches of these standards, the medical staff should be encouraged to share information,

regarding PS. A step forward in enhancing PS is the ISO 9001 certification of hospitals [9].

It has been proven by the World Alliance for Patient Safety that the instruments to measure improvements in medical practice are constantly evolving and advancing [10]. The Institute of Medicine (IOM) declares that patient safety is "indistinguishable from the delivery of quality healthcare" and therefore encourages the use of patient safety reporting systems (PSRS). The latter is very useful in evaluating the causal factors of harm to patients by medical care [11] [12]. Another issue of great importance is the elaboration of validated instruments and systems to measure PS in medical practice worldwide [1]. Experts from all over the globe are focused on creating instruments, able to measure the level of PS safety and to register adverse events/incidents or errors in medical practice [3]. The objective of collecting and analysing data is to ensure continuous learning and improvement quality of medical care. Unfortunately, on a global scale, the spectrum of validated instruments for measuring PS in hospital settings is limited. Moreover, presently, in Bulgaria, no such tools have been introduced and functioning [3].

In our country, in the specialised literature on PS, a single instrument has been described; however, collected data applying it are not comparable to those, from other countries [13].

An important contribution to the evaluation of hospital patient safety culture and quality assurance is the HSOPSC questionnaire, elaborated by the Agency for Healthcare Research and Quality (AHRQ). The questionnaire is based on selected reliable psychometric characteristics and has been validated in more than 20 countries [1] [2]. It contains 42 questions, grouped in 12 different sub-scales and allows reporting and registration of undesirable events and errors. It also provides information about some social and demographic factors as employment place and work position of the respondents. From 2007 to date, international databases offer annually, survey data from more than 600 hospitals in the USA [14].

The present paper aims to illustrate the process of linguistic validation and cultural adaptation of the Hospital Survey on Patient Safety Culture (HSOPSC) to Bulgarian healthcare settings and its test-retest reliability.

## Participants and Methods

The study design is cross-sectional. The translation, linguistic validation, and cultural adaptation of the B-HSOPSC questionnaire were carried out within the frame of the Medical University - Plovdiv's project №11/2016, named: "Introduction a web-based platform for registration and evaluation of hospital patient safety culture and conduction of a representative study for the country." Ethical approval was obtained from the University Research Ethics Committee (№ 05/19.10.2017). Each stage of the recommended protocol is presented in figure 1 and is described in details below. The English source version of the HSOPSC questionnaire was translated into Bulgarian (forward translation) by two independent translators (experienced healthcare professionals), with Bulgarian as a native language and excellent users of English as a second language.

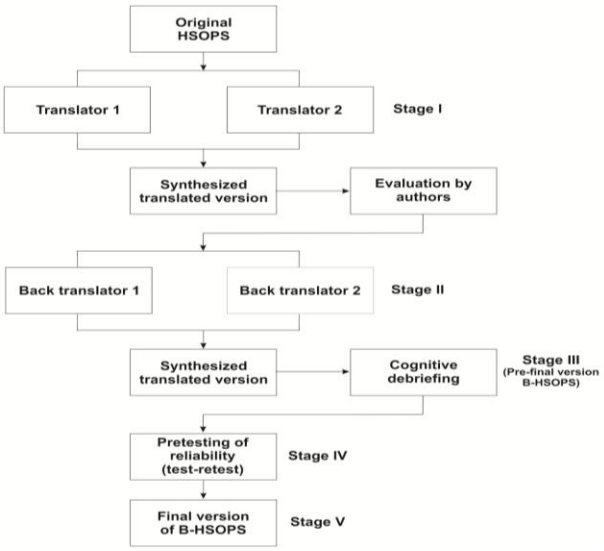


*Figure 1: Stages of cross-cultural adaptation of B-HSOPSC questionnaire*

The translators provided written reports on the decision-making process, the linguistic difficulties, and the encountered problems. The two Bulgarian translations were compared, and a synthesised version was created at a consensus meeting. Additionally, the Bulgarian text was compared with another two translated versions in Slavic languages (the language of both, the Macedonian and the Croatian version is very similar to the target language-Bulgarian). At the next stage, the synthesised version was back-translated into English by two professional translators, who had no access to the original English version of the questionnaire.

Cognitive interviews were carried out using concurrent think-aloud and probing techniques [15], attempting to collect information about potential problems in the B-HSOPSC questionnaire. At the 2-hour cognitive debriefing, the B-HSOPSC questionnaire was administered to an expert committee with 15 members, representing the studied population. The expert group included professionals of various medical specialities, a psychologist, language professionals and translators, and an occupational health specialist [16]. The respondents participating in the cognitive debriefing had equal representation of both genders. Moreover, they were representative of

the major characteristics of the target group and were experts in their professional field. All experts, after completion of the questionnaire, were interviewed by the local project manager. Interviews addressed each item in the B-HSOPSC questionnaire and checked if participants had indicated any difficulty understanding the questions or if they would phrase it differently. The expert committee had to assess whether the questionnaire words and phrases describe the same ideas or subjects in both, the original and the adapted version of the questionnaire. This assessment ensured that all items were properly translated and were relevant in the new setting. During the debriefing process, any discrepancies or uncertainties about the meaning of items were addressed. Participants were encouraged to propose alternative ways of rendering the meaning of the original. Based on the suggestions and interpretations (evaluated for conceptual equivalence) and equivalence (in construct operationalisation), as well as on the identified discrepancies between the original text and its translation, the Bulgarian version of HSOPSC questionnaire, B-HSOPSC, was created.

A general protocol was developed for the translation of instruments into Bulgarian; It included: description of each step in the translation process, a field test-retest study and psychometric characteristics analysis. Pre-test measurement was performed with a convenience sample of 150 respondents from the five university hospitals in Plovdiv. After consenting to participate, the sample group completed the questionnaire at work and returned it to the authors. The B-HSOPSC was resent to the same employees for completion four weeks later. Thus, the reliability of the initial answers was tested.

After analysing the test-retest results, the final version of B-HSOPSC questionnaire was created.

The data from all questionnaires were entered into the appropriate statistical software program. As items were worded in both positive and negative directions, negatively worded items (A5, A7, A8, A10, A14, A16, A17, B3, B4, C6, F2, F3, F5, F6, F7, F9 and F11) first were reverse coded (Table 1). To compare item scale scores obtained during the test and re-test, the Wilcoxon signed-rank test was applied. To evaluate intra-rater reliability, the split-half method was used, and Spearman-Brown coefficient was calculated ($r_{sb}$). An average inter-item correlation of at least 0.50 was regarded as good [17]. The intra-class correlation coefficient (ICC), using the test-retest method, was also used to estimate the inter-rater reliability to check consistency and reproducibility. Internal consistency was assessed using Cronbach's alpha. An alpha value of 0.60 was considered as the lowest acceptable value [17].

Data were processed, using the IBM SPSS Statistics 22 software. The level of statistical significance was set at $P < 0.05$.

## Results

Using standardised procedure, consistency in the content and face validity between the original HSOPSC instrument and the B-HSOPSC were ensured by cross-cultural adaptation [16]. To maintain the instrument content validity at a conceptual level across different cultures, the applicability of original HSOPSC items was checked at a cognitive debriefing interview. The group of interview participants had the following characteristics: the average age of 44.29 years, (SD 8.43); gender ratio M: F (n) was 3:12; employed (n)-15 and the average duration of current job position - 9 years (SD 5.43). The cognitive debriefing interview resulted in a revision of some items in the B-HSOPSC. The phrase in item A 15 *never sacrificed* was replaced by *never ignored*. The possible answers to the questions related to work position were adapted to the national healthcare setting. Questions about the hospital characteristics (teaching status, ownership, and geographic region) were added, as well as questions, investigating the attitude and willingness of respondents to report undesirable events if there is an anonymous communication system that guarantees their privacy. To protect respondents' privacy, other demographic characteristics related to the respondents were not included.

One hundred and fifty questionnaires were distributed, and all of them were returned. Questionnaires with missing data were excluded. Thus, data from 146 questionnaires were analysed.

The high reliability of the instrument was confirmed by the split-half method (0.97) and the ICC-coefficient (0.95). The Spearman-Brown coefficient for most items was satisfactory ($r > 0.70$), except questions A13 and A14.

The overall Cronbach's alpha for the B-HSOPSC questionnaire is 0.918. The internal consistency measured on the structure of 10 sub-scales showed that the Cronbach's alpha was above 0.70 for six of the sub-scales (sub-scales 1, 4, 6, 8, 10, 11), and ranged from 0.60 to 0.69 for the other four (sub-scales 2, 3, 5, 9). Sub-scales 7 *Staffing* and 12 *Overall perceptions of safety* had the lowest coefficients (Table 1).

The number of items and their content remained the same as in the original to a great extent, due to the consensus of the expert committee.

However, after the test-retest analysis, minor changes and adjustments were made to item 13 and item 14. In the course of the cognitive interview, some medical professionals argued that there was lack of conceptual validity for these two items in the Bulgarian setting.

**Table 1: Results from the test-retest reliability of the panel questionnaire among hospital employees (N = 146)**

| Questions | Wilcoxon test | Spearman-Brown coefficient ($r_{sb}$) | Cronbach's A I measu-rement | II measu-rement |
|---|---|---|---|---|
| ***Safety culture dimensions (unit level)*** | | | | |
| **1. Supervisor/manager expectations and actions promoting safety** | | | 0.779 | |
| B1 My supervisor/manager says a good word when he/she sees a job done according to established patient safety procedures | 1.136 | 0.803 | | |
| B2 My supervisor/manager seriously considers staff suggestions for improving patient safety | 0.302 | 0.921 | | 0.805 |
| B3 Whenever pressure builds up, my supervisor/manager wants us to work faster, even if it means taking shortcuts | 0.577 | 0.959 | | |
| B4 My supervisor/manager overlooks patient safety problems that happen over and over | 2.496 | 0.715 | | |
| **2. Organisational learning—continuous improvement** | | | 0.606 | |
| A6 We are actively doing things to improve patient safety | 0.367 | 0.803 | | |
| A9 Mistakes have led to positive changes here | 1.387 | 0.910 | | 0.607 |
| A13 After we make changes to improve patient safety, we evaluate their effectiveness | 0.052 | 0.228 | | |
| **3. Teamwork within hospital units** | | | 0.662 | |
| A1 People support one another in this unit | 0.632 | 0.936 | | |
| A3 When a lot of work needs to be done quickly, we work together as a team to get the work done | 0.879 | 0.928 | | 0.628 |
| A4 In this unit, people treat each other with respect. | 0.977 | 0.743 | | |
| A11 When one area in this unit gets busy, others help out | 1.027 | 0.757 | | |
| **4. Communication openness** | | | 0.776 | |
| C2 Staff will freely speak up if they see something that may negatively affect patient care | 0.351 | 0.919 | | |
| C4 Staff feel free to question the decisions or actions of those with more authority | 1.615 | 0.906 | | 0.770 |
| C6 Staff are afraid to ask questions when something does not seem right | 1.977 | 0.738 | | |
| **5. Feedback and communication about error** | | | 0.611 | |
| C1 We are given feedback about changes put into place based on event reports | 0.877 | 0.773 | | |
| C3 We are informed about errors that happen in this unit | 1.100 | 0.840 | | 0.633 |
| C5 In this unit, we discuss ways to prevent errors from happening again | 0.185 | 0.834 | | |
| **6. Non-punitive response to error** | | | 0.697 | |
| A8 Staff feel like their mistakes are held against them | 1.052 | 0,770 | | |
| A12 When an event is reported, it feels like the person is being written up, not the problem | 1.136 | 0,910 | | 0.748 |
| A16 Staff worry that mistakes they make are kept in their personnel file | 0.243 | 0,903 | | |
| **7. Staffing** | | | 0.304 | 0.331 |
| A2 We have enough staff to handle the workload. | 1.387 | 0.936 | | |
| A5 Staff in this unit work longer hours than is best for patient care | 3.231 | 0.707 | | |
| A7 We use more agency/temporary staff than is best for patient care | 0.416 | 0.769 | | |
| A14 We work in 'crisis mode', trying to do too much, too quickly | 1.464 | 0.318 | | |
| **8. Hospital management support for patient safety** | | | 0.855 | |
| F1 Hospital management provides a work climate that promotes patient safety | 0.577 | 0.979 | | |
| F8 The actions of hospital management show that patient safety is a top priority | 0.000 | 0.952 | | 0.860 |
| F9 Hospital management seems interested in patient safety only after an adverse event happens | 0.707 | 0.952 | | |
| ***Safety culture dimensions (hospital-wide)*** | | | | |
| **9. Teamwork across hospital units** | | | 0.694 | |
| F4 There is good cooperation among hospital units that need to work together | 0.302 | 0.865 | | |
| F10 Hospital units work well together to provide the best care for patients | 0.905 | 0.909 | | 0.653 |
| F2 Hospital units do not coordinate well with each other | 0.992 | 0.835 | | |
| F6 It is often unpleasant to work with staff from other hospital units | 0.511 | 0.832 | | |
| **10. Handoffs and transitions** | | | 0.854 | |
| F3 Things "fall between the cracks" when transferring patients from one unit to another | 0.284 | 0.879 | | |
| F5 Important patient care information is often lost during shift changes | 2.516 | 0.860 | | 0.918 |
| F7 Problems often occur in the exchange of information across hospital units | 0.570 | 0.804 | | |
| F11 Shift changes are problematic for patients in this hospital | 0.577 | 0.909 | | |
| ***Outcome measures*** | | | | |
| **11. The frequency of event reporting** | | | 0.823 | |
| D1 When a mistake is made but is caught and corrected before affecting the patient, how often is this reported? | 0.894 | 0.885 | | 0.864 |
| D2 When a mistake is made but has no potential to harm the patient, how often is this reported? | 0.814 | 0.891 | | |
| D3 When a mistake is made that could harm the patient, but does not, how often is this reported? | 1.363 | 0.839 | | |
| **12. Overall perceptions of safety** | | | 0.331 | |
| A15 Patient safety is never sacrificed to get more work done | 0.486 | 0.913 | | |
| A18 Our procedures and systems are good at preventing errors from happening | 1.291 | 0.872 | | 0.339 |
| A10 It is just by chance that more serious mistakes don't happen around here | 0.720 | 0.868 | | |
| A17 We have patient safety problems in this unit | 1.115 | 0.780 | | |
| | | | - | |
| ***Patient safety grade*** | | | | |
| E1, Please give your work area/unit in this hospital an overall grade on patient safety | 0.577 | 0.974 | | - |
| | | | - | |
| ***Number of events reported*** | | | | |
| G1 In the past 12 months, how many event reports have you filled out and submitted? | 1.732 | 0.963 | | - |

In item A 14 the term *crisis model* was substituted by the phrase *working under conditions of insufficient resources*, and the wording of item A 13 *After we execute changes to improve patient safety, we evaluate their effectiveness* became *After we execute changes to improve patient safety, we evaluate whether they lead to positive results*. At that stage, no changes to sub-scale 7 and sub-scale 12 were made. Following statistical analysis of accumulated data from the future national representative study through a web-based platform and evaluation by the expert committee, some of the questions might be excluded. Eventually, the final version of B-HSOPSC may be amended.

## Discussion

Other researchers, under similar circumstances, have reached unsatisfactory values of Cronbach's alpha in certain sub-scales [18] [19] [20] [21] [22] [23] [24] [25]. Most often, low values are reported in sub-scale 7 *Staffing* (Table 2) [18] [20] [21] [24].

**Table 2: Values of Cronbach's α across original dimensions in the questionnaire and comparison to US, English, Dutch, Croatian, Portuguese, French and Slovenian data**

| Dimensions | № of items | Cronbach's α US | UK | Dutch | Croatian | Portuguese | French | Slovenian | Bulgarian |
|---|---|---|---|---|---|---|---|---|---|
| Supervisor/manager's expectations and actions regarding safety | 4 | 0.75 | 0.68 | 0.70 | 0.79 | 0.72 | 0.83 | 0.74 | 0.81 |
| Organisational learning—continuous improvement | 3 | 0.76 | 0.66 | 0.57 | 0.53 | 0.71 | 0.59 | 0.36 | 0.61 |
| Team work within hospital units | 4 | 0.83 | 0.73 | 0.66 | 0.76 | 0.73 | 0.63 | 0.74 | 0.63 |
| Communication openness | 3 | 0.72 | 0.67 | 0.72 | 0.64 | 0.67 | 0.62 | 0.74 | 0.77 |
| Feedback and communication regarding errors | 3 | 0.78 | 0.80 | 0.75 | 0.74 | 0.76 | 0.64 | 0.72 | 0.63 |
| Non-punitive response to errors | 3 | 0.79 | 0.65 | 0.69 | 0.62 | 0.57 | 0.57 | 0.61 | 0.75 |
| Staffing | 4 | 0.63 | 0.58 | 0.49 | 0.35 | 0.48 | 0.46 | 0.65 | 0.33 |
| Hospital management's support for patient safety | 3 | 0.83 | 0.69 | 0.68 | 0.73 | 0.77 | 0.73 | 0.82 | 0.86 |
| Teamwork across hospital units | 4 | 0.80 | 0.70 | 0.59 | 0.79 | 0.69 | 0.59 | 0.74 | 0.65 |
| Handoffs and transitions | 4 | 0.80 | 0.77 | 0.68 | 0.64 | 0.71 | 0.66 | 0.66 | 0.92 |
| Frequency of event reporting | 3 | 0.84 | 0.83 | 0.79 | 0.91 | 0.90 | 0.84 | 0.88 | 0.86 |
| Overall perceptions of safety | 4 | 0.74 | 0.67 | 0.62 | 0.57 | 0.62 | 0.67 | 0.65 | 0.34 |

In Croatia, researchers report very low values of Cronbach's alpha for sub-scale 7 *Staffing* (0.35), which is similar to our findings [18]. Croatian authors speculate that some items might have been interpreted differently due to some specific national characteristics. They refer particularly to question A7, dealing with issues regarding employment of locum staff through the medical agencies to cope with work overload. As this practice is unusual in most European health systems, question A7 had to be revised and adapted to the specifics of the respective national healthcare system [18]. Eiras at al., show that, once question A7 is excluded, the internal consistency in sub-scale 7 *Staffing* increases from 0.48 to 0.57 [19]. The practice of recruiting locum agency staff is not

popular in Bulgaria, regardless of the fact that it is legally allowed.

Most of the items were found to be valid, yet the staffing sub-scale had rather low reliability, as revealed by research in the USA [26]. Other researchers examining the psychometric properties of the HSOPSC found that the items had acceptable psychometric properties except for the staffing subgroup and questions regarding supervisor/ manager's expectations and actions promoting PS [27].

Our study has some limitations. The convenience sampling method was used for the healthcare professionals' selection in the test-retest study. In fact, the linguistic validation and the cross-cultural adaptation of the B-HSOPSC questionnaire made very few factual amendments to the original. We assume that the result is not skewed regarding overall patient safety culture evaluation.

The present paper examines the development of a translation protocol and the use of cognitive debriefing as part of the cultural adaptation process. We assessed the semantic, idiomatic, experiential, and conceptual equivalence between the source and the target questionnaire. During the field testing, the psychometric characteristics of the questions exhibited good validity and reliability, except for a couple of two items. In the future, the instrument will be administered to the target population in the main study so that the psychometric properties of the instrument can be verified. Therefore, the comparison between our studies results regarding health care professionals' evaluation of patient safety and the results of other similar studies possible.

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# Epidemiology of Hand Burn in Albania 2011-2016

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**Abstract**

**INTRODUCTION:** Hand burns occur commonly both as part of larger burn injuries as well as isolated injuries.

**AIM:** To give an overview of epidemiologic features and outcome of burn hand patients who admitted to our Service. This study was performed at University Hospital Center "Mother Teresa" Tirana which is the only tertiary hospital in Albania.

**MATERIAL AND METHODS:** This prospective study included all patients who had combustion of the hands solely or hands accompanied with burns to other areas of the body, treated and followed up at our service during the years 2011-2016.

**RESULTS:** Of the 333 included burn patients, 64% were males. The median age of patients is 25.9 years. About half of patients belong to the age group 20-60 years (49.5%) and only 10.2% belong to the age of 60 years. In most of the cases (73.6%), the burn of hands is associated with the burn of the other anatomical region, mostly forearm.

**CONCLUSION:** The surgical treatment is used for less 30% that of patients. The undesirable results of the burn of hands are presented in the 33% of the patients. The contractures were the main unfavourable outcome of the burned hand.

## Introduction

Burns is the fourth most frequent traumatic cause in the world, following accidents, crashes or violence [1]. Burns are a major cause of global damage. The World Health Organization estimates an incidence of 1% and more than 300,000 people die each year from combustion-related fires [2].

In high-income countries, there is a reduction in the incidence of burns, the severity of burns, hospitalisation and mortality rates. But the prevalence of combustion is higher in developing countries than in developed countries, and this is related to the survival rate of burning patients in developed countries closely related to providing first aid and appropriate treatment after initial assessment [3] [4].

The adults are most likely to burn at home, outdoors or at work. Burning in adult males occurs more often outdoors and at work, while women usually live at home. At home, the most common cause is cooking since it is the most common activity. Greater older people often experience burns in the bathroom and then in the kitchen, while children are more often burned in the home environment (84%), and in 80% of cases of burns, children are rich. Due to damage to the skin and other organs, burning can lead to open wounds, disabilities, deaths and major economic consequences, deeply psychological and emotional complications [3].

Therefore, burning patients require not only acute treatment but also subsequent rehabilitation, reconstruction and long-term anti-scar therapy. Although 90% of combustion is preventable, burns

remain a major public health problem [5]. To improve the effects of preventive measures, it is necessary to recognise epidemiologic features and the unwanted frequency outcome in post burning phase.

## Material and Methods

In this prospective study were included all patients who had combustion of the hands solely or hands accompanied with burns to other areas of the body, treated and followed up at the Burns and Plastic Service of the University Hospital Center "Mother Teresa", Tirana, Albania during the years 2011-2016.

For each patient were collected demographic data (age in years, sex), anatomical region, an association of burned hand with other burned areas, the treatment used (surgical or conservative) unwanted post-burning outcomes (contractures, ulcerations, etc.). This study was accepted by the ethical committee of Tirana University of Medical Sciences, Tirana, Albania. All continuous variables were presented as means±SD, and the frequencies of categorical variables were presented as percentages. Chi-square test was used to compare the proportions of categorical variables and student *t*-test to compare the mean of continuous variables. AP value<0.05 was considered significant.

## Results

The study included 333 patients with the burning of hands (Table 1).

**Table 1: Summary statistics of age by gender**

| Gender | Age, years | | | | | | |
|---|---|---|---|---|---|---|---|
| | N | Mean | SD | Min | Max | Median | IQR |
| Female | 119 | 27.8 | 23.2 | 1 | 91 | 21.8 | 4.7 – 43.9 |
| Male | 214 | 31.2 | 21.4 | 0 | 91 | 27.6 | 13.0 – 50.5 |
| Total | 333 | 29.9 | 22.1 | 0 | 91 | 25.9 | 11.5 – 49.1 |

Of the subjects selected, 214 (64%) are males, and 36% are females. The median age of women in years, of men, is 27.5 years, and the median age total patients' population is 25.9 years. In total, almost half of patients belong to the age group 20-60 years (49.5%) and only 10.2% belong to the age of 60 years (Table 2). There is a statistically significant difference in the distribution of patients by age group and gender, with males predominating in the age group 20-60 years (55.1%) ($p < 0.01$).

**Table 2: Distribution of patients by age group and gender**

| Anatomical region | N | % |
|---|---|---|
| Hand and forearm | 113 | 34.0 |
| Hand and face | 81 | 24.3 |
| Hand only | 88 | 26.4 |
| Hand and different regions | 51 | 15.3 |
| Total | 333 | 100.0 |

Based on the anatomical region, it is noted that the hand solely is affected in almost 26.4% of cases, the hand and forearm in 34% of cases, hand and face and 24.3% of cases. In 15.3% of cases, the patients had burns of the body, the gluteal region and the lower extremities (Table 3).

**Table 3: Distribution of cases according to an anatomical region**

| Anatomical region | N | % |
|---|---|---|
| Hand and forearm | 113 | 34.0 |
| Hand and face | 81 | 24.3 |
| Hand only | 88 | 26.4 |
| Hand and different regions | 51 | 15.3 |
| Total | 333 | 100.0 |

Of the 333 patients with burned hands, only 98 (29%) of them underwent surgical treatment of the wounds (95% CI 24.4–34.1).

In total, after treating 333 patients with hand burn, 102 patients or one in three patients showed undesirable results such as contractures of various levels, ulcerations, syndactyly or keloid (Table 4).

**Table 4: Distribution of unwanted effects**

| Unwanted outcome | N | % |
|---|---|---|
| Dorsal web-space contracture | 28 | 28.1 |
| Volar contracture | 16 | 6.6 |
| Fingers' contracture | 22 | 22.9 |
| Ulceration/wounds | 27 | 27.1 |
| Syndactyly | 7 | 7.4 |
| Keloid | 2 | 2.1 |

In most cases, undesirable outcomes are dorsal web-space contracture (28%, and ulceration/wounds (27.1%) ($p < 0.01$). Syndactyly and Keloid are rarely found.

## Discussion

Investigating combustion epidemiology and unwanted outcomes are important for assessing the effects of preventive and treatment measures on burning. The findings of our study represent the epidemiological situation in Albania, considering that the plastic and burns department at the university hospital centre is the only tertiary service specialised in the treatment of combustion. Burns occur at all ages, including pediatric and advanced age, defining burning an unintended injury with a

very broad age range. Burning is a disability that is often encountered in working age. This study's data show that the average age is approximately 30 years and that the age group most affected is 20-60 years old. The age of over 60 is less affected by the fact that in this age group the individual is less active and less exposed to the risk factors for combustion. Men have a higher percentage of burns in all age groups and this is explained by the fact that they are more exposed to different etiologic factors of burning such as fire, electricity etc. while women are more exposed to cooking facilities with a low likelihood of burning [3] [6] [7] [8] [9]. Thus, adult males are more likely to have burns of hands according to the experience at our clinic. Among women, burning is most common in the age group <20 years. This finding is supported by the fact that the average age of women who have had burns is lower. According to the World Health Organization, this is explained by the fact that young girls are often involved in housework and caring for younger children by placing them in the kitchen facilities and increasing the risk of burns due to carelessness [3].

In most cases, hand burning is accompanied by burns in the forearm, and only one in four people have solely hand burns. Cases, when burns of the hand are associated with burns to other anatomic regions, are rarer. Often, the left hand is most affected than right one, and rarely both hands. Surgical treatment was applied in 29% of patients, and the undesired outcome was evidenced in nearly one-third of the cases involved in the study, which is consistent with studies reported in the literature [10] [11]. The most common unwanted post-burn outcomes were contractures and ulcerations/wounds. In 50% of cases were reported most commonly dorsal and finger contractures and less are keloids or syndactyly. The findings of the study are similar to *Salisbury's et al.,* study [12] on post burning deformities in upper extremities with hand and finger contractures being more frequent, as well as the study conducted in Kosovo in which the contractures also rank high as the most common unwanted outcome of burned hand [13].

In conclusion, burns are injuries commonly encountered in adults. According to gender, males are more likely to have burns while among women burn occurs more frequently under the age of 20 years. Combustion of the hands is usually associated with burning injuries to other anatomic areas of the body, especially the forearm. Most hand burns do not require surgical treatment, but almost one in three patients who have suffered from hand burns experience unwanted results in the post-burning stage. According to our study, the most common unwanted outcome is hand or finger contractions in almost half of the cases.

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# Mathematical Model for Forecasting the Influence of Atmospheric Pollution on Population Morbidity in Stara Zagora Municipality (Bulgaria)

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**Abstract**

**AIM:** This paper aims to create a mathematical model for forecasting the morbidity of the population in the Republic of Bulgaria and the Stara Zagora Municipality in particular as a consequence of the atmospheric pollution.

**SUBJECTS AND METHODS:** This model is based on a formula which determines the correlation between the average annual concentrations of atmospheric pollutants $SO_2$, $PM_{10}$, Pb aerosols, $NO_2$ and $H_2S$) and the morbidity of the population based on the number of people who visited their GPs in a relation with a chronic health problem or emergency condition and the number of hospitalisations in two age groups (newborn to 17 years olds and 18 and older) as well as for the entire population in the period 2009-2013, making it possible to predict morbidity levels.

**RESULTS:** The expected morbidity level predictions based on the number of people who visited their GPs in Municipality are lower, while hospitalisation level predictions are higher. This model has been created and tested and is applicable in all residential areas.

**CONCLUSIONS:** A new, very sensitive, mathematical model has been created and tested (average margin of error from 0.61% to 2.59%) and is applicable in all residential areas.

## Introduction

Stara Zagora Municipality is situated in the central part of South Bulgaria, in the Upper Thracian Plain, on the south hills of Sredna Sarnena Gora mountain (N 42° 25 ', E 25° 37'). It covers an area of 1019.1 square km and is located at 240 m above sea level. It comprises 52 towns and villages with a total of 156,662 residents, 137,834 of whom live in the city of Stara Zagora. Stara Zagora Municipality is amongst the most industrially developed municipalities in Bulgaria, which is the main cause of air, water and soil pollution. One of the key threats to public health associated with the environment in Stara Zagora is atmospheric air pollution. Its protection is governed by the Clean Air Act [1] as well as by its associated regulations – Regulation No. 14/1997 [2]; Regulation No. 11/2007 [3]; Regulation No. 12/2010 [4], where all Bulgarian rules and regulations are harmonised with those of the EU (Directive 2008/50/EC). Atmospheric air quality monitoring and control are carried out throughout the year by the Regional Inspectorate of Environment and Water Directorate.

In the past 20 years, the atmospheric air in Stara Zagora Municipality has been amongst the most polluted in the country [5] [6] [7]. The atmospheric air quality in the municipality is contingent upon the operation of numerous local (industrial enterprises, domestic heating, heavy traffic) and regional (the

three thermal power stations of the Maritza East power complex situated 40 km south of Stara Zagora and Zmeevo, a military testing ground 7 km to the southwest) emission sources. These sources mainly generate sulphur dioxide ($SO_2$), breathable $PM_{10}$, nitrogen dioxide ($NO_2$), lead aerosols and hydrogen sulphide ($H_2S$). Two trans-European corridors go through the territory of the municipality. These characteristics coupled with weather conditions and the topography of the area facilitate the migration of pollutants and affect the morbidity of the population. An ecological task of top priority is to prevent atmospheric air pollution from the major pollutants ($PM_{10}$, sulphur and nitrogen oxides, ozone), given their role as a risk factor in the aetiology, pathogenesis and spreading of some diseases (cardiovascular diseases, respiratory diseases etc.)

The purpose of this study is to analyse air pollution and its impact on the morbidity amongst the population of Stara Zagora Municipality in the period 2009 - 2013, as well as to create a mathematical model of predicting morbidity levels.

## Subjects and Methods

On the territory of Stara Zagora Municipality, sample-taking and analyses of the atmospheric pollutants (sulphur dioxide, $PM_{10}$, nitrogen oxides, lead aerosols and hydrogen sulphide) are conducted in an automatic measuring station, two differential optical absorption spectroscopic systems and one unit for the manual taking of samples. Standards in compliance with European law are used in the analysis of the pollutants. Information about the morbidity based on the number of people who visited their GPs in a relationship with a chronic health problem or emergency condition and the number of hospitalisations has been obtained from the Regional Health Inspectorate – form 365, 1A, appendices 5 and 6. The study covers the two age groups (from 0 to 7-year-olds, 18 and older) and the entire population of Stara Zagora Municipality.

The influence of atmospheric pollution on the morbidity of the population in Stara Zagora Municipality in the period 2009-2013 is based on disease class according to the International Classification of Diseases, revision X.

The correlation between the examined atmospheric pollutants and morbidity can be calculated using the following formula:

$$Y_i = e^{\varepsilon i} K_0 \prod_{j=1}^{n} X_{i;j}^{Kj}$$

Where:

$Y_i$ - the dependant variable (result = the number of predicted diseases);

$i$ - the year;

$n$ – number of factors;

$X_{i;j}$ - the value of factor j for the period i;

$K_j$ – powers (coefficients) – the constants of each dependence;

$K_0$ - the module (the equaliser of the dimensions of each dependence);

$\varepsilon_i$ - statistical error.

Module $K_0$ and the powers $K_j$ are obtained using an integral exponential function by applying the least squares method used by Frenkely and described in Multifactor correlation model for labour productivity [8].

In this particular case, the factors (pollutants) are $X_{i;1}$-$SO_2$, $X_{i;2}$-$PM_{10}$, $X_{i;3}$-$NO_2$, $X_{i;4}$-Pb aerosols). The magnitude of each factor ($X_i$) is calculated in μg/m$^3$.

## Results

The analysis of systemically controlled pollutants ($SO_2$, $PM_{10}$, $NO_2$, $H_2S$ and lead aerosols) by months and years for the period 2009-2013. Showed that the mean monthly concentrations of $SO_2$, $NO_2$, $H_2S$ and lead aerosols did not exceed the permissible levels. The only observed pollutant in which there are cases of exceeding mean monthly concentrations relative to the threshold value is $PM_{10}$. At only one of the measurement points, all $PM_{10}$ average annual $PM_{10}$ values for 2009, 2010 and 2011 are above the limit value of 40 μg/m$^3$. Contributing to this is probably the lively traffic on the boulevard where the points are located. About the annual average concentrations, the results for all atmospheric pollutants indicate that they are within the boundaries of the norm and safety for human health. The complex assessment of atmospheric pollution in the municipality of Stara Zagora shows a slight fluctuation of the aggregate indices by years, without any sudden changes, and confirms the tendency to reduce the systemically observed pollutants: sulfur dioxide, $PM_{10}$ nitrogen oxides, lead aerosols and hydrogen sulphide for the period 2009-2013.

As regards the morbidity based on the number of people who visited their GPs in a relationship with a chronic health problem or emergency condition, the greatest share for the entire population take the cardiovascular diseases (22.99%). They are more frequent (27.77%) in the age group of 18 and older, or more as compared to the age group 0-17 (0.97%). Second are respiratory diseases

(17.34%) for the entire population. In age group 0-17, the relative share is rather high, 47.20%. Third, come to the diseases of the genitourinary system (9.76%).

As regards incidence of diseases that require hospitalisation, the leading diseases with almost the same share are the diseases of the digestive system (11.90%) and cardiovascular diseases (11.85), followed by respiratory diseases (10.86%) and genitourinary systems (8.88%). In age group 0-17, the greatest share is the share of the respiratory diseases (36.93%). Most common in this disease class is pneumonia (82.44%). Children most frequently suffer from infectious diseases where disturbed immune balance is observed. Cardiovascular diseases are most common amongst adults (14.26%).

During the examined period, there is a tendency in Stara Zagora Municipality towards decreasing the incidence of diseases based on the number of people who visited their GPs in a relationship with a chronic health problem or emergency condition and an increase based on hospitalisation as compared to the base year (2009).

The correlation between atmospheric pollution and the morbidity of the population as well as tracking the influence of each pollutant on morbidity is determined using the values of the mathematical expression $X_{i;j}^{Kj}$ based on the mathematical model used. The data analysis after the computations shows that $PM_{10}$ has the greatest influence on the morbidity of the population in Stara Zagora Municipality based on the number of people who visited their GPs in a relationship with a chronic health problem or emergency condition and the number of hospitalisations. The correlation is particularly strong in the age group 0-17 by the number of people who visited their GPs in a relationship with a chronic health problem or emergency condition ($r = 0.762$, $p < 0.05$).

Sulphur dioxide exhibits a positive correlation with morbidity based on the number of hospitalisations in the age group 18 and older at the beginning of the studied period (2009-2013) ($r = 0.430$, $p < 0.05$), and at the end of the period it is negative. Lead aerosols too have a pronounced influence on morbidity based on the number of hospitalisations amongst the population aged 18 and older in Stara Zagora Municipality ($r = 0.501$, $p < 0.05$).

We found the additive action of some pollutants as $SO_2$+$PM_{10}$ on malignant diseases for the whole population by hospitalisation [9] [10] [11] [12] [13] and cardiovascular diseases for the whole population is affected by the additive effect of $PM_{10}$+Pв aerosols [14] [15] [16].

After applying the Least squares method on the morbidity based on the number of hospitalisations and on the number of people who visited their GPs in a relationship with a chronic health problem or emergency condition amongst the entire population in Stara Zagora Municipality, the following coefficients are obtained as an independent variable:

$K_0\, e^{19.7723}$ $K_1$ - 0.1039 $K_2$ - 1.8949 $K_3$ - 1.5106 $K_4$ - 0.2254

The following formula will be used to calculate the predicted future morbidity count:

$$Y_{prognosis} = e^{19:7723} X_{i,1}^{0:1039} X_{i,2}^{1:8949} X_{i,3}^{1:5106} X_{i,4}^{0:2354}$$

The result calculated based on the formula above makes it possible to forecast morbidity with a significant precision-the average margin of error for the studied period ranges from 0.61 to 2.59%. The expected morbidity level predictions based on the number of people who visited their GPs in a relationship with a chronic health problem or emergency condition in Stara Zagora Municipality are lower, while hospitalisation level predictions are higher as compared to the numbers obtained during the period of the study (Table 1).

**Table 1: Registered and projected morbidity of the population in Stara Zagora Municipality by the number of people who visited their GPs in a relationship with a chronic health problem or emergency condition and the number of hospitalisations for the period 2009-2013**

| Year | Number of registered diseases | Number of projected diseases | Error for the year % |
|---|---|---|---|
| | Diseases by the number of people who visited their GPs in a relationship with a chronic health problem or emergency condition | | |
| 2009 | 333425 | 105396 | 0.68 |
| 2010 | 339482 | 196226 | 0.42 |
| 2011 | 402741 | 108831 | 0.72 |
| 2012 | 321726 | 141328 | 0.56 |
| 2013 | 321968 | 104115 | 0.67 |
| | The average margin of error for the period = 0.61% | | |
| Year | Number of registered diseases | Number of projected diseases | Error for the year % |
| | Diseases by the number of hospitalisations | | |
| 2009 | 34038 | 105396 | 2.09 |
| 2010 | 37277 | 196226 | 4.26 |
| 2011 | 37194 | 108831 | 1.92 |
| 2012 | 32019 | 141328 | 3.41 |
| 2013 | 45777 | 104115 | 1.27 |
| | The average margin of error for the period = 2.59% | | |

## Discussion

The majority of cardiovascular diseases can be explained by the fact that people older than 65 are more vulnerable as regards hypertonic disease, cerebrovascular diseases and ischemic heart disease. These diseases, in particular, indirectly associated with air pollution, seasons and climate factors, increase the possibility of the occurrence of thrombosis [17] [18] [19]. The respiratory system is affected to no lesser extent, and data shows that it is the most sensitive to atmospheric pollutants and children are the most sensitive group [20] [21] [22]. This is correlated to the immature immunity, high vulnerability and the body's predisposition to different respiratory diseases, especially in the early childhood years. Frequent pneumonia in hospitalised children

are influenced by meteorological factors, which is confirmed by the studies of some authors [23] [24]. It is established that atmospheric pollutants have a suppressive effect on the production of immunoglobulin for this age group [25] [26] [27].

Similar to our observations of the effect of nitrogen dioxide on the respiratory system, described Nickmilder M. et al., 2007 which establish the inflammatory changes in the airways, but they bind them to the low-level contamination with nitrogen oxides and ozone [28].

Numerous studies of this age group show a positive relationship even between low concentrations of lead aerosols and damage to hemopoiesis, nervous tissue, hearing, changes in neurobehavioral functions, and intellectual [29] [30] [31] [32]. Reports of blocking the bioactivity of porphyrin synthesis enzymes, impaired haem- synthesis and the development of anemia by the action of lead is found in research by de Carvalho RP et al., 2006; Zimmermann M. B. et al., 2006; Muwakkit S. et al., 2008; Rondó P. K. et al., 2011, Park S. et al., 2014 [33] [34] [35] [36] [37]. A lot of studies show a relationship between atmospheric pollution and malignant diseases. The analyzing of the data in morbidity by the number of people who visited their GPs in a relation with a chronic health problem or emergency condition of the groups from 0 to 17 years, 18 and more and the whole population shows high correlation dependence between sulphur dioxide and the increasing of bladder and lung cancer leading to high mortality [38] [39] [40] [41]. For the influence of $PM_{10}$ on all diseases, report Raaschou-Nielsen O. et al., 2013; Ponomareva L. A et al. V.V. Inogamova, 2013; Jevtić M. et al., 2014 [42] [43] [44]. Some factors like quality of water, food and feeding can affect the occurrence of diseases of the genitourinary system.

Using the mathematical model formula, it is possible to calculate the predicted value of the number of diseases based on all average annual concentrations of the pollutants for the years in question. The formula is a multiplication product because all pollutants together affect the morbidity and since the concentrations of each factor (pollutant) change over time, an integral exponential function is included too. The calculation of the average development rate for the different pollutants makes it possible to predict the level of atmospheric pollution for each coming year and, hence, the morbidity for the same period. The decrease in atmospheric pollution for the studied period is associated with the commissioning and effective operation of desulphurisation in Maritza East power complex, the efforts directed toward maintaining the road infrastructure, the gasification of Stara Zagora City, reduced use of wood and coal for heating in the municipality, the use of more environmentally-friendly fuels. Determining the projected morbidity levels confirms the tendencies in the dynamics and structure of morbidity based on the number of people who visited their GPs in a relationship with a chronic health problem or emergency condition and the number of hospitalisations amongst the population in Stara Zagora Municipality for the period 2009-2013.

In conclusion, the main methods of medical, mathematical statistics (regression, correlation analysis etc.) do not make it possible to cover fully and precisely the actual correlations between the different factors and the result. In fact, these connections are non-linear which is why it is necessary to use a multi-factor correlation model with non-linear dependences. The functions used in the current study constitute a connection between two factors (atmospheric pollutant and morbidity) and exclude the interaction of all factors. The proposed dependence provides a theoretical model of the interaction, which is quite close to the real one. Determining the dependences makes it possible to realise and control the fluctuations of the result through the impact of one factor or another. A disadvantage of the model can be assumed to be the implicit nature of the functional interdependencies. In reality, this does not have a considerable effect on the practical application of the model in every residential area.

Using the created mathematical model and the atmospheric pollution data, it is possible to forecast the morbidity in every residential area.

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# Prevalence Refractive Errors among Medical Students of Qassim University, Saudi Arabia: Cross-Sectional Descriptive Study

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**Keywords:** Errors of refraction; Myopia; Hyperopia; Young adults

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**Abstract**

**OBJECTIVE:** To study the prevalence of various errors of refraction among the medical students studying at the college of medicine, Qassim University, Saudi Arabia.

**METHODS:** This is a cross-sectional descriptive study conducted at Qassim University clinics over a period of two months. The study population comprised 162 male and female students from different academic years. The students were selected randomly so that around 35-40 students were taken from each academic class. The selected study population was explained the objectives of the study and a written consent form that stated the purpose, methods, risks, benefits, and the assurance of the confidentiality of the data was obtained from each student. After giving the consent, each subject was examined by auto refractometer. The examination was carried out by an optometrist without using cycloplegia. Both right and left eyes were thoroughly examined by auto refractometer and on the average three readings of the refraction measurements were taken. The readings were recorded on a data sheet of every individual, and the Statistical analysis was performed using Statistical Package for Social Sciences (SPSS).

**RESULTS:** One hundred and sixty-two (162) students with a mean age of 22.44 years, Std 1.661 and a range of 8 (19-27) were included in the study. Of the total number, 111 (68.51%) were males and remaining 51 (31.48%) were females. Of the total sample, only 1 (0.617%) student had diabetes mellitus, and 6 (3.70%) students gave a history of previous ocular surgery. Myopia was found to be the commonest error of refraction 53.7% with hyperopia next to it.

**CONCLUSION:** Myopia is found to be a common error of refraction in young adults. A regular checkup is essential to timely correct the error and to prevent deterioration of the vision.

## Introduction

Eyes have been defined as the ‘windows of the soul’. The International Agency for the Prevention of Blindness and World Health Organization have been declared this by starting the global initiative known as ‘VISION 2020’ for reducing of preventable blindness [1] [2]. Errors of refraction particularly myopia, have now become common and are continuously on rising globally [2]. Poor vision is a major limitation for a sound and enlighteningly supporting school/college condition for understudies in various areas of the world. Visual impedance because of uncorrected refractive errors is exceptionally common among youthful grown-ups and is the second most common reason behind treatable visual hindrance [4]. The world's driving and most easily cured solution for poor vision among youthful grown-ups are refractive errors [5]. Pascolini D, [6] mentioned that visual weakness is a noteworthy medical issue and 80% of the reasons for this infirmity are viably preventable. Refractive errors now are turning into a huge issue in numerous countries globally. The incidence rate of myopia is beginning to increment particularly in Asian nations achieving pestilence levels [7]. Bourne RR et al., [8] claim uncorrected errors of refraction to be among the most

common causes of visual disability all over the world. Refractive errors can be a weight on nations' economy particularly the developing ones. In any case, it can be remedied by utilising a contact lens or power glasses. Many investigations have demonstrated a solid connection between the level of intelligence with years of school participation and the seriousness of myopia [9] [10] [11] [12]. A larger population is seemingly unaware of the problem, and this leads to a progressive visual inadequacy which seriously affects their potential. This applies very clearly to the young adults who are studying in their schools, colleges, and universities and suffer because of poor vision due to common errors of refraction [4] [5] [6] [7] [8] [9] [10] [11] [12].

Therefore, the present study was designed to study the prevalence of various errors of refraction among the medical students studying at the college of medicine, Qassim University, Saudi Arabia. The data collected from this study help to increase the awareness of refractive errors and to enhance vision associated promotions to reduce the refractive errors not only among medical students but also students from other colleges.

## Methods

It's a cross-sectional study conducted over two months in a sample of 162 students from the college of medicine, Qassim University, KSA. The induction of the study population was by random selection from different academic years. The selected study population was explained the objectives of the study and a written consent form that stated the purpose, methods, risks, benefits, and the assurance of the confidentiality of the data was obtained from each student. After obtaining consent from the participants, each participant was examined by an auto refractometer (Auto Refractometer ARK-510A, NIDEK, Aichi, Japan) as described previously [13] [14]. Briefly, three measurements were taken of each participant's refractive status for their both eye with an auto refractometer. Refractive error measurements were recorded in sphere, negative cylinder, and cylinder axis format.

The readings were recorded on a data sheet of every individual, and the Statistical analysis was performed using Statistical Package for Social Sciences (SPSS). Also, a detailed history was taken about co-morbidities and history of previous surgery for the correction of the errors if any. All calculations of refractive error status were based on the non-cycloplegic auto refractometer readings. Spherical equivalent (SE) was calculated as sphere plus half cylinder. Myopia was defined as SE of at least -0.75 diopters (D) in either eye. Myopes were divided into three refractive error sub-groups based on their refractions (SE): low myopia (SE between -0.75 and -2.99 D), moderate myopia (SE between -3.00 and -5.99 D), and high myopia (SE equal to or more myopia than -6.00 D). Hyperopia was defined as SE+1.00 D or positive and emmetropia as a spherical equivalent value between SE -0.75 D and SE+1.00 D in either eye. Astigmatism was defined as -1 Cylinder or more.

## Results

This study comprises exclusively 162 students from different academic years of college of medicine, Qassim University. Among them, 111 (68.5%) were males, and the rest 51 (31.5%) were females. Only 2.65% students had diabetes mellitus, and 3.7% were reported to have previous ocular surgery. The demographic details of all studied subjects are given in Table 1.

**Table 1: Demographics details of studied subjects**

| Subjects | Data |
|---|---|
| Age, years | 22.4 ± 1.661 |
| Mean ± SD; (range) | (19–27) |
| Gender: | |
| Males | 111 (68.5%) |
| Females | 51 (31.5 %) |
| Co-Morbidities: | |
| Diabetes Mellitus | 01 (2.65%) |
| Previous ocular surgery | 06(3.70%) |

In this study, we have taken all five academic year medical students and the breakups of students selected from each academic year are summarised in Table 2.

**Table 2: Academic year of MBBS students**

| Academic Year | Number | Percentage(%) |
|---|---|---|
| First year | 31 | 19.35 |
| Second year | 40 | 24.69 |
| Third year | 36 | 22.22 |
| Fourth year | 25 | 15.43 |
| Fifth year | 30 | 18.51 |
| Total | 162 | |

Various errors of refractions detected in the study population regarding their frequency are shown in Table 3.

**Table 3: Frequency of errors of refraction**

| Error | Frequency | Percentage (%) |
|---|---|---|
| Myopia | 87 | 53.7 |
| Hyperopia | 06 | 3.7 |
| Astigmatism | 02 | 1.2 |
| Emmetropia | 67 | 41.3 |
| Total | 162 | |

The most successive error of refraction was observed to be nearsightedness which was found in 87 (53.7%) examine subjects in our sample followed by Hypermetropia in 6 (3.7%) and Astigmatism in residual 2 (1.2%) subjects. The remaining 67 (41.3%) study subjects were emmetropes. We further

characterised myopic participants into three different categories based upon the severity of myopia. Our results show that mild myopic condition was the highest among all the myopic participants with the frequency of 58 (66.7%), followed by the moderate myopic conditions, which was found with the frequency of 22 (25.3%). However, the severe myopic condition was found in only in 7 myopic participants with the percentage of 8.0%. The results of all studied 87 myopic patients are summarised in Table 4.

**Table 4: Degree of the myopic condition in among the medical students**

| Degree of myopia | Frequency | Percentage (%) |
|---|---|---|
| Mild Myopia (SE between -0.75 and -2.99D) | 58 | 66.66 |
| Moderate Myopia (SE between -3.00 and -5.99D) | 22 | 25.28 |
| Severe Myopia (SE equal or more than -6.00D) | 07 | 8.04 |
| Total | 87 | |

## Discussion

Refractive errors can be perceived through normal examination of patients who present to ophthalmologic centres, or through vision screening of the majority allowed to move around voluntarily. The previous approach may work exquisitely in developed nations with wellbeing cognizant individuals. Vision screening programs are a vital necessity in underprivileged communities all around the world. Vision screening is generally normally done on schoolchildren, which is a valuable methodology for perceiving potentially treatable visual assortments from the standard, incorporating visual need in light of refractive goof and related amblyopia.

Ghaderi et al., [9]. Also mention in their study about rising incidence of errors of refraction in younger age children. It is now well reported that myopia is the most common error of refraction worldwide and it continues on the rise, and now it becomes a major social and economic burden of the affected individuals globally [15].

The prevalence of myopia in the United States appears to be significantly increased in 1999-2004 as compared with 30 years before, whereas Indian population was reported to have myopic condition approximately 20% [16] [17]. As far as the incidences of myopia in the specific cities are a concern, the reports from the cities like Singapore, Hong Kong Taiwan, *etc.* showed myopia is more common and on the rise [18]. Recently, Pan et al., [19] performed a meta-analysis on age-specific prevalence of myopia among Asian population and was found to be increasing with age [19].

This study was conducted on 162 medical students from all five academic years of Qassim University, Saudi Arabia and the data showed an increased incidence of errors of refraction among them. The majority of them were affected by myopia followed by hyperopia, astigmatism and emmetropia. Our results are fully supported by the previous study conducted in Singapore medical students, which showed the occurrence rate of the myopic condition more than 82% as compared to the frequency of other errors of refraction [20]. Not only have these, but our study is also supported by another study conducted on medical students of Malaysia, where myopia was also found to be higher as compared with other errors of refraction [7].

Furthermore, our findings in medical students are also supported by another study performed among medical students of Norway, where myopia was again reported to be higher [21]. Interestingly, it is also important to the point that not only medical students are affected by myopia, but the reports showed that students at school levels were also affected by myopia [10] [11] [12], these findings further strengthen our results. An investigation of Sydney schoolchildren indicated ethnic contrasts in myopia prevalence; kids of East Asian ethnicity had a higher rate of myopia than European Caucasian kids [22].

Increasing levels of myopia increment the danger of vision hindrance and specifically, high nearsightedness is related to the danger of genuine and perpetual visual inability due to related sight-undermining problems. This study highlights the various degrees of myopia with high myopia being the most dangerous for vision as progression can have deleterious effects on visual acuity. Erdinest et al., [23] have recently proposed various treatment options for controlling the progression of myopia in the young population. Many other similar studies have postulated dangers of progressive myopia to cause various vision-threatening complications like retinal detachment, choroid atrophy, glaucoma etc. [24] [25] [26] [27] [28].

Another cross-sectional survey conducted in KSA included 21 primary schools with of 5176 children (mean age 9.5 ± 1.8 years) [29]. A study involved 504 medical students selected from hail university Saudi Arabia also show results in line with our study [30].

All these related studies have screened for myopia among younger children who may reduce the attribute ability of myopia occurrence. The various errors are correctable visual disorders that can cause blindness and there is a deep concern about the rising incidence of these problems all over the world.

In conclusion, the errors of refraction are the most common correctable causes of blindness all over the world. An action towards screening programs is suggested to pick the population affected to take timely measures to correct the error before it can lead to vision-threatening complications.

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# Establishing Cardiopulmonary Resuscitation Services in Sub-Saharan Africa: A Survey of Suggestions Made by Health Care Workers in Cross River State, Nigeria

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**Keywords:** Cardiopulmonary resuscitation; Services, awareness; Infrastructure; Capacity building; Sub-Saharan Africa; Health workers

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**Abstract**

**BACKGROUND:** Rising trend in Non-Communicable Diseases (NCDs) in developing countries often result in sudden death, which are largely preventable through effective cardiopulmonary resuscitation (CPR). Most communities in Sub-Saharan Africa, however, lack access to CPR services, due to a deficiency in requirements for the establishment of such services. These requirements can be grouped into a triad of awareness, infrastructure and capacity building.

**AIM:** This study was aimed at assessing the perceived need and recommendations for improvement in CPR services in Cross River State.

**METHODS:** Proportionate sampling was used to recruit healthcare workers in this cross-sectional study. Data was obtained using semi-structured open-ended questionnaire consisting of recommendations for improving CPR services. Responses were coded and grouped into three essential areas. Data were entered and analysed using SPSS version 20.0.

**RESULT:** Two hundred and twenty-nine (229) questionnaires were completed; mean age of respondents was 42.1 ± 11.2 years. The commonest cadre of healthcare worker was nurses (135, 59.0%). One, two, and three areas of suggestions were made by 55.5%, 37.1%, and 7.4% of respondents, respectively. Suggestions included training of health care workers on CPR (111, 48.5%) and provision of resuscitation equipment (95, 41.5%). Sixty-five respondents (29.3%) recommended creating awareness and means of contact, while some respondents recommended capacity building (132, 57.6%) and resuscitation infrastructure set-up (149, 65.1%).

**CONCLUSION:** Healthcare workers perceive an urgent need for the establishment of CPR services in our health facilities and communities. There is need to address the triad of awareness, infrastructure and capacity building for the establishment of CPR services peculiar to Sub-Saharan Africa.

## Introduction

Sudden unexpected death following a witnessed cardiac arrest can be reversed through prompt and effective bystander cardiopulmonary resuscitation (CPR) [1]. This is especially so if it is due to ventricular fibrillation, and the chain of survival including immediate recognition, CPR and prompt defibrillation are instituted by a trained provider [2]. Unfortunately, most communities in Sub-Saharan Africa, lack access to CPR services, due to lack of a triad of awareness, infrastructure and capacity building.

This study was aimed at assessing the perceived need and suggestions for improvement in CPR services among healthcare workers in Cross River State.

## Methods

Cross-sectional study design using proportionate sampling technique was used to recruit healthcare workers, from healthcare institutions across the geopolitical regions of Cross River State.

The semi-structured open-ended questionnaire was used to obtain data, essentially consisting of suggestions or recommendations for improving CPR services in their region. Responses were coded and grouped into three essential areas for the establishment of CPR services. These areas were Awareness creation, Provision of infrastructure and Capacity building. Data were entered and analysed using SPSS version 20.0.

## Result

Three hundred and thirty-seven health care workers were recruited, but 229 completed their questionnaire, yielding a response rate of 68.0%. Most respondents (162, 70.7%) were female, with a female: male ratio of 1:0.4. Mean age was 42.1 ± 11.2 years, ranging from 19-77 years old. Approximately half of the respondents (117, 51.1%) were within 31-50 years old (Table 1).

One-tenth of respondents worked in private health institution (19, 8.3%) or Non-Governmental Organization (5, 2.2%), while the majority (205, 89.5%) were government healthcare workers. Majority (135, 59.0%) were nurses, followed by doctors (24, 10.5%) and pharmacists (17, 7.4%). Mean some years of practice were 17.3 ± 11.7 years, ranging from 1-43 years. One hundred and forty respondents (61.1%) had previously received training on CPR.

**Table 1: Socio-demographic characteristics of respondents (N = 229)**

| Variable | Frequency | Percent |
|---|---|---|
| Sex | | |
| Male | 67 | 29.3 |
| Female | 162 | 70.7 |
| Total | 229 | 100 |
| Age groups (in years) | | |
| ≤20 | 2 | 0.9 |
| 21-30 | 51 | 22.3 |
| 31-40 | 47 | 20.5 |
| 41-50 | 70 | 30.6 |
| 51-60 | 52 | 22.7 |
| 61-70 | 6 | 2.6 |
| 71-80 | 1 | 0.4 |
| Total | 229 | 100 |
| Institution type | | |
| Government | 205 | 89.5 |
| Private | 19 | 8.3 |
| Non-Governmental Organization | 5 | 2.2 |
| Total | 229 | 100 |
| A cadre of healthcare worker | | |
| Nurse | 135 | 59.0 |
| Doctor | 24 | 10.5 |
| Pharmacist | 17 | 7.4 |
| Physiotherapist | 12 | 5.2 |
| Laboratory scientist | 9 | 3.9 |
| Community health worker | 9 | 3.9 |
| Administrative staff | 5 | 2.2 |
| Others | 18 | 7.9 |
| Total | 229 | 100 |

Approximately one-fifth of respondents (45, 19.7%) recommended improvement in public awareness on CPR, while 32 (14.0%) recommended activation and/or publicity of medical emergency contact phone number(s) (Table 2).

**Table 2: Suggestions/recommendations related to awareness & access (N = 229)**

| Suggestion | Frequency | Percent |
|---|---|---|
| Improvement in public awareness on CPR | | |
| Yes | 45 | 19.7 |
| No | 184 | 80.3 |
| Total | 229 | 100 |
| Activation & publicity of emergency contact | | |
| Yes | 32 | 14.0 |
| No | 197 | 86.0 |
| Total | 229 | 100 |

The commonest suggestion made, related to capacity building was the training of healthcare workers (111, 48.5%), followed by training of general public (21, 9.2%) on CPR. Other areas related to the capacity building were rarely suggested, including patient education (1, 0.4%), availability and use of CPR guidelines (2, 0.9%) and intersectoral/interdisciplinary collaboration (5, 2.2%) (Table 3).

**Table 3: Suggestions/recommendations related to capacity building (N = 229)**

| Variable | Frequency | Percent |
|---|---|---|
| Training of healthcare worker | | |
| Yes | 111 | 48.5 |
| No | 118 | 51.5 |
| Total | 229 | 100 |
| Training of general public | | |
| Yes | 21 | 9.2 |
| No | 208 | 90.8 |
| Total | 229 | 100 |
| Patient education | | |
| Yes | 1 | 0.4 |
| No | 228 | 99.6 |
| Total | 229 | 100 |
| Availability and implementation of guidelines | | |
| Yes | 2 | 0.9 |
| No | 227 | 99.1 |
| Total | 229 | 100 |
| Close monitoring and alertness of patients | | |
| Yes | 7 | 3.1 |
| No | 222 | 96.9 |
| Total | 229 | 100 |
| Collaboration (intersectoral & interdisciplinary) | | |
| Yes | 5 | 2.2 |
| No | 224 | 97.8 |
| Total | 229 | 100 |

Common suggestions related to resuscitation infrastructure were the provision of resuscitation equipment (95, 41.5%), provision of resuscitation medications and oxygen (29, 12.7%), and having a response team (31, 13.5%) (Table 4). Five respondents (2.2%) suggested the provision of incentives for resuscitation workers, while one respondent (0.4%) suggested having a geographic mapping of the state.

One, two, and three areas of suggestions were made by 55.5%, 37.1%, and 7.4% of respondents, respectively. One hundred and two respondents (44.5%) made suggestions in at least two areas (table 5). Sixty-five respondents (29.3%) made suggestions related to awareness and contact, while most respondents made suggestions related to capacity building (132, 57.6%) and building of resuscitation infrastructure (149, 65.1%).

## Discussion

Public awareness of their role in the chain of survival has been shown to increase their confidence and willingness to resuscitate sudden cardiac arrest (SCA) victims. It also improves the outcome of such resuscitation effort. A study in Spain revealed that 94.7% respondents consider training the general population on CPR to be very important [3].

**-Table 4: Suggestions/recommendations related to resuscitation infrastructure set-up (N=229)**

| Variable | Frequency | Percent |
|---|---|---|
| Build resuscitation infrastructure | | |
| Yes | 19 | 8.3 |
| No | 210 | 91.7 |
| Total | 229 | 100 |
| Have mobile resuscitation van/ambulances | | |
| Yes | 17 | 7.4 |
| No | 212 | 92.6 |
| Total | 229 | 100 |
| More accident and emergency staffing and service points | | |
| Yes | 26 | 11.4 |
| No | 203 | 88.6 |
| Total | 229 | 100 |
| Provide resuscitation equipment | | |
| Yes | 95 | 41.5 |
| No | 134 | 58.5 |
| Total | 229 | 100 |
| Provide resuscitation medications / oxygen | | |
| Yes | 29 | 12.7 |
| No | 200 | 87.3 |
| Total | 229 | 100 |
| Have response team | | |
| Yes | 31 | 13.5 |
| No | 198 | 86.5 |
| Total | 229 | 100 |
| Geographic mapping of the state | | |
| Yes | 1 | 0.4 |
| No | 228 | 99.6 |
| Total | 229 | 100 |
| Provide incentives for resuscitation workers | | |
| Yes | 5 | 2.2 |
| No | 224 | 97.8 |
| Total | 229 | 100 |

The same study found that though 37% had received training, only 20.2% considered themselves able to respond appropriately with bystander CPR. This emphasises the need for training and retraining. To ensure prompt defibrillation, bystanders are taught to call for help using an emergency response number. This toll-free number should be easy to remember and well known to the public.

**Table 5: Distribution of areas of suggestions/recommendations made (N=229)**

| Variable | Frequency | Percent |
|---|---|---|
| Makes a suggestion(s) related to awareness/contact | | |
| Yes | 67 | 29.3 |
| No | 162 | 70.7 |
| Total | 229 | 100 |
| Makes a suggestion(s) related to capacity building | | |
| Yes | 132 | 57.6 |
| No | 97 | 42.4 |
| Total | 229 | 100 |
| Makes a suggestion(s) related to building resuscitation infrastructure | | |
| Yes | 149 | 65.1 |
| No | 80 | 34.9 |
| Total | 229 | 100 |
| Number of areas suggested | | |
| One | 127 | 55.5 |
| Two | 85 | 37.1 |
| Tree | 17 | 7.4 |
| Total | 229 | 100 |
| Suggest at least two areas | | |
| Yes | 102 | 44.5 |
| No | 127 | 55.5 |
| Total | 229 | 100 |

Three digit numbers such as 112 in the United Kingdom and 911 in North America are easily remembered in the panicky atmosphere usually created by SCA. Respondents in this study believe that such should be the case in our setting and where such a number exists, it should be adequately publicised.

Many respondents indicated their need for capacity building by suggesting regular training of healthcare providers in CPR. Re-certification in basic life support (BLS) and advanced cardiac life support (ACLS) is the basis for the renewal of practising licenses in many countries for health workers. This should be made compulsory particularly for those working in Accident/Emergency and Intensive Care Units (ICU). Training also includes the ethics of resuscitation regarding when to withhold CPR [4]. Anthony-Pillai expressed a personal view about a high court decision around cardiopulmonary resuscitation (CPR) [4]. This opinion identified that the judge failed to recognise the statutory role given to clinicians in identifying when treatment is life sustaining. In failing to recognise the role of the clinician, he felt the ruling in Winspear risks the likelihood of inappropriate CPR attempts.

Given the critical need for bystander CPR, it is not surprising that some respondents in this study suggested the need to train citizens as a way of improving resuscitation service in the State [5]. A study by Weisfeldt and colleagues revealed that training lay responders and involving them in the use of Automated External Defibrillators (AED) in high-risk public settings nearly doubled the survival after the out-of-hospital cardiac arrest. These results reinforce the importance of strategically expanding community-based AED programs [6]. Bystander CPR and early first responder defibrillation were significantly associated with increased survival in a study reported by researchers in Canada [7]. The same researchers reported 56.1% of cardiac arrests in their series to have occurred at home. This further strengthens the importance of lay citizens CPR capacity building as a way of promoting appropriate immediate response for improved outcome.

Patient education though rarely suggested by the respondents' plays a role in directing resuscitation service. A study by Chu and colleagues highlighted the need to train relatives of high-risk patients on CPR [8]. Knowledgeable patients sometimes express their autonomy by accepting CPR in the event of cardiac arrest or refusing it through advance directives and the Do not attempt resuscitation (DNAR) order [9] [10] [11]. After interviewing Cardiac arrest survivors, their families, friends, neighbours and co-workers, Schneider in their study concluded that equipping high-risk patients and their families with AEDs is a viable method of increasing their survival in case of a recurring cardiac arrest [12]. Capacity building is thus essential not just for health workers but also the lay public especially high-risk patients and their relatives.

From the year 2000 to 2010, the International Liaison Committee on Resuscitation (ILCOR) published guidelines on resuscitation. These guidelines were adopted by various resuscitation bodies to suit their infrastructure and local capacity. The American Heart Association Basic Life Support (BLS) and Advanced Cardiac Life Support (ACLS) algorithms are very popular. Similarly, the European Resuscitation Council has her algorithms [13] [14]. There is a need for Low and Medium-Income Countries to develop suitable algorithms adaptable to the realities of near absence of pre-hospital care and first responders to out of hospital SCA situations [15]. Intersectoral/interdisciplinary collaboration has been shown to improve CPR outcome. In Seattle, Firefighters who were more likely to arrive the scene of cardiac arrest within the first 4 critical minutes were commissioned to be the first responders, and this gave rise to the concept of Citizens CPR. Such collaboration will be an advantage in LMIC. Aguilera-Campos et al. found that police arrived at the scene of SCA before the ambulance [16]. Similarly, a study in Canton of Ticino found the use of an App by Emergency dispatchers recruited both the traditional (police and fire brigade) first responders and lay responders leading to more prompt initiation of CPR with better outcomes [17].

In this study, most respondents suggested the need for availability of resuscitation infrastructure. Equipment as Automated External Defibrillators (AED), Self-inflating Bags must be available and readily accessible to all healthcare providers. Also, BLS and ACLS ambulance vehicles are required for pre-hospital care and inter-hospital transfers. This is unfortunately not the case in many LMIC. There is a need for political will in making resuscitation equipment available for improved outcome. Emergency drug carts, an oxygen source and a means of giving it should also be available.

The need for a response team both for out of the hospital and an in-hospital cardiac arrest has been proven to increase survival. This recommendation was made by 13.4% of our respondents. Some hospitals do not just have a cardiac arrest team but a Medical Emergency Team (MET) that watches out for patients with physiological derangement who are likely to deteriorate to a cardiac arrest. Early Warning Scores have been used in such instances to enhance the early recognition arm of the chain of survival [18] [19]. Roberts and Baxter in separate studies comparing pre and post introduction of MET found a reduction in cardiac arrests, postoperative complications, and hospital mortality as well as better ICU resource utilisation. Geographic mapping will be useful when there is an active medical emergency response team for out of hospital cardiac arrests. Only one responder in this series recommended this. Researchers in Sao Paulo confirmed this in their study [20].

It is worrisome that no respondent mentioned the need for dispatcher directed CPR. This is a common practice in developed countries where a trained dispatcher receives the distress call, dispatches the ambulance to the scene of SCA and guides the caller through appropriate CPR technique [21]. Not surprisingly most respondents want to build their capacity and have better resuscitation infrastructure. This will improve the service for most of the patients who come to us in the hospital and for those outside. There is a call by respondents for an emergency response number. It is time for the operators of the global system of mobile technologies to add to their corporate social responsibility the creation of a simple toll-free number for emergency use and publicise it.

In conclusion, healthcare workers perceive an urgent need for the establishment of organised resuscitation services in our health facilities and communities. However, only a few of these potential future healthcare leaders and policymakers, have an understanding of all the areas or requirements for establishment of such services in our setting. There is need to create awareness among healthcare workers, citizens, patients and their relatives. There is also an urgent need for political will in ensuring availability of resuscitation infrastructure and building the capacity of healthcare workers.

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# Can Health Belief Model Predict Breast Cancer Screening Behaviors?

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**Keywords:** Breast cancer; Health belief model; Screening methods

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**Abstract**

**BACKGROUND:** Breast cancer is the second cause of cancer-related death among women. Prevention programs insist on the early diagnosis and screening to reduce the mortality rate.

**AIM:** The study was conducted to determine the predictors of breast cancer screening behaviours based on the health belief model.

**MATERIAL AND METHODS**: The present cross-sectional study was conducted by involving 304 women ranging from 20 to 65 years of age, living in East Guilan cities, the North of Iran, in 2015 using two-stage cluster sampling. The research instrument was Champion's Health Belief Model Scale. The data were analysed based on Regression test by using SPSS software version 18.

**RESULTS:** The results showed perceived benefits (ExpB = 1.118, p = 0.009), self-efficacy (ExpB = 1.122, p = 0.001) and the perceived barriers (ExpB = 0.851, p = 0.001) as the predictors of breast self-examination. In addition, the study revealed that the two components of perceived benefits (ExpB = 1.202), and the perceived barriers were the predictors of mammography (ExpB = 0.864) (p = 0.001). None of the health belief model components showed a role to predict clinical breast examination (P > 0.05).

**CONCLUSION:** The present study highlights the need for educational programs, which should focus on increasing breast self-exam skills and understanding the benefits of healthy behaviours and eliminating their barriers.

## Introduction

Breast cancer is one of the most common types of cancers [1]. This cancer is considered as the first common cancer among Iranian women [2] and the third cause of cancer leading death in women [3]. In almost 70 percent of cases, the disease is detected at the end stage, and hence the treatment becomes difficult. Detection of breast cancer in early stage led to an almost complete cure, and with timely diagnosis and effective treatment, the survival rate can be up to 90 percent [4] [5]. Therefore, a detailed screening program can effectively detect the early stages of the disease and prevent malignancy in the advanced stages [6].

The proposed breast cancer screening methods are mammography, clinical breast examination (CBE) and breast self-examination (BSE) [7]. Previous studies indicated that the self-examination is the most important step in identifying cancer in early stages [8]. It also causes women to pay more attention to changes in their breasts and to go faster for clinical examination and mammography [9]. However, despite its high efficacy in reducing mortality, various research findings showed that the adoptions of such behaviours by women in different populations are low. Several factors may affect the

performance or non-performance of breast screening behaviours that are essential to identify them to reinforce these behaviours. In health education, various theoretical models used to study the health behaviour such as the Champion Health Belief Model (CHBM) introduced by Champion in the 1980s. This model has been widely used by researchers [8]. According to this model, behavioral beliefs and modifying factors are effective in shaping behavior and when a woman is susceptible to breast cancer (perceived susceptibility) and aware of the threat of disease on their health (perceived severity) and also know the benefits of screening methods (perceived benefits) than its barriers (perceived barriers), she most likely will follow the screening methods [9].

Considering the risk factors for breast cancer, the main emphasis of breast cancer prevention programs is focused on early detection and screening to reduce mortality [10]. According to the previous studies, the health behaviours in using clinical examination, self-examination and mammography are low [11], and there is not sufficient information about the predictive factors of breast cancer screening behaviours, especially in northern areas of Iran. Therefore, in this study, the researchers decided to use CHBM to predict the behaviour of breast cancer screening among the women of East Guilan cities. It is hoped that the results of this research could help improve health decisions for designing effective educational interventions to reduce breast cancer.

## Material and Methods

This article is part of a larger cross-sectional study was conducted on women of East Guilan, the North of Iran, in 2015.

The sample size estimated 304 by using the sample size formula with a confidence level of 95%. Inclusion criteria were age between 20 to 65 years living in East Guilan cities (Lahijan, Astaneh, Langerud, Roudsar) and exclusion criteria were breastfeeding, pregnancy and having breast cancer.

The data were collected through two-stage cluster sampling. The research instrument was Champion's Health Belief Model Scale (CHBMS). The questionnaire contains 57 items that were answered based on the five degrees Likert scale. Each item has 5 response choices ranging from strong disagreement (1 point) to strong agreement (5 points). The HBM subscales were included the perceived susceptibility (3 items), Seriousness (7 items), BSE Benefits (6 items), BSE Barrier (9 items), BSE self-efficacy (confidence) (10 items), health motivation (7 items), benefits of mammography (6 items), barriers of mammography (9 items). All the items had five response choices ranging from strongly disagree = 1 to agree = 5 strongly. Higher scores express more agreement with health beliefs except for barriers to mammography [12].

The reliability of this scale has been tested in different populations and calculated between 0.6-0.89 using Cronbach's Alpha coefficients [13]. Reported Cronbach alpha coefficients for a Farsi version of HBM ranged from 0.72-0.84 [12].

Data were analysed by descriptive statistics and regression test using SPSS software version 18.

The present study and its protocol were approved by the Institutional Human Ethics Committee of Lahijan Islamic Azad University. The researcher entered the research setting only after proper information to the participants about the purpose of the study. The written consent was obtained from all the participants by ensuring that the questionnaires were anonymous. Each participant was completely free to participate in the study.

## Results

The results regarding demographic characteristics showed the age distributions were almost equal in all three age groups (20-30, 31-40 and 41-65 years of age) are shown in Table 1 below. The majority of women (78%) were married, with higher education (41.1%), housewives (75%), and with moderate family's income (92.4%). The majority of the participants (67.3%) had not regular checks, were with no history of breast cancer (95%), and had no family history of breast cancer (90.8%) (Table 1).

**Table 1: Distribution of demographic variables among the women ranging from 20 to 65 years of age, living in East Guilan cities (N=304)**

| Variable | | N | % | Variable | | N | % |
|---|---|---|---|---|---|---|---|
| Age group (years) | 20-30 | 100 | 32.9 | History of benign breast lumps | Yes | 15 | 5 |
| | 31-40 | 105 | 34.5 | | No | 289 | 95 |
| | 41-65 | 99 | 32.6 | The family history of breast cancer | Yes | 28 | 9.2 |
| Education | Illiterate | 20 | 7.2 | | No | 276 | 90.8 |
| | Primary-Secondary | 62 | 20.4 | Having children | Yes | 215 | 70.7 |
| | High school diploma | 97 | 31.9 | | No | 89 | 29.3 |
| | Academic | 125 | 41.1 | Regular check-up | Yes | 100 | 32.7 |
| Job status | Housewife | 228 | 75 | | No | 204 | 67.3 |
| | Employed | 76 | 25 | Menopause | Yes | 254 | 83.6 |
| Family income | Less than average | 23 | 7.6 | | No | 50 | 16.4 |
| | Average | 145 | 47.7 | Lactation history | Yes | 202 | 66.4 |
| | More than average | 136 | 44.7 | | No | 102 | 33.6 |
| Marital status | Single | 55 | 18.1 | | | | |
| | Married | 237 | 78 | | | | |
| | Widow and divorced | 12 | 3.9 | Health insurance | Yes | 252 | 82.9 |
| | | | | | No | 52 | 17.1 |

Data expressed as Frequency (n) and Percentage (%).

The predictor factors of screening behaviours (BSE, CBE and mammography), according to the logistic regression are shown in Tables 2, 3 and 4.

In relation to the predictor factors of breast self-examination (BSE), Table 2 shows that by increasing only one point in the perceived benefits score, the probability of self-examination significantly increase to 0.112 times (p = 0.009) and one point increase in the self-efficacy score, increases the probability of self-examination to 0.115 times with a significant increase (p = 0.001). The findings also indicate that by increasing in perceived barriers, the possibility of self-examination will be reduced to -0.161 times with a significant decrease (p = 0.001). On the other hand, women who have more self-efficacy and perceived benefits and less perceived barriers have more rates of making BSE. However, among the other subscales (perceived susceptibility, perceived seriousness and health motivation) there was no difference between the two groups regarding performing or not performing the self-examination (P > 0.05).

**Table 2: Comparison of performance and prediction of BSE based on the HBM among the investigated women (N=304)**

| Components of HBM | BSE | | B | Exp (B) | P.value |
|---|---|---|---|---|---|
| | Yes Mean ± SD | No Mean ± SD | | | |
| Perceived susceptibility | 6.88±2.52 | 6.68±2.82 | 0.094 | 1.099 | 0.102 |
| Perceived seriousness | 20.40±5.32 | 20.80±5.99 | 0.006 | 1.006 | 0.838 |
| Perceived benefits | 23.31±3.30 | 20.74±4.51 | 0.112 | 1.118 | 0.009** |
| Perceived barriers | 14.30±4.42 | 18.32±5.48 | -0.161 | 0.851 | 0.001*** |
| Self-efficacy | 30.12±4.44 | 26.29±6.34 | 0.115 | 1.122 | 0.001*** |
| Health motivation | 26.67±4.35 | 24.95±5.31 | 0.013 | 1.013 | 0.696 |

Note: BSE = breast self-examination; HBM = Health Belief Model; Data expressed as mean ± S.D, B (coefficient) and Exp(B) ( the exponentiation of the B coefficient, which is an odds ratio); Significant difference of values is indicated by **p < 0.01 and ***p ≤ 0.001.

As for the predictor factors of clinical breast examination, Table 3 shows that by increasing in HBM subscales, despite changes as increase or decrease in results, they could not significantly affect the probability of clinical breast examination. In other words, the results indicated that none of the health belief model subscales has a role in predicting clinical breast examination performance.

**Table 3: Comparison of performance and prediction of CBE based on the HBM among the investigated women (N=304)**

| Components of HBM | CBE | | B | Exp (B) | P.value |
|---|---|---|---|---|---|
| | Yes Mean ±SD | No Mean ±SD | | | |
| Perceived susceptibility | 6.77±2.86 | 6.74±2.67 | 0.036 | 1.036 | 0.542 |
| Perceived seriousness | 20.37±5.75 | 20.74±5.75 | -0.006 | 0.994 | 0.823 |
| Perceived benefits | 22.71±4.54 | 21.39±4.19 | 0.018 | 1.018 | 0.675 |
| Perceived barriers | 16.11±5.88 | 17.03±5.32 | -0.033 | 1.033 | 0.307 |
| Self-efficacy | 28.51±6.45 | 27.48±5.84 | 0.018 | 1.018 | 0.525 |
| Health motivation | 26.57±6.36 | 25.30±4.54 | 0.001 | 1.001 | 0.987 |

Note: CBE = clinical breast examination; HBM=Health Belief Model; Data expressed as mean ±S.D, B (coefficient) and Exp(B)( the exponentiation of the B coefficient) ;Significant level= *p < 0.05.

About the predictor factors of mammography, Table 4 shows that by increasing one point in the perceived benefits score, the probability of mammography will be increased to 0.184 times with a significant increase (p = 0.001). The findings also indicate that by only one point increase in the perceived barriers score, the possibility of performing mammography is significantly reduced to -0.146 times (p = 0.001). In other words, women with more perceived benefits and less perceived barriers, have more rates to perform mammography. However, among the other subscales (perceived susceptibility, perceived seriousness, self-efficacy and health motivation) there was no difference between the two groups about performing mammography (P > 0.05).

**Table 4: Comparison of performance and prediction of mammography based on the HBM among the investigated women (N=304)**

| Components of HBM | Mammography | | B | Exp (B) | P.value |
|---|---|---|---|---|---|
| | Yes Mean ±SD | No Mean ± SD | | | |
| Perceived susceptibility | 7.46±2.87 | 6.64±2.67 | 0.123 | 1.131 | 0.108 |
| Perceived seriousness | 21.27±5.96 | 20.56±5.71 | 0.035 | 1.036 | 0.348 |
| Perceived benefits | 22.54±5.93 | 21.12±5.01 | 0.184 | 1.202 | 0.001*** |
| Perceived barriers | 22.10±5.95 | 26.30±6.26 | -0.146 | 0.864 | 0.001*** |
| Self-efficacy | 28.22±5.18 | 27.64±6.11 | 0.018 | 1.018 | 0.525 |
| Health motivation | 26.00±6.05 | 25.53±4.87 | -0.037 | 0.963 | 0.418 |

Note: HBM = Health Belief Model; Data expressed as mean ± S.D, B (coefficient) and Exp(B)( the exponentiation of the B coefficient); a Significant difference of values is indicated by ***p ≤ 0.001.

## Discussion

The present study aimed to predict the factors affecting the breast cancer screening behaviours in women from 20 to 65 years of age living in East Guilan cities.

The results demonstrated that self-efficacy and perceived benefits predict breast self-examination (BSE) directly and perceived barriers affect inversely. However, other components of HBM (perceived susceptibility, perceived seriousness, and health motivation) could not predict breast self-examination.

Different studies have been reported various results about predictive factors of breast cancer screening behaviours. The results of Hasani's study (2011), who aimed to predict HBM factors among the women referred to health centres in Bandar Abbas are by the present study. They also observed perceived benefits and self-efficacy were predictors of breast self-examination and perceived barriers were inverse predictors [8].

This is in contrast to the study carried out by Sahraee et al. (2013) whose study found that self-efficacy impact directly and perceived severity had the opposite effect on breast self-examination [14]. Mahmoudi et al., (2011) showed that perceived susceptibility, perceived seriousness, health motivation, self-efficacy; perceived benefits predicted directly breast self-examination and perceived barriers

were its inverse predictor [15]. Nourizade et al., (2010) observed a significant correlation between perceived seriousness and mammography [16]. Ghourchaei et al., (2013) reported an inverse relationship between perceived seriousness and BSE. Similarly, we also showed a correlation between the health belief model components and clinical breast examination. However, a significant relationship was observed between perceived susceptibility and mammography [17].

In the present study, perceived benefits and self-efficacy were the main predictors of breast self-examination. Perceived benefits mean positive results by avoiding disease exposure. For example, the smallest suspicious mass can be detected by monthly breast self-examination [8]. Self-efficacy is person's confidence in her ability to carry out successful and accurate BSE and diagnose the suspected tumour. Therefore, educational programs should be focused on proper planning and training to increase women's self-efficacy about breast self-examination. On the other hand, reducing the BSE barriers could also have been an important role in predicting breast self-examination [8].

The results of the present study also showed that perceived benefits were directly predictor of mammography and perceived barriers were its reverse predictor. However, other components (perceived susceptibility, perceived seriousness, self-efficacy and health motivation) were not predictors of mammography. This is in agreement with various previous studies [18] [19] [20] [21]. But the results of Taymoori and colleague (2014) and Noroozi et al. (2011) reported self-efficacy as the most important predictor of mammography cannot support our results [2] [22] directly.

However, reducing barriers can increase perceived benefits, and self-efficacy affect indirectly on perceived benefits and perceived barriers to do mammography [22]. In the Iranian women's culture, barriers to mammography include pain, anxiety, fear of radiation, and the absence of clinical signs of breast cancer [23] [24]. Therefore, proper planning is recommended to minimise these factors and provide background to encourage women to do health behaviours. It is possible to promote women's health literacy by providing the booklet or educational pamphlet. Also, it can be achieved through the creation of health campaigns and networks. Moreover, it is suggested that health care centres reduce barriers by providing easy access to screening examination for clients.

The overall findings of this study showed that self-efficacy, perceived benefits and perceived barriers could predict BSE behaviour and the perceived benefits and barriers could predict mammography. So educational interventions must be considered to improve health behaviours as skills for BSE. Further, increasing understanding of the benefits and the elimination of barriers to health behaviours through correct training is required. Also, proper planning is recommended for the implementation of educational interventions to promote screening programs.

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