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The Role of TNF-α induced MSCs on Suppressive Inflammation by Increasing TGF-β and IL-10

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Keywords: TNF-α; MSC; TGF-β; IL-10

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Abstract

BACKGROUND: Mesenchymal stem cells (MSCs) may serve as immunoregulators by producing various anti-inflammatory molecules. Under sufficient level of TNF-α, MSCs become activated and adopt immune-suppressive phenotype (MSCs type-2) by releasing various anti-inflammatory molecule including TGF-β and IL-10. However, the ability of MSC itself to produce IL-10 under TNF-α stimulation and the correlation of TGF-β production of MSCs to IL-10 level remains to be elucidated.

AIM: In this study, MSCs were activated with various TNF-α doses to determine the increase of IL-10 and TGF-β level as well as its correlation.

MATERIAL AND METHODS: This study used post-test only control group design, by using 3 study groups, consist of 1 control (C) and 2 treatments (T) (TNF-α = 5 and 10 ng/mL) with triplicate induced in MSC for 24 hours, then the levels of IL-10 and TGF-β were measured by using ELISA assay.

RESULTS: The results of this study showed a significant increase of TGF-β and IL-10 levels ($p < 0.05$) at TNF-α 5 and 10 ng/mL dose of TNF-α. Moreover, there was a significant negative correlation between TGF-β and IL-10 level on 5 and 10 ng/mL dose TNF-α treatment.

CONCLUSION: Based on our study, we conclude that the 5 ng/mL dose of TNF-α is a sufficient dose for MSCs to suppress the inflammatory milieu. The higher increase of TGF beta is due to the controlled inflammation by IL-10.

Introduction

Mesenchymal stem cells (MSCs) are classically defined as multipotent cells expressing the surface markers of CD73, CD90, CD105, and lacking the expression of CD45, CD34, CD14 or CD11b, CD79a or CD19 and Human Leucocyte Antigen (HLA) class II. MSCs also can differentiate into osteocytes, chondrocytes and adipocytes under standard *in-vitro* differentiating conditions [1]. They can be isolated from the bone marrow, mobilised peripheral blood, cord blood, umbilical cord (UC), placenta, adipose tissue, dental pulp, and even the fetal liver and lungs. On the other side, the autologous use has several limitations in decreased growth and [2] differentiation capacity [3] [4] of cell numbers and age-related changes. UC-MSCs show a gene expression profile more pluripotent and stemness than BM-MSCs [5] [6]. MSCs display profound immunomodulatory properties by suppressing excessive inflammatory responses of a variety of immune disorders.

Several studies have reported that MSCs actively interact and communicate with innate and adaptive immune cells to ameliorate immune disorders [7] [8] [9]. Several clinical studies have

shown that MSCs-based therapy effectively controls in various autoimmune disease, including Systemic Lupus Erythematosus (SLE), Graft versus Host Disease (GvHD), Rheumatoid Arthritis (RA), inflammatory bowel disease and multiple sclerosis.

Furthermore, the ability of immunosuppressive of MSCs is regarding the production of cytokines such as TGF-β, IDO, NO, PGE2, IL-10 and TSG-6 [10] [11] [12]. IL-10 and TGF-β1 serve as potent anti-inflammatory cytokines in controlling excessive inflammatory responses. Specifically IL-10 attenuates pro-inflammatory signals by inhibiting pro-inflammatory cytokines release particularly IFN-γ, IL-2, and TNF-α [13], while TGF-β1 ameliorates immune disorder by generating of CD4 + CD25 + FoxP3 + T_{reg} [14].

TNF-α is an active stimulator molecule in enhancing the secretion of various inflammatory cytokines. The previous study reported that TNF-α-activated MSCs suppress inflammation by inducing IL-10 production in macrophage cells but MSCs its self-were not the source of IL-10 due to MSCs from IL-$10^{-/-}$ mice were still effective in improving the survival of mice with sepsis [15]. A similar study has also described that IL-10 level decrease after 24 hours of intravenous infusion of mouse MSCs [16]. On the other side, TGF-β as immunosuppressive molecules constitutively produced by MSCs [17] also involved in promoting the T-cell production of IL-10 through direct activation of IL-10 promoter via Co-Smad4 [18]. Under sufficient level of TNF-α MSCs become activated and adopt immune-suppressive phenotype (MSCs type-2) by releasing various anti-inflammatory molecule including TGF-β and IL-10 [19]. However, the ability of MSC itself to produce IL-10 under TNF-α stimulation is yet unclear. Moreover, the correlation of TGF-β production of MSCs to IL-10 level remains to be elucidated.

Therefore, in the present study, we explored the ability of MSCs in vitro in producing IL-10 and TGF-β at 5 and 10 ng/mL TNF-α for 24-hour incubation.

Material and Methods

Adult 19-day pregnant Wistar rats, weighing 350-450 g, were provided by the animal husbandry department. The animal was used according to good animal practices, and animal experiments were approved by our local animal care.

The umbilical cords were collected from the fetuses 19-day pregnant Wistar rats under general anaesthesia. The blood vessels were removed from umbilical cord, then the tissue parts under aseptic conditions were cut into smaller pieces and transferred to a T25 culture flask containing DMEM (Sigma-Aldrich, Louis St, MO) supporting with 10% Fetal Bovine Serum (FBS) (Gibco™ Invitrogen, NY, USA), 1% penicillin (100 U/mL)/streptomycin (100 µg/mL) (Gibco™ Invitrogen, NY, USA). The UC tissues were incubated at 37°C in a humid atmosphere consisting of 5% CO_2. The medium was renewed every 3 days, and after reaching 80% confluency (14 days), the cells were passaged. MSCs-like at passages 4–5 were used for the following experiments.

MSCs-like surface antigens were analysed by flow cytometric analysis at the fourth passage. The cells were subsequently incubated in the dark with fluorescein isothiocyanate (FITC)-conjugated, Allophycocyanin (APC)-conjugated or phycoerythrin (PE)-conjugated monoclonal antibodies, including CD105, CD90 and CD73. FITC- APC- and PE-conjugated isotypes were used as negative controls. The analysis was performed using BD Pharmingen™ (BD Bioscience, Franklin Lakes, NJ, USA) at 4°C for 30 min. The cells were washed twice with 1% BSA/PBS, resuspended in 200 µL 1% BSA/ PBS and analysed by a flow cytometer (BD Biosciences, San Jose, CA, USA).

To characterise the isolated cells, we further performed the osteogenic differentiation assay at the fourth passage. Osteogenesis was induced by osteogenic induction medium containing 10 mmol/L β glycerophosphate, 10^{-7} mol/L/ 0.1 µM dexamethasone, 50 µmol/L ascorbate-2-phosphate (Sigma-Aldrich, Louis St, MO) and supporting with 10% FBS (Gibco™ Invitrogen, NY, USA) in DMEM (Sigma-Aldrich, Louis St, MO) at 37°C and 5% CO_2. Calcium deposition was shown by Alizarin Red staining (Sigma-Aldrich, Louis St, MO) after 21 days incubation.

MSCs (5 x 10^4 cells/well) was supplemented by TNFα recombinant (5 and 10 ng/mL (BioLegend, San Diego, CA)) in 24-well plate using DMEM (Sigma-Aldrich, Louis St, MO) then incubated for 24 hours at 37°C with 5% CO_2. Each experiment was performed in triplicate. The TNF-α recombinant medium was collected and analysed for TGFβ and IL-10 levels using ELISA assay.

The levels of both TGF-β and IL-10 released in the culture supernatants from the various treatment groups were measured by specific ELISA. Briefly, according to the manufacturer's instructions (Fine Test, Wuhan, China), TGF-β and IL-10 were calculated according to a standard curve constructed for each assay, and each assay was performed in triplicate. The colourimetric absorbance was recorded at a wavelength of 450 nm.

Data are presented as the means ± standard deviation. All calculations were carried out using IBM SPSS 22.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. The statistical significance of

the differences between the groups was assessed using one way-ANOVA and continued with Duncan post-hoc analysis. Correlation between IL-10 and the TGF-β level was done using one-tailed Pearson's test. *P* values: **, *P* < 0.001.

Results

Isolation of UC-MSCs was performed based on the capacity to plastic attachment under standard culture condition. Isolated cells were cultured for 2-3 weeks in monolayer and used for differentiation analysis after 4 to 5 passages. The UC-MSCs were initially characterized by their elongated fibroblastic cellular phenotype (Figure 1a). Moreover, Osteogenesis was confirmed at day 21 of culture by immunodetection with Alizarin Red staining (Figure 1b).

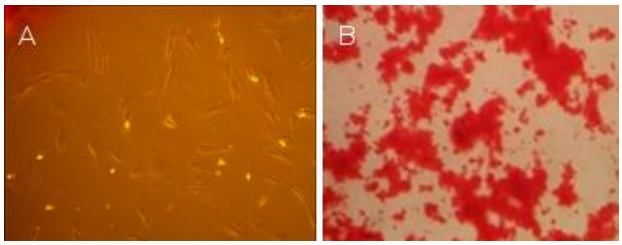

Figure 1: a) UC-MSCs characterisation was based on their peculiar fibroblast-like (spindle shape) morphology; b) and osteogenic differentiation with Alizarin Red staining appears red colour

The specific marker of UC-MSCs expression cultured in the media was evaluated as presented in Figure 2. We have characterised the expression pattern of UC-MSCs by flow cytometric analysis with the positive MSCs markers CD73, CD90 and CD105.

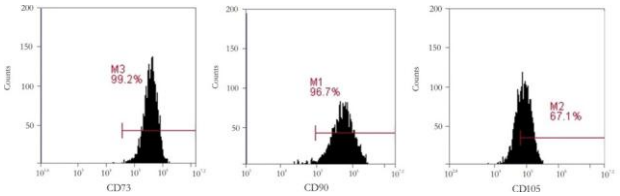

Figure 2: Detection by flow cytometric demonstrates positive expression of three UC-MSCs markers. Populations are 99.2% positive for CD73, 96.7% positive for CD90 and 67.1% positive for CD105

We subsequently quantify levels of IL-10 and TGF- β (Figure 3) by ELISA in triplicate. The analysis of TGF-β concentration showed that there was significantly increase in all treatments (p < 0.001) at 5 ng/mL (64.09 ± 2.25 ng/mL) and 10 ng/mL (92.78 ± 1.28 ng/mL) TNF-α dose. Furthermore, the IL-10 concentration on all treatments showed the significantly increased (p < 0.001) at 5 ng/mL (533.12 ± 3.92 ng/mL) and 10 ng/mL (513.42 ± 4.31 ng/mL) TNF-α dose. In other side, we found the negative correlation between TGF-β and IL-10 level on 5 and 10 ng/mL dose TNF-α treatment (p = 0.007, r = -0.933).

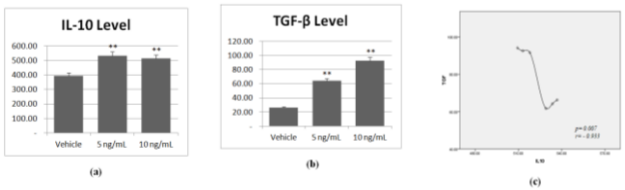

*Figure 3: a) ELISA assays for two treatment groups with doses 5 and 10 ng/mL showed the highest concentration of TGF-β level at 10 ng/mL TNF-α dose (92.78 ± 1.28 ng/mL), **, P < 0.001; b) Furthermore, the optimum concentration of IL-10 level in MSCs medium was 5 ng/mL TNF-α dose (533.12 ± 3.92 ng/mL). **, P < 0.001 vs vehicle control; c) Moreover, the data demonstrated that there was significant negative correlation between TGF-β and IL-10 level on 5 and 10 ng/mL dose TNF-α treatment*

Discussion

Inflammation is a cell protective response to eliminate various pathogens and preserve host integrity. MSCs as sensors of inflammatory may create both anti-inflammatory and proinflammatory effects when interacting to cell innate of the immune system or exposed by the various cytokine. Previous studies have shown that MCSs respond to inflammatory milieu by polarising either into MSCs type-2 with an immune-suppressive phenotype or MSCs type-1 with proinflammatory profile depend on Toll-Like Receptors (TLRs) type activation [7] [9] [21] [22]. Several studies have reported that immunosuppressive of MSCs occur through releasing various anti-inflammatory molecule including IL-10, ILra-1, PGE_2, IDO, NO, TGF-β and TSG-6. The release of its anti-inflammatory molecules may occur through co-cultures of MSCs along with immune cells or under TNF-α, IL-1 and IFN-γ stimulation [15] [20]. The capacity of MSCs type-2 in suppressing the excessive inflammation and ameliorating immune disorders has opened new perspectives in clinical research, particularly in autoimmune disease.

Our studies demonstrated that MSCs released IL-10 level was significantly increase at 5 ng/mL and 10 ng/mL TNF-α (Figure 3a, P < 0.001). These data suggest that stimulation of low-dose TNF-α (5 ng/mL) can promote the polarisation of MSCs into MSCs type-2. Under sufficient dose of TNF-α, MSCs upregulate the expression of TLR-3 leading to release of the various anti-inflammatory molecule including IL-10 [8] [9]. The binding of TNF-α to TNFR-1 of MSCs resulted in the activation of NF-κβ and ERK signalling which produces cyclooxygenase-2 (COX2) and upregulates TLR-4 expression. TLR4-primed MSCs population which are known as MSCs type-1 exhibit a proinflammatory profile, including upregulation of COX2 which increase PGE_2 secretion. Specifically,

PGE_2 bound to EP_2 and EP_4 receptors of MSCs leading to the shift from MyD88-dependent proinflammatory (MyD88-independent pathway) to TRIF-TRAM mediated anti-inflammatory signal by a P110δ isoform of PI3k kinase resulting in IL-10 secretion [23]. These facts suggest that MSCs are polarised into MSCs type-1 at initial stimulation of TNF-α characterised by COX2 secretion and then repolarise into MSCs type-2 along with the accumulation of inflammatory signal inside MSCs (Figure 4).

In this study, the IL-10 level tends to decrease at 10 ng/mL than 5 ng/mL dose of TNF-α. A higher dose of TNF-α cause downregulation of MSCs type-2 signalling and induce apoptotic program through TNF-related apoptosis-inducing ligand-receptor 2 (TRAIL-R2) pathway. The similar study has reported that TNF-α may induce apoptotic through TRAIL-R2 pathway [24]. TRAIL-R2 is a specific cell surface receptor belongs to the TNF receptor superfamily [25] that also expressed in MSCs [26] [27]. Upon binding TNF-α, TRAIL-R2 initiates the recruitment of Fas-associated protein with death domain (FADD) and procaspase-8 to form the death-inducing signalling complex (DISC) then activates downstream caspase-3 and leads to apoptosis [28]. These suggest that the apoptosis pathway may be activated by higher doses of TNF-α.

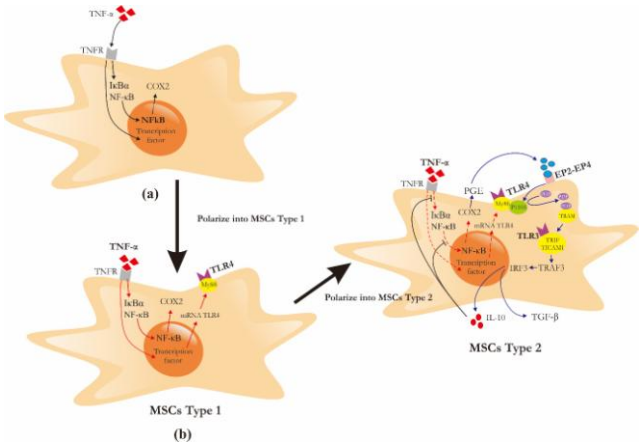

Figure 4: Schematic for MSCs polarisation under TNF-α stimulation; a) TNF-α binding to TNFR-1, then activating NF-κβ and ERK signalling which produces COX2 and (b) upregulate TLR-4 expression leading to polarise MSCs into MSCs type-1 that exhibit proinflammatory profile; c) COX-2 upregulation resulting in the increase of PGE_2 secretion. The binding accumulation of PGE_2 to EP_2 and EP_4 receptors of MSCs triggering the shift of MyD88-dependent proinflammatory (MyD88-independent pathway) to TRIF-TRAM mediated anti-inflammatory signal by a P110δ isoform of the PI3k kinase, then repolarise to MSCs type 2 (anti-inflammatory phenotype) and inducing TRAF3-IRF3 resulting in IL-10 secretion. IL-10 inhibit NF-κβ/IKκβ cytoplasmic and NF-κβ nucleic then control the inflammatory milieu leading to the strong TGF-β secretion

We also found that the significant increase of TGF-β level was about 4-fold than IL-10 level (Figure 3b, $P < 0.001$) and shown a negative correlation between IL-10 and TGF-β production (Figure 3c). These finding suggest that MSC rapidly respond to inflammatory milieu by polarising into immune-suppressive phenotype (MSCs type-2). The release of IL-10 has shown that the inflammation milieu is under control by MSCs, thus MSCs strongly produce other cytokine or growth factor including TGF-β production. This observation supports other studies revealing that IL-10 was elevated at 6 and 12 hour and then it decreased at 24 hour [15]. TGF-β is pleiotropic cytokines that is a well-known immunosuppressive molecule with important roles in immunoregulation [15] [19]. TGF-β inhibited inflammatory cytokine-induced iNOS expression in a SMAD3-dependent manner. In this study, we explore MSCs without the co-culture with the immune cells, thus we don't know exactly how the MSCs may interact and suppress the activated immune cells particularly in the case of an autoimmune disease.

Based on our study, we conclude that the 5 ng/mL dose of TNF-α is a sufficient dose for MSCs to suppress the inflammatory milieu. The higher increase of TGF-β is due to the controlled inflammation by IL-10.

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Vascular Endothelial Growth Factor 936 C/T Gene Polymorphism in Indonesian Subjects with Diabetic Polyneuropathy

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Keywords: *VEGF +936 C/T* polymorphism; VEGF-A level; Diabetic polyneuropathy

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Abstract

AIM: This study aimed to confirm the role of f *VEGF* gene *936 C/T* polymorphism and Diabetic Polyneuropathy (DPN) in the Indonesian population as well as to investigate its relationship with VEGF-A level and the role of vascular risk factors.

MATERIAL AND METHODS: This was a cross-sectional study involving 152 subjects. Clinical symptoms and signs of DPN were examined using DNE and DNS scoring followed by nerve conduction study. All subjects underwent anthropometric, clinical examination and laboratory procedures to obtain body mass index, HbA1C level, lipid profile, Polymorphism of *+936 C/T VEGF* gene (PCR-RFLP technique), and VEGF-A plasma level (ELISA). Statistical analysis using a t-test or Mann-Whitney was performed to assess continuous data and Chi-square for categorical data. Multivariate logistic regressions were also performed to determine the relationship between independent variables and DPN.

RESULTS: Sixty-nine (45.4%) fulfilled the diagnostic criteria of DPN. There was a significant association between *CT + TT* genotype and DPN (OR 0.35 95%CI 0.16-0.79 p = 0.01). Multivariate logistic regression showed that plasma VEGF-A level (OR = 1.003; 95% CI = 1.000-1.007; p = 0.03), diabetes duration (OR = 1.108; 95% CI = 1.045-1.175; p = 0.001), and *CT+TT* genotype (OR = 0.347; 95%CI = 0.148-0.817; p = 0.013) were associated with DPN. Sub-group analysis on subjects with HbA1C level ≥7% showed that VEGF-A (OR = 1.011; 95%CI = (1.004-1.017; p = 0.03), diabetes duration (OR = 1.245; 95% CI = 1.117-1.388; p < 0.001), *CT + TT* genotype (OR = 0.259; 95%CI = 0.074-0.911p = 0.035), with an adition of HDL (OR = 0.916; 95% CI = 0.857-0.978; p = 0.009) were significant predictors of DPN while LDL (OR = 1.017; 95% CI = 1.000-1.035; p = 0.053) acted as modifying factor.

CONCLUSION: It appeared that *CT + TT* genotype of *VEGF +936* gene might act as a protecting factor for DPN while VEGF-A, diabetes duration, HDL, and LDL acted as risk factors especially on subjects with HbA1C level ≥ 7.

Introduction

Diabetic polyneuropathy (DPN) is the most common complication of diabetes mellitus affecting the peripheral nervous system. It is associated with high morbidity and mortality rate and contributes to a decrease in quality of life [1] [2]. The global prevalence of DPN is 10% in the first year after the diagnosis and increases to approximately 50% after diagnosed with diabetes mellitus for 25 years [1] [3]. According to a study performed by Soewondo et al., (2010) [4], it is predicted that around 63.5% of Indonesian diabetic patients treated in tertiary hospitals met the clinical diagnostic criteria of DPN. Data about risk factors associated with this fact is not well documented. Understanding this fact is crucial in the context of early detection and prevention.

Growth factor imbalance as well as high blood glucose level and oxidative stress are believed to have a role in the pathogenesis of DPN as found in animal models. Of all those growth factors, special attention should be paid to the circulating Vascular Endothelial Growth Factor-A (VEGF-A). It is predicted of having a potentially neuroprotective capacity, but its

role is highly influenced by glucose level and oxidative stress. A previous study by Deguchi et al. (2009) found that VEGF level increased in the active phase of DPN but decreased in the advance phase [5]. This fact raised some doubt whether VEGF has a potentially beneficial role, or in reverse, is involved in the pathogenesis of DPN.

It is believed that the genetic factor plays an important role in the development of DPN. Of all genetic variants associated with circulating VEGF level, the polymorphism of *936 3'UTR* of *VEGF* gene has been studied and has shown various results. Zhang *et al.,* (2014) found that the *CC* genotype was associated with an increased risk of developing DPN while *CT* and *TT* genotype was associated with a decreased risk. *CC* genotype also associated with a higher level of serum VEGF-A level [6]. On the other hand, Kim et al. (2009) found that there was no relationship between *936 C/T VEGF* gene polymorphism with DPN although TT genotype was associated with higher plasma VEGF-A level [7].

Vascular risk factors such as high levels of HbA1C, total cholesterol, triglyceride and low level of HDL as well as obesity have also been associated with the risk of developing DPN [6] [8]. These suggest that DPN is a multifactorial condition, a combination of various factors such as metabolic, vascular, and genetics.

Methods

This was a cross-sectional study evaluating the relationship between polymorphism of *VEGF* gene *936 C/T 3'UTR* with DPN. It was conducted in Atma Jaya Academic Hospital, Jakarta between September 2017 and March 2018. Participants were recruited consecutively and had undergone general and neurological clinical examinations, blood tests, and nerve conduction studies. Body mass index, blood pressure, results of the genetic test to evaluate polymorphism of *VEGF* gene *936 C/T 3'UTR*, plasma VEGF-A level, HbA1C, lipid profile, and results of nerve conduction studies were documented. DNA analyses were performed in the Biomedical Laboratory of the School of Medicine Unika Atma Jaya Jakarta and the Laboratory of Medical Parasitology, Faculty of Medicine, Public Health, and Nursing Universitas Gadjah Mada Yogyakarta. The plasma VEGF-A level analysis was performed in Prodia Lab Jakarta, and other laboratory tests were done in the Clinical Pathology Department, School of Medicine, Unika Atma Jaya and Atma Jaya Academic Hospital. Nerve Conduction Studies (NCS) were performed in the Diagnostic Installation of Atma Jaya Academic Hospital.

The inclusion criteria of the subjects were (1) adults diagnosed with type-2 diabetes mellitus aged < 67 years, (2) willing to participate in the study and signed informed consent. The exclusion criteria were having (1) a diagnosis of chronic kidney disease and/or on routine hemodialysis, (2) a history of cancer or chemotherapy, (3) an acute exacerbation of chronic osteoarthritis, (3) peripheral arterial disease-defined by a manual ankle-brachial index of > 0.8, (4) acute stroke or myocardial infarction; (5) arrhythmias and or on cardiac pacemaker, (6) severe polyneuropathy (unrecordable NCS) or diabetic ulcer and/or history of diabetic ulcer, (7) a history of erectile dysfunction, constipation, and chronic diarrhea.

This study was approved by the Medical and Health Research Ethics Committee (MHREC), Faculty of Medicine, Public Health, and Nursing Universitas Gadjah Mada.

Diagnosis of DPN was established when the respondent had a positive result for at least one of the diagnostic evaluation. Diagnostic evaluation in this research was performed using (1) Diabetic Neuropathy Symptom (DNS) & Diabetic Neuropathy Examination (DNE) scoring and (2) NCS. DPN is considered positive if DNS score ≥ 1 and DNE score > 3 while findings from NCS suitable to PDN in this research is (1) ≥ 1 abnormality on ≥ 2 separate nerves or (2) ≥ 1 abnormality on at least 2 nerves which must include the sural nerve [9] [10] [11]. General and neurological examinations as well as DNS and DNE scoring were performed by one specifically-trained physician and conducted before the NCS examination. NCS examinations were performed by a neurologist trained in EMG and Evoked Potentials.

The HbA1C examination was performed using Boronat affinity binding method. Whole blood samples were mixed with reagents on Nycocard kit and read using a Nycocard™ Reader. Total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglyceride levels were assessed using the Insert kit from Labtest on an automated biochemical analyser.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)

DNA isolation from venous EDTA blood was performed using *Wizard® Genomic DNA Purification Kit* (Promega) product code A1120. The sequence for upstream and downstream primers were [12]:

- *forward*: 5'-AAG GAA GAG GAG ACT CTG CGC3.'
- *reverse*: 5'- TAT GTG GGT GGG TGT GTC TAC AGG-3.'

DNA amplification using PCR technique was performed using the kit Go Taq® Green Master Mix (Promega). PCR product was then digested using NlaIII enzyme with CutSmart^{R} buffer (New England Biolabs). Digested product and marker were analysed in 2% agarose gel using FloroSafe staining. After the electrophoresis process (110 V for 30 minutes), the result was then photographed and analysed. Digested products were (Figure 1):

1) *CC* genotype: 1 band with product length 198 bp

2) *CT* genotype: 3 bands with product length198 bp, 114 bp, and 84 bp

3) *TT* genotype: 2 bands with product length 114 bp, dan 84 bp.

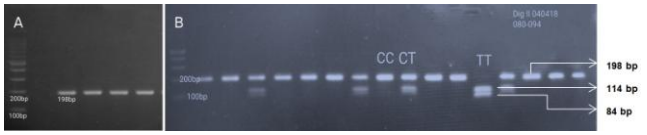

Figure 1: A) Electrophoresis of PCR product showing 1 band (198bp); B) Electrophoresis of Digested PCR Product. CC genotype consists of 1 band (198 bp), CT genotype consists of 3 bands (198 bp, 114 bp, and 84 bp) and TT genotype consists of 2 bands (114 bp and 84 bp). Marker 100 bp on lane 1

Plasma VEGF-A level was detected using ELISA method using ab119566-VEGFA Human ELISA kit from Abcam. Plasma was obtained from centrifuged venous EDTA blood (1500 rpm in 5 minutes) and stored in a refrigerator (-70°C) before the procedure. All steps on the ELISA procedure was conducted according to the protocol written on the kit.

All data were analysed using SPSS version 22. Association between polymorphism of *VEGF* gene *936 C/T* and DPN was determined using Chi-square test. Association between numerical variables with genotype and DPN were evaluated using t-test if the data showed normal distribution and Mann-Whitney U test if not. Logistic regression analysis was then performed to evaluate the relationship between DPN and predictive factors, including genotype and vascular risk factors.

Results

There were 152 patients eligible as study participants. Ninety-eight participants (64.55%) were women, and 54 (35.5%) were men. More than half of the participants were 51-60 years (55.9%). Most of the participants (63.2%) were diagnosed with type-2 diabetes for more than 5 years. Of all participants, 45.4% fulfilled the diagnosis of DPN according to the protocol of this study. Majority of the respondents had HbA1C level ≥ 7% (65.1%). Hypertension was found in 88 (57.9%) of subjects, and 33 (21.7%) were obese. Fifty-two subjects (34.2%) had a cholesterol level of > 200 mg/dl, 108 (71.1%) had an LDL level of > 100 mg/dL, 95 (62.5%) had an HDL level < 40 mg/dL, and 100 (65.8%) had a triglyceride level > 150 mg/dL (Table 1).

Table 1: Baseline characteristics of study subjects

Characteristic	n	%
Sex		
Men	54	35.5
Women	98	64.5
Age (years)		
<40	7	4.6
41-50	26	26.0
51-60	85	55.9
>60	34	22.4
Duration of diabetes diagnosis (years)		
≤ 5	96	63.2
>5	56	36.8
DPN Status		
DPN (-)	83	54.6
DPN (+)	69	45.4
HbA1C (%)		
< 7	53	34.9
≥ 7	99	65.1
Hypertension		
No	88	57.9
Yes	64	42.1
Body Mass Index (kg/m^2)		
< 30, n= 119 (78.3%)	33	78.3
≥ 30, n= 33 (21.7%)	119	21.7
Total cholesterol (mg/dL)		
< 200	100	65.8
≥ 200	52	34.2
LDL (mg/dL)		
< 100	44	28.9
≥ 100	108	71.1
HDL (mg/dL)		
< 40	95	62.5
≥ 40	57	37.5
Triglyceride (mg/dL)		
< 150	52	34.2
≥ 150	100	65.8
Genotype Frequency		
CC	115	75.6
CT	34	22.4
TT	3	2

The frequency of *CC* genotype was 75.6%, *CT* genotype 22.4% and *TT* genotype 2%. Genotype frequency fulfilled Hardy-Weinberg Equillibrium (χ2 = 0.07; p = 0.787). There was a significant relation between *CT+TT* of *VEGF +936* genotype and diabetic neuropathy (OR 0.35; 95%CI 0.16-0.79; p = 0.01) (Table 2).

Table 2: The relationship between genotype of VEGF gene 936 C/T polymorphism and DPN

		DPN		OR (95% CI)	P
		DPN (-) n (%)	DPN (-) n (%)		
Genotype	*CC*	56 (67.5)	59 (85.5)	1	Reference
	CT+TT	27 (32.5)	10 (14.5)	0.35 (0.16-0.79)	0.01*

Bivariate analysis of predictor variables in numerical data showed that the duration since diabetes diagnosis (in years) had a significant association with DPN [5(0-35) vs 3(0-25); p = 0.01] and body mass index had a significant association with *CC* genotype [27.23 ± 4.66 vs 25.28 ± 3.93; p = 0.023)]. Another variable such as HbA1C level, total cholesterol, LDL, HDL, triglyceride, and body mass index failed to show statistical significance. One subject is excluded in analysis involving VEGF-A level due to an extreme outlier. A trend towards a higher level of plasma VEGF-A level in DPN group when

compared with non DPN group was observed [159.50 (20.40-886.00) vs 136.10 pg/mL (17.50-631.40)] as well as between *CT+TT* vs CC genotype [166.10 (17.50-522.50) vs 133.50 (20.40-886.00)] but not statistically significant (Table 3).

Table 3: Bivariate analysis on the association of predictor variables with DPN and genotype of VEGF Gene 936 C/T polymorphism

Variable	DPN Status		p	Genotype		
	Non-DPN	DPN		*CC*	*CT+TT*	P
Diabetes	3.00	5.00	0.001	4.00	4.00	0.755
Diagnosis duration (years)	(0-25)	(0-35)		(0-35)	(0-25)	
HbA1C (%)	7.5 (5.0-15.0)	8.0 (4.0-15.0)	0.110	7.8 (4.9-15.0)	7.6 (4.0-14.9)	0.526
Total Cholesterol (mg/dL)	190 (123-287)	189 (92-346)	0.517	189 (92-346)	193 (123-249)	0.854
LDL (mg/dL)	115 ± 30	120 ± 39	0.347	119 ± 35	112 ± 33	0.305
HDL (mg/dL)	43 (30-85)	42 (30-83)	0.119	42 (30-83	44 (30-85)	0.149
Tryglyceride (mg/dL)	129 (65-636)	131 (67-330)	0.530	130 (67-636)	131 (65-265)	0.940
BMI (kg/m^2)	26.95 ± 4.73	26.53 ± 4.37	0.573	27.23 ± 4.66	25.28 ± 3.93	0.023
VEGF – A (pg/mL) (n = 151)	136.1 (17.50-631.40)	159.50 (20.40-886.00)	0.202	133.50 (20.40-886.00)	166.10 (17.50-522.50)	0.171

Multivariate logistic regression showed that plasma VEGF-A level (OR = 1.003 (1.000-1.007); p = 0.03), duration since diabetes diagnosis (OR = 1.108; 95%CI = 1.045-1.175; p = 0.001), and *CT+TT* genotype (OR = 0.347; 95%CI = 0.148-0.817; p = 0.013) were associated with DPN (Table 4).

Table 4: Multivariate logistic regression of to predict DPN in all subjects (n = 151)

Predictors	B	SE	Wald	OR (95% CI)	p
VEGF-A	0.003	0.002	4.684	1.003 (1.000-1.007)	0.03
CT+TT Genotype	-1.057	0.436	5.865	0.347 (0.148-0.817)	0.015
Diagnosis duration	0.103	0.030	11.805	1.108 (1.045-1.175)	0.001

Sub-group analysis on subjects with HbA1C level ≥ 7 (Table 5) indicated a similar relationship, showing that VEGF-A (OR = 1.011; 95%CI = (1.004-1.017; p = 0.03), diabetes duration (OR = 1.245; 95% CI = 1.117-1.388; p < 0.001), *CT+TT* genotype (OR = 0.259; 95%CI = 0.074-0.911, p = 0.035), with an adition of HDL (OR = 0.916; 95% CI = 0.857-0.978; p = 0.009) were significant predictors of DPN while LDL (OR = 1.017; 95% CI = 1.000-1.035; p = 0.053) acted as modifying factor.

Table 5: Multivariate logistic regression to predict DPN in subjects with HbA1C ≥ 7 (n = 98)

Predictors	B	SE	Wald	OR (95% CI)	p
VEGF-A	0.011	0.003	10.061	1.011 (1.004-1.017)	0.001
CT+TT Genotype	-1.349	0.614	4.433	0.259 (0.074-0.911)	0.035
Diagnosis duration	0.219	0.055	15.682	1.245 (1.117-1.388)	<0,001
HDL	-0.088	0.034	3.735	0.916 (0.857-0.978)	0.009
LDL	0.017	0.009	0.628	1.017 (1.000-1.035)	0.053

Discussion

Of all 152 subjects, in regards with polymorphism of *VEGF* gene *936 C/T*, we found that the frequency of *CC* genotype was 75.6% and *CT+TT* genotype was 24.4%. The frequency of genotype variation (*CC* vs *CT+TT*) for polymorphism of *VEGF* gene *936 C/T* (rs3025039) in general population is 75.1% vs 24.9%, East Asia 68.9% vs 31.1%, and South Asia 79.1% vs 20.1% [13]. In this study, it was observed that the genotype frequency of *VEGF* gene *936 C/T* polymorphism was close to the general population. Hardy-Weinberg analysis showed $\chi 2$ = 0.07; p = 0.787 meaning that the genotype frequency fulfilled the Hardy-Weinberg Equilibrium.

This study found that the *CT+TT* genotype of *VEGF* gene *936 C/T* polymorphism is associated with the decreased odds of DPN, hence can be considered as having a protective effect. This finding is consistent with a previous study performed by Zhang et al. (2014) [6], in which the *CC* genotype was correlated with a higher level of plasma VEGF-A. The exact role of *VEGF* gene *936 C/T* polymorphism in DPN remains controversial. Another study performed by Kim et al. (2009) [7] found that there was no significant relationship between *VEGF* gene *936 C/T* polymorphism and DPN although DPN and *TT* genotype correlated with higher plasma VEGF-A level. Another Single Nucleotide Polymorphism (SNP) regarding *VEGF* gene at the position of *7C/T* found to be associated with DPN. The allele *C* was considered probably related to increased risk of developing DPN while allele *T* might be protective [14]. The exact mechanism of the potentially protective effect of *T* allele in this SNPs is still unclear [6] [14].

Although this study failed to reach a statistical significance, a higher median of VEGF-A level was observed in subjects with DPN and *CT+TT* genotype. These findings support the results of the study by Kim et al*.,* (2009) [7]. It is presumed that the *VEGF* gene 936 C/T polymorphism is somehow related with the AP-4 binding protein of VEGF gene which determines the level of circulating VEGF. Deguchi et al., (2009) revealed that the circulating VEGF level increased in the early and symptomatic stage of DPN and decreased later at an advanced stage [5]. The positive relationship between VEGF-A level and DPN from multivariate logistic regression analysis was found in this study supports this finding.

Another interesting finding from this study is the significant association between polymorphism of *VEGF* gene *936 C/T* with body mass index. *CC* genotype had a significantly higher BMI compared with *CT + TT* genotype (27.23 ± 4.66 vs 25.28 ± 3.93; p = 0.023) (Table 2). The previous study on obstructive sleep apnea failed to prove the relationship between *VEGF* gene *936 C/T* polymorphism with obesity [15]. High VEGF-A level and VEGF-A overexpression are potentially protective towards obesity and insulin resistance. VEGF-A also has a role on the thermogenesis of brown adipose tissue (BAT) which plays a role in the development of obesity [16]. This study found that the level of VEGF-A on *CT + TT* genotype is higher compared with CC genotype. Although the difference was not statistically

significant, this could somehow explain the relationship between *VEGF* gene *936 C/T* polymorphism with BMI. More studies are still needed to confirm this finding

The increase in VEGF expression in the early stage of DPN is a response to hypoxia and oxidative stress. The target of this process is the endothelial cells, with proliferation and angiogenesis/ neovascularisation as a result. VEGF through VEGFR2 also plays the neuroprotective role together with other angioneurins such as IGF1, TGF beta 1, EPO, FGF2, HGF, EGF, and PGRN. These substances are believed to have a positive effect on remyelination and neurogenesis. These facts support the concept that VEGF has a dual effect, involved in pathogenesis as well as protecting the nerves in DPN [5] [17]. The fact that VEGF-A had a positive but small effect on the relationship with the occurrence of DPN could be explained by this concept.

The regression model in this study showed that the interaction of *VEGF* gene *936 C/T* polymorphism with diagnosis duration and vascular factors especially blood lipids plays an important role in the development of DPN. In subjects with HbA1C ≥ 7%, LDL, HDL, and duration of diabetes diagnosis had a positive effect on the development of PDN. These findings have a clinical/practical implication, meaning that prevention strategy can be focused on individuals with high HbA1C, high LDL level, low HDL level, and longer duration of diabetes diagnosis.

Dislipidemia considered as an important risk factor of DPN [18]. Lipid levels are related with the accumulation of sorbitol, formation of oxidised lipids and PARP, and activation of lipoxygenase on peripheral nerve fibres. Free fatty acids cause toxicity on neuronal cell and Schwann's cells and also trigger the release of proinflammatory cytokines causing neuronal inflammation. Lipids are also related to insulin resistance of which in type-2 diabetic patients may worsen the hyperglycemic state. The peripheral nerve tissue expressed oxidised LDL (oxLDL) scavenger receptor. The receptors internalise oxLDL and glycated LDL which triggers inflammatory signals, causing NADPH oxidase inactivation which may cause increased oxidative stress [19] [20]. Lower HDL level has been proven to be associated with DPN and means that management to dislipidemia plays an important role in preventing and treatment of DPN [21].

To our knowledge, this is the first study in regards to *VEGF* gene *936 C/T* polymorphism in a diabetic subject in Indonesia. There were some limitations to this study. Outliers of the VEGF-A level might show that excluding conditions, of which some of them relied only on medical record, such as acute on chronic OA, history of cancer, acute stroke and acute myocardial infarction were not enough to avoid extreme values of VEGF-A level. It is necessary to add the items on exclusion criteria such as smoking (active or passive), alcoholism, and other situation that could interfere with VEGF-A level. The diagnostic criteria in this study were likely only to accommodate mild DPN. Hence the result of this study can only be applied to those group. This study did not compare the genotype frequency between diabetic and normal subjects. Nevertheless, some previous studies have proved that polymorphism of *VEGF* gene *936 C/T* did not associate with diabetes mellitus [6] [22].

The *CT+TT* genotype of *VEGF* gene *936 C/T* polymorphism is potentially protective, while the *CC* genotype may be associated with a higher risk of developing DPN. A trend of higher VEGF-A level in DPN and *CT + TT* genotype was observed. This study also suggested that VEGF-A has a potential role in the pathogenesis of DPN, especially from the vascular aspect. Vascular risk factors, especially HbA1C and LDL, and HDL, as well as diagnosis duration, acted as modifying factors which interact with *VEGF* gene *936 C/T* polymorphism in the development of DPN.

Further study is needed to reveal the relationship between *VEGF* gene *936 C/T* polymorphism and the physiological function of peripheral nerve in DPN such as quantitative sensory testing or neuroesthesiometer value of each nerve. This is important to find out the influence of *VEGF* gene *936 C/T* polymorphism on the physiology of peripheral nerve to reveal its protective mechanism. Further study towards other genetic variants related to glycemic and metabolic function, ethnicity, especially the ones presumably related to nerve structure and function is needed to find the strongest genetic factors associated with DPN.

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Correlation between 25-Hydroxyvitamin D and Lipid Profile among Children with Beta Thalassemia Major

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Abstract

BACKGROUND: Beta thalassemia major is associated with lipid profile abnormalities, presented as a lower level of total cholesterol (TC), low-density lipoproteins (LDL), high-density lipoprotein (HDL), and higher triglyceride level; increasing risk for cardiovascular complications. The previous studies indicated that Vitamin D give a positive impact on the lipid profile in healthy children population. However, its role needs to be determined in a high-risk group of children with beta-thalassemia major.

AIM: To determine the correlation between vitamin D (25-OHD) and lipid profile among children with beta-thalassemia major.

METHODS: A cross-sectional study was conducted in a general tertiary hospital in Medan, Sumatera Utara, Indonesia from January to March 2018. Subjects were children aged below 18-year-old with beta-thalassemia major. The measurement of vitamin D (25-OHD) level and 10-12 hour overnight fasting serum lipid profile including total cholesterol, triglyceride, HDL, and LDL were performed. The analysis was done using Pearson's correlation and Fisher test. P value < 0.05 was considered significant.

RESULTS: Forty-five subjects were enrolled in this study, with serum ferritin level ranged from 1017 to 13372 ng/mL. The prevalence of vitamin D deficiency (a 25-OHD level less than 20 ng/mL) in this study was 40%, with mean value at 20.6 (SD 5.3) ng/mL. The markers for cardiovascular risk were observed to be elevated, both in Atherogenic Index Plasma (0.32 ± 0.25) and TC: HDL ratio (4.2 ± 1.5). Statistical analysis revealed that Vitamin D had positive correlation with total cholesterol ($r = 0.302$, $p = 0.044$) and HDL ($r = 0.297$, $p = 0.048$). There was no significant correlation between both vitamin D and triglyceride ($p = 0.305$), or vitamin D and LDL ($p = 0.727$).

CONCLUSION: Vitamin D correlated positively with total cholesterol and HDL in children with beta-thalassemia major. Positive correlation to HDL indicated a beneficial effect of vitamin D to reduce the risk of cardiovascular complication.

Introduction

Thalassemia beta was caused by a genetic disorder in globin β chain production. Complete loss of globin β chain presented as a β-thalassemia major [1]. Patients with β-thalassemia major need regular blood transfusion, arising complications from iron accumulation in heart, liver, and endocrine organs [2] [3]. It has been shown that children with β-thalassemia major develop premature atherosclerosis, as a result of chronic hemolysis and iron deposition in the blood vessels, along with dyslipidemia [3]. Children with β-thalassemia have abnormalities in lipid profile, presented as a lower level of total cholesterol (TC), low-density lipoproteins (LDL), high-density lipoprotein (HDL), and higher triglyceride level [4].

Dyslipidemia in patients with β-thalassemia was caused by several factors; such as plasma dilution due to anaemia, increased erythropoietic activities followed by increased cholesterol uptake by macrophage and histiocyte in the reticuloendothelial system, liver impairment due to iron deposition, hormonal disturbance, and decreased extra-hepatic lipolysis activity [5]. The main factors contributing to dyslipidemia in a patient with β-thalassemia major are iron overload and oxidative stress [4].

The prevalence of Vitamin D deficiency in patients with beta-thalassemia major was reported as 5-87% worldwide [6]. It was more prevalent in children with beta-thalassemia aged above 10 years old [7] [8].

Vitamin D deficiency is associated with increased liver iron concentration, which impairs the hydroxylation process of Vitamin D by a 25-hydroxylase enzyme in the liver [9]. Vitamin D has been associated with the stiffness of blood vessels, which increased the risk of cardiovascular diseases and atherosclerosis [10].

Some previous studies revealed that vitamin D is associated with favourable lipid profile in children population [11]. Kelishadi et al. reported that vitamin D had an inverse correlation with total cholesterol, triglyceride, and LDL in healthy children population. Besides, there was a positive correlation between vitamin D level and HDL level [11]. Its favourable effect on lipid profile was associated with an increased level of calcium, parathyroid hormone suppression, and decreased insulin resistance [12] [13]. Vitamin D also maintain an adequate concentration of apolipoprotein A-1, which influences the formation of HDL [14]. The study by Hirschler reported that high dose vitamin D supplementation of 100.000 IU per month for 2 months is associated with a significant increase of HDL level and decreased triglyceride level in children [15]. However, the favourable effect of vitamin D to lipid profile in a high-risk group of children with beta-thalassemia major has not been extensively studied yet.

The objective of this study is to determine the correlation between vitamin D (25-OHD) and lipid profile among children with beta-thalassemia major

Methods

This study was an analytic observational study with a cross-sectional design. The data were collected from patients with β-Thalassemic Major in Haji Adam Malik General Hospital, Medan, Indonesia who receive a regular blood transfusion in one-day care ward from January until March 2018. The inclusion criteria were patients with β-Thalassemic Major diagnosed by haemoglobin electrophoresis, received regular blood transfusion, aged 1-18 years old, and serum ferritin level more than 1000 ng/ml. The exclusion criteria were patients with β-Thalassemic Major who already consumed vitamin D supplement or anti dyslipidemia drugs. The minimum sample required in this study was 42 subjects, calculated using the hypothesis test formula for correlation study with the power of 90 (β 10%).

The independent variables of this study was 25-hydroxyvitamin D, which was classifed as deficiency (less than 20 ng/mL), insufficiency (20-30 ng/mL), and normal (30-80 ng/ml). The dependent variable data were lipid profile, including total cholesterol, triglyceride, High Density Lipoproteins (HDL) and Low Density Lipoproteins (LDL). Triglyceride was classifed as normal (< 150 mg/dL) and high (≥ 150 mg/dL). Total cholesterol was classifed as normal (< 170 mg/dL), borderline (170–199 mg/dL), and high (≥ 200 mg/dL). HDL was classified as normal (> 40 mg/dL) and low (≤ 40 mg/dL). LDL was classified as normal (< 110 mg/dL), borderline (110-129 mg/dL), and high (≥ 130 mg/dL) [16].

The baseline data were obtained from subjects who fulfilled inclusion and exclusion criteria, including age, sex, weight, and length, the presence of organomegaly, Mid Upper Arm Circumference (MUAC), and serum ferritin within last 3 months before the study. The nutrition status of the subjects was plotted according to Weight for Length chart from WHO Child Growth Standard 2006 for children under 5 years old and CDC 2000 growth chart for children 5-18 years old.

The subject was requested to have 10-12 hours fasting before blood sampling, in which 3 ml of venous blood were extracted and collected in a serum tube. Measurement of 25-hydroxyvitamin D was performed via *chemiluminescence microparticle immunoassay* (CMIA) method. Measurement of triglyceride level was performed via glycerol phosphate oxidase method and cholesterol level via the enzymatic method. Measurement of HDL level was performed via accelerator selective detergent method and LDL level via the liquid selective detergent method. Instrument Architect (ABBOTT laboratories, USA) was used to perform all laboratory examinations. Pearson's correlation and Fisher exact test were used for statistical analyses by *SPSS Statistics ver. 20* software. Statistical significance was considered at p value less than 0.05. This study was approved by Ethics Committee of Faculty of Medicine, Universitas Sumatera Utara and Haji Adam Malik General Hospital.

Results

Out of 49 patients with β-thalassemia major, there were 45 subjects who fulfilled the inclusion and exclusion criteria. Four patients were excluded since the serum ferritin level was below 1000 ng/mL. There was a female preponderance of 56% in the subjects. The median age of the subjects was 10.9 years old, with the youngest subject of 1.6 years old and the oldest subject of 17.9 years old. The nutrition status of 47% of subjects was malnourished, and most of the subjects were stunted at 62% (Table 1).

All subjects had serum ferritin level above

1000 ng/mL with a median of 2000 ng/ml. This study reported the mean level of 25-hydroxyvitamin D in children with a β-thalassemia major at 20.6 ng/mL, with 55.6% of the subjects were in the insufficiency range (20-30 ng/mL).

Table 1: Clinical, Anthropometry, and Laboratory Parameters of Children with β-thalassemia major (n = 45)

Parameter	Value
Sex, n (%)	
Male	20 (44)
Female	25 (56)
Age (year), median (min-max)	10.9 (1.6 – 17.9)
Weight (kg), median (min-max)	24 (10 – 61)
Height (cm), mean (SD)	121.8 (± 23.53)
Mid upper arm circumference (cm), mean (SD)	17.28 (± 2.52)
Nutrition Status, n (%)	
Well-nourished	21 (47)
Malnourished	22 (49)
Overweight	2 (4)
Height for Age, n (%)	
Normal	16 (36)
Stunted	28 (62)
Severely Stunted	1 (2)
Serum Ferritin (ng/mL), median (min-max)	2000 (1017-13371.95)
Haemoglobin pre transfusion (g/dL), mean (SD)	6.9 (± 1.3)
25-hydroxyvitamin D (ng/mL), mean (SD)	20.6 (± 5.3)
Normal, n (%)	2 (4.4)
Insufficency, n (%)	25 (55.6)
Deficiency, n (%)	18 (40)
Triglyceride (mg/dL), median (Min-Max)	112 (64 - 287)
Normal, n (%)	34 (75.5)
High, n (%)	11 (24.5)
Total Cholesterol (mg/dL), mean (SD)	93.1 (± 21.4)
Normal, n (%)	45 (100)
Borderline, n (%)	0 (0)
High, n (%)	0 (0)
LDL (mg/dL), median (Min-Max)	57 (24 - 173)
Normal, n (%)	41 (91.2)
Borderline, n (%)	2 (4.4)
High, n (%)	2 (4.4)
HDL (mg/dL), mean (SD)	22.2 (± 6.1)
Normal, n (%)	0 (0)
Low, n (%)	45 (100)
Atherogenic Index Plasma, mean (SD)	0.32 (± 0.25)
Ratio Total Cholesterol: HDL, median (min-max)	4.4 (2.9-10.9)

Min = Minimum value; Max = Maximum value; SD = Standard Deviation.

The median of triglyceride and LDL were within normal range, at 112 mg/dL and 57 mg/dL respectively. The mean level of total cholesterol was within normal range at 93.1 mg/dL. However, the mean level of HDL was below the normal range at 22.2 mg/dL. Majority of the subjects had a normal level of triglyceride and LDL, at 75.5% and 91.2% respectively. All subjects in this study had a normal level of total cholesterol and low level of HDL. The markers for a predictor of cardiovascular diseases, Atherogenic Index Plasma (AIP) and total cholesterol to HDL ratio (TC: HDL), were shown in Table 1. Atherogenic Index Plasma was calculated by the formula log(Triglyceride: HDL), and the mean value was elevated at 0.32 (SD 0.25). The median value of TC: HDL was elevated too at 4.4 (range 2.9-10.9).

Table 2: Correlation between vitamin D and Lipid Profiles

		Triglyceride	Total Cholesterol	HDL	LDL
25-hydroxyvitamin D	r	-0.156	0,302	0,297	0,054
	p	0,305	0,044	0,048	0,727
	n	45	45	45	45

Pearson's test Confidence Interval 95%; r = correlation coefficient.

The correlation between 25-hydroxyvitamin D and lipid profile was shown in Table 2. Transformation data of triglyceride and LDL level were performed to make normal data distribution. Hence the Pearson's correlation was used in statistical analyses. Table 2 showed that there was no significant correlation between 25-hydroxyvitamin D and triglyceride level (p-value at 0.305) and LDL level (p-value at 0.727).

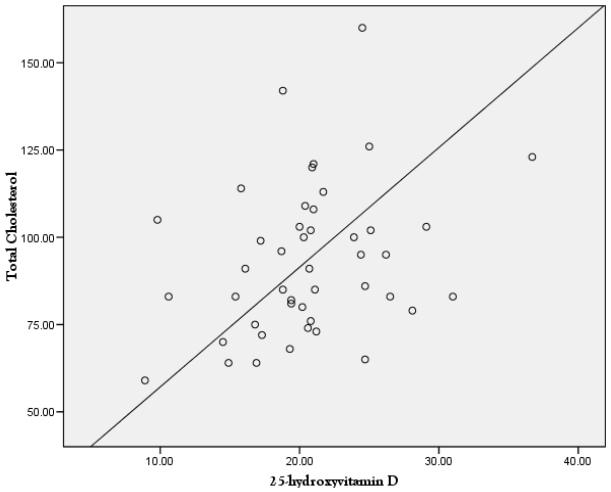

Figure 1: Scatter graph for correlation between 25-hydroxyvitamin D and total cholesterol

There was a significant correlation between 25-hydroxyvitamin D and total cholesterol level (p-value at 0.302). The correlation had a weak positive correlation coefficient (r = 0,302) as depicted in Figure 1. Besides, there was a weak positive correlation between 25-hydroxyvitamin D and HDL level (r=0,297) as depicted in Figure 2.

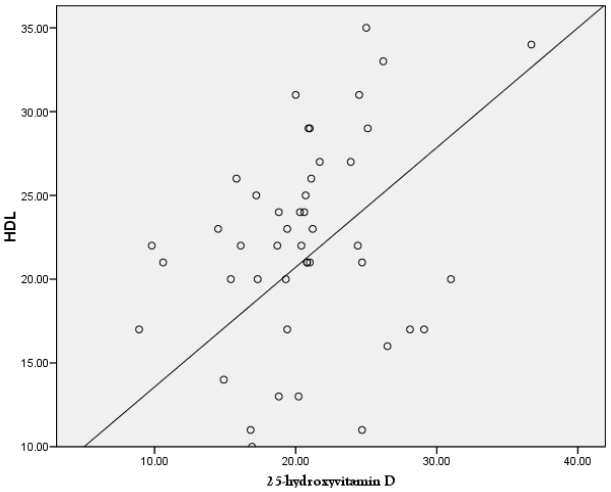

Figure 2: Scatter graph for correlation between 25-hydroxyvitamin D and HDL

Discussion

This study reported the prevalence of vitamin D deficiency at 40% and vitamin D insufficiency at 55.6% in children with beta-thalassemia major. This

result is in concordance with the previous study in Mesir by Fahim, who reported 37% of children with beta-thalassemia major had vitamin D deficiency, and another 54% had vitamin D deficiency [17]. The previous study in Indonesia by Fadillah reported a higher prevalence of vitamin D deficiency at 86% [18].

Up to 50-90% of Vitamin D is synthesised in the skin as the result of sun exposure, while the rest come from the diet. Vitamin D deficiency in a patient with beta thalassemia has been associated with liver iron accumulation, interfering with vitamin D hydroxylation [19]. Body iron accumulation in a patient with beta-thalassemia was monitored by serum ferritin measurement [18]. In this study, all subjects had high ferritin serum ranged from 1017 up to 13371.95 ng/mL, regardless of routine chelation therapy.

Serum iron deposition in the skin can cause hyperpigmentation, leading to the decreased conversion of 7-dehydrocholesterol to vitamin D3 in the skin. In tropical countries, vitamin D deficiency might occur as the children with beta-thalassemia was not allowed to play outside [6].

Dyslipidemia in children with beta-thalassemia major presented as a lower level of total cholesterol, HDL, and LDL; and higher triglyceride level compared to healthy child population [20] [21] [22]. Some factors contributing to dyslipidemia in thalassemia are plasma dilution due to anaemia and increased erythropoietic activity [5]. In this study, subjects had a low level of haemoglobin before blood transfusion at 6.9 g/dL (SD 1.3 g/dL). Increased erythropoietic activity will increase cholesterol uptake by macrophage and histiocyte within the reticuloendothelial system [5].

In this study, the mean or median level of total cholesterol, triglyceride, and LDL were still within normal range. Meanwhile, the HDL level was below the normal range (< 40 mg/dL). The mean value of HDL in this study at 22.2 (SD 6.1) mg/dL were much lower than a previous study by Fahim at 48.3 (SD 24.7) mg/dL [4]. Lipid profile abnormality which found in this study was low HDL level.

This study was not in agreement with previous studies, which reported that children with a β-thalassemia major in India, Egypt, and Jordania had high triglyceride level (> 150 mg/dL) [21] [22] [23] [24]. The decrease of triglyceride level was associated with decreased extra-hepatic lipolysis activities in children with beta-thalassemia [22] [23]. Another study in Iran and Italy reported that there was no significant difference between children with β-thalassemia major and healthy children control [25] [26]. The data from Southeast Asian is not available yet, prompting to further studies in the future.

All subjects in this study had HDL level below 40 mg/dL. The decrease of HDL level is associated with quick cleaning of activated monocyte and macrophage in a patient with beta-thalassemic major [24]. Pearson's statistic analyses in this study revealed a significant weak positive correlation between vitamin D and HDL level (r = 0,297); increase of vitamin D level was followed by an increase of HDL level. It was in agreement with the previous study in adult subjects by Saeidlou [13]. Vitamin D establish an adequate concentration of apolipoprotein A-1, which serves as a precursor of HDL formation [14]. The correlation coefficient in this study is more significant than the previous study in healthy children subjects by Kelishadi (r = 0.156) [11]. The studies in Iran dan Argentina reported that vitamin D supplementation 1000 IU daily or 100.000 IU per month was associated with an increase of HDL level [10] [15]. Hence, vitamin D supplementation in children with β thalassemia major is hypothesised to increase HDL level significantly.

In addition to low HDL level, subjects had a higher ratio of total cholesterol to HDL (TC: HDL) at 4.4. The upper limit for TC: HDL ratio is 3.5 based on Adult Treatment Panel III [20]. It is in agreement with the previous study by Ashar, who reported an increase of TC: HDL ratio at 5.7 in children with beta-thalassemia; increasing the risk of coronary heart diseases [20]. Another cardiovascular marker in this study, *Atherogenic Index Plasma* (AIP), was elevated at 0.32 (SD 0.25). Atherogenic Index Plasma value at more than 0.24 is a significant predictor for cardiovascular diseases [27]. The previous study by Sherief reported that high AIP level at 0.45 (SD 0.12) correlated with premature atherosclerosis in a patient with beta-thalassemia [5].

This study revealed the positive correlation between 25-hydroxyvitamin D and total cholesterol (r = 0,302). In contrast, the previous meta-analysis in healthy children population revealed a negative correlation between 25-hydroxyvitamin D and total cholesterol (r = -0,086) [11]. Vitamin D maintains a role in increasing calcium absorption in the digestive tract, through the formation of Calbindin (Calcium Binding Protein). Calbindin influences the influx of calcium into brush border of epithelial cells in the digestive tract through the diffusion process [28]. It was hypothesised that calcium binds the fatty acids from the diet into an insoluble form, which impair fat absorption in the digestive tract [12].

In children with beta-thalassemia major, iron overload and oxidative stress induce hypocholesterolemia [4]. In human, calcium acts as an inhibitor to the absorption of iron in the digestive tract. The absorption of iron in the digestive tract was conducted via Divalent Metal Transporter 1 (DMT 1) and Ferroportin (FPN) in the apical membrane. The interaction between calcium and DMT1 will inhibit the absorption of iron in the digestive tract [29]. It is hypothesised that iron absorption inhibition reduced iron overload and oxidate stress, which explained the positive correlation between 25-hydroxyvitamin D and total cholesterol in this study.

The strength of this study is the first study to

present the correlation between 25-hydroxyvitamin D and lipid profiles among a high-risk group of children with β-thalassemia major. There was an increase of markers for cardiovascular in the children with the β-thalassemia major; prompting to increase awareness of early cardiovascular diseases related to lipid profiles abnormalities. The limitation of this study is its inability to reveal a causal correlation between 25-hydroxyvitamin D and lipid profiles. The positive correlation between 25-hydroxyvitamin D and HDL need to be examined in the experimental study.

In conclusion, there is a significant positive correlation between 25-hydroxyvitamin D and lipid profiles in children with beta-thalassemia major, including total cholesterol and HDL level; but not with triglyceride and LDL level. Positive correlation to HDL indicated a beneficial effect of vitamin D to reduce the risk of cardiovascular complication. Further studies were needed to assess its causal correlation.

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Overview of MDM2 and B-RAF Expression in Gastric Lesions

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Abstract

BACKGROUND: Globally, gastric cancer (GC) it is the fourth most common cancer and the third cause of cancer-related deaths. Overexpression of MDM2 and B-RAF appeared to be increased in malignancy and associated with poor prognosis in several human tumours, but their role in gastric cancer remains controversial.

AIM: We had investigated the immunohistochemical expression of MDM2 and B-RAF in 136 gastric lesions with/without *H. pylori* association.

MATERIAL AND METHODS: Studied specimens include chronic gastritis (32), intestinal type GC (70), diffuse GC (22) and gastrointestinal stromal tumours (GIST) (12).

RESULTS: MDM2 expression increased significantly in intestinal GC compared to other groups ($p < 0.001$), while B-RAF expression increased significantly in GIST compared to other groups ($p < 0.001$). *H. pylori* increased expression of MDM2 in intestinal GC cases but did not affect B-RAF expression. MDM2 expression correlated with high grade of tumor differentiation ($p < 0.001$), deep invasion ($p < 0.05$), nodal metastases ($p < 0.05$) and distant metastases ($p < 0.1$) in intestinal GC, while B-RAF expression did not correlate with TNM stage ($p < 0.1$).

CONCLUSION: MDM2 up-regulation was more frequent in intestinal GC, while B-RAF up-regulation was more frequent in GIST compared to other groups; MDM2 expression in intestinal GC was correlated with *H. pylori* association, high grade of differentiation, deep invasion, nodal and distant metastases, meanwhile, B-RAF expression was correlated with high-grade intestinal GC but did not correlate with *H. pylori* or TNM stage. The possible role of both MDM2 and B-RAF in predicting progression of gastric tumours and prognosis deserves further investigations.

Introduction

Worldwide, Gastric cancer (GC) is the fourth most common cancer in men (8.5%) and the third cause of cancer-related deaths (10.1%). In the female, it is the fourth most common cancer (4.8%) and the third cause of cancer-related deaths (7.2%) [1]. Although the incidence of gastric cancer has gradually decreased over the last half-century, the prognosis of advanced gastric cancer remains poor and gastric cancer-related mortality rates remain unacceptable in many areas [2].

Gastric carcinogenesis is a multistep and multifactorial process. The intestinal type of gastric cancer is often related to environmental factors such as Helicobacter pylori infection, diet, and lifestyle, while the diffuse type is more often associated with genetic abnormalities [3].

The *Helicobacter pylori* (*H. pylori*) bacterium is responsible for 5.5% of all infection-associated cancers and is the major cause of gastric cancer in consequence of chronic inflammation [4]. Persistent gastric mucosa inflammation results in chronic gastritis and progresses through a multistep process to gastric atrophy, intestinal metaplasia, dysplasia, and finally carcinoma [5].

In Egypt, infection with *H. pylori* is common, and acquisition of infection occurs at a very young age [6]. Also, gastric cancer is the 13th most common cancer in men (1.8%) and the 10th cause of cancer-related deaths (2.2%). In the female, it is the 14th most

common cancer (1.5%) and the 11th cause of cancer-related deaths (2.2%). For both sexes, it is the 12th most common cancer (1.6%) and 11th cause of cancer-related deaths (2.2%) [7].

Several biological markers are tested as potential predictors of the gastric carcinoma outcome, and some of them are essential to developing a malignancy. *MDM2* (Murine double minute 2) is an oncogene that has been mapped to chromosome 12q13–14 and encodes a 90 kDa cellular oncoprotein. The gene structure on the human chromosome was identified in 1992 [8]. It binds to, and negatively regulates, transactivation of p53 and was then itself found to be a transcriptional target of p53, defining a negative feedback loop of p53 tumour suppressor gene [9]. The *MDM2* oncogene played an important role in cancer progression as overexpression of *MDM2* in tumour cells induced cell proliferation and inhibits cell apoptosis [10]. Several studies have shown that *MDM2* overexpression was associated with poor survival and was a useful predictive factor for poor prognosis in humans with hepatocellular carcinoma and breast carcinomas [11] [12].

V-RAF murine sarcoma viral oncogene homolog B1 (*B-RAF*) is a member of the RAF family of protein kinases which has three members: *A-RAF*, *B-RAF* and *Raf-1* [13]. All RAF proteins are serine/threonine kinases located in the RAS/RAF/MEK/ERK cascade as downstream effectors of RAS and can phosphorylate and activate MEK, which in turn activates ERK. *B-RAF* is the most potent activator of MEK [14] [15] and is the only one known to be activated by mutation in human cancer [16]. They are mainly found in melanoma, thyroid papillary carcinoma and colorectal tumours with microsatellite instability [17].

In this study, we investigated immunohistochemical expression of *MDM2* and *B-RAF* in chronic gastritis and malignant gastric lesions; and their correlation with *H. pylori* association, tumour location, grade, and TNM stage in Egyptian patients.

Material and Methods

This study was conducted on 136 archival gastric paraffin blocks from Pathology Department of Theodor Bilharz Research Institute. All samples had been obtained as endoscopic biopsies or gastrectomy specimens. The study protocol was approved by the Ethics committee of Theodor Bilharz Research Institute, for the protection of human subject and adopted by the 18th world medical assembly, Helsinki, Finland (2013).

Our studied lesions were classified into four groups: chronic gastritis: 32 specimens; intestinal GC: 70 specimens; diffuse GC: 22 specimens; GIST: 12 specimens.

Gastric tissue sections were stained by Hematoxylin-eosin for routine diagnosis, grading and staging of tumours. Giemsa stain was used to detect *H. pylori* in gastric sections.

Immunohistochemistry for *MDM2* and *B-RAF* was performed on tissue sections cut from the paraffin blocks at 4µm onto positively charged slides (Superfrost Plus, Menzel-Glaser, Germany) and stained on an automated platform (Dako Autostainer Link 48) using: anti-human *MDM2* monoclonal primary antibodies (Clone MSP14, NeoMarkers, Fremont, CA, USA) and anti-B-RAF pV600E (Spring Bioscience, Pleasanton, CA; purchased from Zytomed Systems, Berlin, Germany) at 1:200 dilution. Heat-induced antigen retrieval was used for 30 min at 97°C in the high-PH EnVision™ FLEX Target Retrieval Solution.

For each setting, positive and negative control slides were included. As a negative control, gastric tissue was processed, but the primary antibodies were not added and instead add non-immune immunoglobulin G (IgG; DAKO, Glostrup, Copenhagen, Denmark). The positive control was a section of liposarcoma for *MDM2* and colorectal carcinoma for *B-RAF*.

All sections were assessed and scored. The sections were examined by using light microscope [Scope A1, Axio, Zeiss, Germany]. Photomicrographs were taken using a microscope-camera [AxioCam, MRc5, Zeiss, Germany]. All procedures were done at the pathology department of Theodor Bilharz Research Institute, Cairo, Egypt.

Scoring of *MDM2* immunostaining was performed semiquantitatively, using digital images and 22-in monitor with hardware calibration capabilities. Staining was considered to be negative (0) if no staining was seen within a tumour, weakly positive (1+) if focal staining was seen, and strongly positive (2+) if there was diffuse staining in more than 80% of tumour cells [18]. Nuclear staining could be detected in very few cases, and the vast majority of positive cases showed only cytoplasmic staining.

The intensity of cytoplasmic immunostaining was scored from zero to 3 (0: no staining, 1: weak, 2: moderate and 3: strong) [19]. Cases with moderate and strong immunostaining were considered positive [20].

We have also counted the percentage of cells with positive expression in 5 successive high power fields.

The immunohistochemical results were analysed using SPSS version 20 (IBM Corporation, Armonk, New York, USA). Data are presented as the mean ± S.D. Two-tailed Student's *t*-tests and one-way

ANOVA were used to evaluate the data. Comparison of difference in percentage between groups was evaluated using two-tailed Fischer's exact test. Differences were considered statistically significant at $P < 0.05$.

Results

Different studied gastric lesions were more common in males (73.5%) than females (26.5%). The differences were statistically significant ($p < 0.05$) in cases of chronic gastritis and intestinal GC, while non-significant in cases of diffuse GC and GIST ($p > 0.05$) (Table 1).

Table 1: Gender in different studied lesions

Lesion	Gender		Total no. (%)
	Female no. (%)	Male no. (%)	
Chronic gastritis	4_a (11)	28_b (28)	32 (23.5)
Intestinal GC	24_a (66.7)	46_b (46)	70 (51.5)
Diffuse GC	6_a (16.7)	16a (16)	22 (16.2)
GIST	2_a (5.6)	10a (10)	12 (8.8)
Total	36	100	136

GC: gastric cancer, GIST: gastrointestinal stromal tumor.

Endoscopically, cases of chronic gastritis represented usually as diffuse mucosal lesions, cases of intestinal and diffuse GC represented as fungating or ulcerative lesions and usually located at the gastro-oesophagal junction (GEJ) or pylorus, while GIST cases represented as mass lesions. No significant differences were found considering endoscopic appearance or location of studied gastric lesions (Table 2).

Table 2: Endoscopic appearance and location of studied gastric lesions

		Lesion				Total
		Chronic gastritis no. (%)	Intestinal GC no. (%)	Diffuse GC no. (%)	GIST no. (%)	
Endoscopic appearance	Diffuse	32_a (100)	0_b	0_b	0_b	32 (23.5)
	Fungating	0_a	64_b (91.4)	20_b (90.9)	0_a	84 (61.8)
	Mass	0_a	0_a	0_a	8_b (66.7)	8 (5.9)
	Ulcer	0_a	6_a (8.6)	2_a (9.1)	0_a	8 (5.9)
	Wall thickening	0_a	0_a	0_a	4_b (33.3)	4 (2.9)
	Unavailable	32_a (100)	$56_{b,c}$ (80)	14_c (63.6)	$12_{a,b}$(100)	114(83.8)
Anatomic site	Cardia	0_a	2_a(2.9)	0_a	0_a	2(1.5)
	Diffuse	0_a	2_a(2.9)	0_a	0_a	2(1.5)
	Fundus	0_a	4_a(5.7)	0_a	0_a	4(2.9)
	GEJ	0_a	$4_{a,b}$(5.7)	4_b(18.2)	$0_{a,b}$	8(5.9)
	Pylorus	0_a	2_a(2.9)	4_a(18.2)	0_a	6(4.4)
Total		32	70	22	12	136

GC: gastric cancer, GEJ: gastro-esophageal junction, GIST: gastrointestinal stromal tumor.

Cases of intestinal GC and diffuse GC showed the significantly higher percentage of *H. pylori* positivity compared to chronic gastritis and GIST ($p < 0.05$) (Table 3).

All studied chronic gastritis and GIST cases were negative for *MDM2* expression. *MDM2* positivity was identified in 31.4% of intestinal GC and 9.1% of diffuse GC, with the statistically significant difference between intestinal GC and other groups ($p < 0.001$) as well as between diffuse GC and both chronic gastritis and GIST ($p < 0.05$).

Table 3: Association between *H. pylori* and different studied lesions

H. pylori	Lesion				Total no. (%)
	Chronic gastritis no. (%)	Intestinal GC no. (%)	Diffuse GC no. (%)	GIST no. (%)	
Positive	12_a (37.5)	44_b (62.9)	$14_{a,b}$(63.6)	$6_{a,b}$ (50)	76 (55.9)
Negative	20_a (62.5)	26_b (37.1)	$8_{a,b}$ (36.4)	$6_{a,b}$ (50)	60 (44.1)
total	32	70	22	12	136

GC: gastric cancer, GIST: gastrointestinal stromal tumor.

On the other hand, *B-RAF* positivity was identified in all studied GIST cases, 22.9% of intestinal GC and 6.2% of chronic gastritis cases, while all diffuse GC were negative, with statistically significant difference comparing GIST to other groups ($p < 0.001$) and comparing intestinal GC to chronic gastritis and diffuse GC ($p < 0.05$) (Table 4).

Table 4: *MDM2* and *B-RAF* immunoreactivity in different lesions

Lesion	*MDM2*		*B-RAF*		Total
	Negative no. (%)	Positive no. (%)	Negative no. (%)	Positive no. (%)	
Chronic gastritis	32 (100)	0	30 (93.8)	2	32
Intestinal GC	48 (68.6)	22 (31.4)**	54 (77.1)	16 (22.9)#	70
Diffuse GC	20 (90.9)	2 (9.1)*	22 (100)	0	22
GIST	12 (100)	0	0	12 (100)**	12
Total	112	24	106	30	136

GC: gastric cancer, GIST: GIST: gastrointestinal stromal tumor; ** Significant difference with other groups ($p < 0.001$); * Significant difference with chronic gastritis and GIST ($p < 0.05$); #Significant difference with chronic gastritis and diffuse GC ($p < 0.05$).

Mean percentage of *MDM2* positive cells and intensity of expression were significantly higher in intestinal GC followed by diffuse GC compared to chronic gastritis and GIST cases ($p < 0.001$), while mean percentage of *B-RAF* positive cells and the intensity of expression were significantly higher in GIST followed by intestinal GC compared to chronic gastritis and diffuse GC cases ($p < 0.001$) (Table 5).

Table 5: Expression of *MDM2* and *B-RAF* (mean percentage of positive cells and intensity of expression) in different studied lesions

Lesion (no.)	*Mdm2*		*B-raf*	
	Percent Mean ± Std. Error of mean	Intensity Mean ± Std. Error of mean	Percent Mean ± Std. Error of mean	Intensity Mean ± Std. Error of mean
Chronic gastritis (32)	0.50 ± 0.35	0.06 ± 0.04	2.31 ± 1.32	0.19 ± 0.09
Intestinal GC (70)	8.51 ± 1.28	0.94 ± 0.08	15.49 ± 3.12	0.74 ± 0.10
Diffuse GC (22)	1.45 ± 0.70	0.27 ± 0.13	0.00 ± 0.00	0.00 ± 0.00
GIST (12)	0.00 ± 0.00	0.00 ± 0.00	86.67 ± 1.42	2.67 ± 0.14
p value	P < 0.001	P < 0.001	P < 0.001	P < 0.001

GC: gastric cancer, GIST: GIST: gastrointestinal stromal tumor.

For statistical purposes, we separately studied the relation between clinic-pathological features of intestinal GC cases and immunohistochemical expression results of *MDM2* and *B-RAF*.

As regards the endoscopic appearance of intestinal GC; fungating lesions exhibited a higher percentage of *MDM2* positive cells and *MDM2* intensity of expression, while ulcerative lesions

exhibited a higher percentage of *B-RAF* positive cells and *B-RAF* intensity of expression. However, these relations did not reach a significant difference between examined groups (p > 0.1) (Table 6).

Table 6: Relationship between the expression of MDM2 and B-RAF with the Endoscopic appearance of intestinal GC

Endoscopic appearance (no. Of lesions)	*MDM2*		*B-RAF*	
	Percent	Intensity	Percent	Intensity
	Mean ± Std. Error of mean		Mean ± Std. Error of mean	
Fungating (64)	8.94 ± 1.38	0.97 ± 0.08	15.38 ± 3.33	0.72 ± 0.10
Ulcer (6)	4.00 ± 1.26	0.67 ± 0.21	16.67 ± 9.01	1.00 ± 0.37
P value	P > 0.1	P > 0.1	P > 0.1	P > 0.1

Considering the tumour location, the mean percentage of *MDM2* positive cells and intensity of expression were significantly higher in tumours with the diffuse location, followed by GEJ compared to other sites (p < 0.001). On the other hand, **the** mean percentage of *B-RAF* positive cells and intensity of expression were higher in tumours at GEJ followed by fundus compared to other sites; the difference was statistically significant for *B-RAF* intensity score (p < 0.001) but non-significant for *B-RAF* per cent (p > 0.1) (Table 7).

Table 7: Relationship between the expression of MDM2 and B-RAF with anatomical site of intestinal GC

Anatomical site (no. Of lesions)	*MDM2*		*B-RAF*	
	Percent	Intensity	Percent	Intensity
	Mean ± Std. Error of mean		Mean± Std. Error of mean	
Undefined (56)	5.18 ± 0.50	0.79 ± 0.06	14.71 ± 3.51	0.68 ± 0.10
Cardia (2)	5.00 ± 0.00	1.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Diffuse (2)	40.00 ± 0.00	3.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Fundus (4)	17.50 ± 7.22	1.00 ± 0.00	20.00 ± 11.58	1.00 ± 0.58
GEJ (4)	29.00 ± 12.12	2.00 ± 0.58	45.00 ± 1443	2.50 ± 0.29
Pylorus (2)	15.00 ± 0.00	1.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
P value	P < 0.001	P < 0.001	P > 0.1	P < 0.001

GEJ: gastro-oesophageal junction.

Regarding *H. pylori* association, the mean percentage of *MDM2* positive cells and intensity of expression were higher in *H. pylori*-associated intestinal GC compared to *H. pylori* non-associated tumours, without a statistically significant difference (p > 0.1). On the contrary, mean percentage of *B-RAF* positive cells and intensity of expression were higher in *H. pylori* non-associated intestinal GC, without statistical significance (p > 0.1) (Table 8).

Table 8: Relationship between the expression of MDM2 and B-RAF with H. pylori association of intestinal GC

H. Pylori (no. Of lesions)	*Mdm2*		*B-raf*	
	Percent	Intensity	Percent	Intensity
	Mean ± std.error of mean		Mean ± std.error of mean	
Positive (44)	9.41 ± 1.85	1.00 ± 0.11	14.55 ± 3.87	0.73 ± 0.13
Negative (26)	7.00 ± 1	0.85 ± 0.07	17.08 ± 5.36	0.77 ± 0.14
P value	P > 0.1	P > 0.1	P > 0.1	P > 0.1

Mean percentage of *MDM2* positive cells and intensity of expression were significantly higher in high grade intestinal GC compared to low grade ones (p < 0.0001 & p < 0.01 respectively), and in high stage compared to lower stages; the difference was statistically significant for *MDM2* intensity score (p < 0.05) and non-significant for *MDM2* percent (p > 0.05), additionally percentage of *MDM2* positive cells and intensity of expression increased significantly with increasing lymph node stage (p < 0.05 and < 0.0001 respectively) and with presence of distant metastases; the difference was statistically significant for *MDM2* intensity score (p < 0.05) and non-significant for *MDM2* percent (p > 0.01) (Table 10).

Table 9: Relationship between the expression of *MDM2* and *B-RAF* with intestinal GC grade of differentiation

Grade (no. Of lesions)	*MDM2*		*B-RAF*	
	Percent	Intensity	Percent	Intensity
	Mean ± Std. Error of mean		Mean ± Std. Error of mean	
High (12)	21.83 ± 5.77	1.50 ± 0.34	25.83 ± 8.28	1.17 ± 0.37
Low (58)	5.76 ± 0.52	0.83 ± 0.05	13.34 ± 3.33	0.66 ± 0.09
P value	P < 0.0001	P < 0.01	P > 0.1	P < 0.05

In addition, mean percentage of *B-RAF* positive cells and the intensity of expression were higher in high-grade intestinal GC compared to low-grade tumours; the difference was statistically significant for *B-RAF* intensity score (p < 0.05) and non-significant for *B-RAF* per cent (p > 0.1) (Table 9), moreover, these parameters were higher in T3 intestinal GC compared to T2 and T4 without statistical significance (p > 0.1) (Table 8). Also, *B-RAF* parameters were higher in N1 stage compared to N0 and N3 and in M0 compared to M1without statistical significance (Table 10).

Table 10: Relationship between the expression of MDM2 and B-RAF in intestinal GC with TNM stage

Item (no. Of lesions)	*MDM2*		*B-RAF*	
	Percent	Intensity	Percent	Intensity
	Mean ± Std. Error of mean		Mean ± Std. Error of mean	
T				
2 (12)	2.50 ± 0.75	0.50 ± 0.15	13.83 ± 7.62	0.67 ± 0.22
3 (38)	9.21 ± 2.08	1.00 ± 0.12	18.84 ± 4.85	0.79 ± 0.13
4 (20)	10.80 ± 1.76	1.00 ± 0.00	10.10 ± 3.76	0.70 ± 0.18
P value	P > 0.05	P < 0.05	P > 0.1	P > 0.1
N				
0 (28)	5.14 ± 1.09	0.57 ± 0.10	7.07 ± 3.40	0.50 ± 0.12
1 (26)	8.15 ± 1.29	1.00 ± 0.00	22.46 ± 5.67	0.92 ± 0.15
3 (16)	15.00 ± 4.52	1.50 ± 0.22	18.88 ± 7.64	0.88 ± 0.27
P value	P < 0.05	P < 0.0001	P > 0.05	P > 0.1
M				
0 (52)	8.04 ± 1.47	0.85 ± 0.08	17.00 ± 3.67	0.85 ± 0.12
1 (18)	9.89 ± 2.60	1.22 ± 0.15	11.11 ± 5.95	0.44 ± 0.12
P value	P > 0.1	P < 0.05	P > 0.1	P > 0.05

Each subscript letter denotes a subset of gender categories whose column proportions do not differ significantly from each other at the 0.05 level.

Each subscript letter denotes a subset of lesion categories whose column proportions do not differ significantly from each other at the 0.05 level

Each subscript letter denotes a subset of lesion categories whose column proportions do not differ significantly from each other at the 0.05 level.

Discussion

Gastric cancer is still a serious public health problem in the world. The high mortality rate that is seen globally is mainly due to the advanced stage at

diagnosis with the availability of few biomarkers for early detection [21].

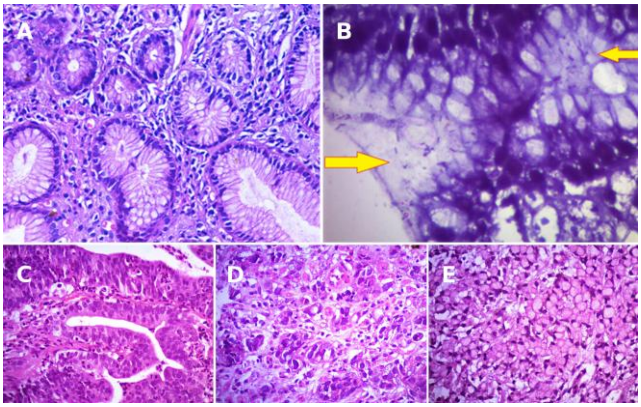

Figure 1: Sections from gastric tissue showing: A) A case of chronic gastritis (H&E stain X200); B) Helicobacter pylori microorganisms in relation to surface epithelium of gastric mucosa (arrows) (Giemsa stain X 400); C) A case of intestinal type gastric adenocarcinoma of low grade (H & E stain X 200); D) A case of high-grade gastric adenocarcinoma; intestinal type (H & E stain, X 200); E) A case of diffuse gastric carcinoma of signet-ring type (H & E stain X 200)

In the present work, male predominance was reported which is similar to the worldwide trend (2:1) [22], as 73.5% of gastric lesions belonged to males compared to 26% belonged to females, with incidence 2.8:1. A percentage lower than ours reported by Gaballah et al., [23] and Darwish et al., [24] who reported male to female ratio of 1.2:1 and 1.3: 1 respectively.

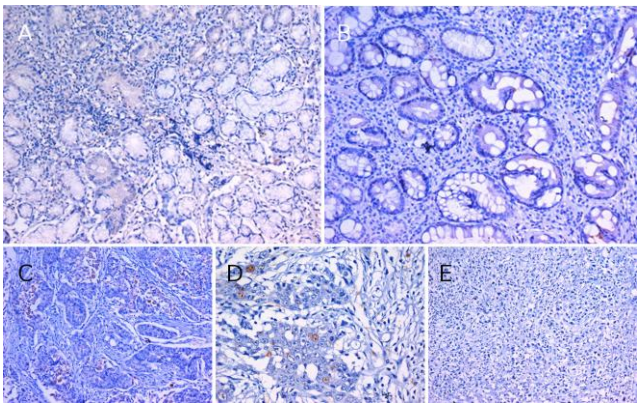

Figure 2: IHC using anti-MDM-2 monoclonal antibody in gastric sections: A) A case of chronic gastritis negative for MDM2 expression (X 200); B) A case of chronic gastritis with intestinal metaplasia negative for MDM2 (X 200); C) Sections in intestinal-type gastric carcinoma, low grade, showing mild focal nuclear expression of MDM2 (X 100); D) Section in intestinal-type gastric carcinoma, high grade, showing mild focal expression of MDM2 (X 200); E) Section in signet-ring type gastric carcinoma, negative for MDM2 expression (X 100)

The International Agency for Research on Cancer (IARC) classified *H. pylori* bacterium as a Group I carcinogen [25] *H. pylori is* a pathogen that colonises the gastric epithelium and causes chronic inflammation and considerably increases the risk of developing GC [26]**.** Our study showed that *H. pylori* were significantly associated with intestinal-type and diffuse GCs compared to GISTs and chronic gastritis, this comes by previous reports [27] [28] [29].

Endoscopically, our studied data sheet showed that cases of chronic gastritis usually represented as diffuse mucosal lesions, cases of intestinal and diffuse GC represented as fungating or ulcerative lesions, while GIST cases represented as mass lesions. Anatomically, no significant difference was detected considering the location of studied gastric lesions. Anatomical site of most of our studied lesions had not been mentioned. However, GEJ was the most frequent site mentioned for GCs; and this could be related to gastro-oesophageal reflux.

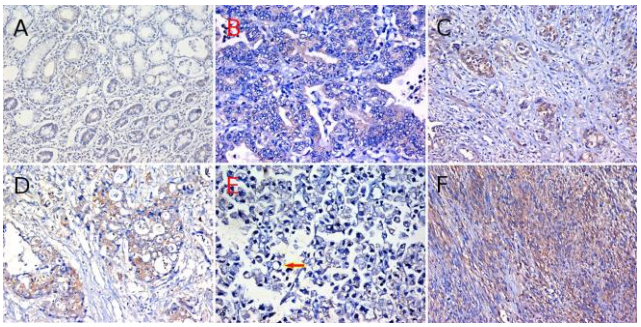

Figure (3): IHC using anti-B-RAF monoclonal antibody in gastric sections expressed as brown cytoplasmic staining (X 200): A) A case of chronic gastritis showing negative B-RAF expression (X 200); B) A case of intestinal type gastric adenocarcinoma, low grade, showing mild focal B-RAF expression (X 200); C) & D) Sections in intestinal-type gastric adenocarcinoma, high grade, showing moderate B-RAF expression (X 200); E) A case of diffuse gastric carcinoma (signet ring pattern) showing negative B-RAF expression (X 200); F) A case of gastrointestinal stromal tumour (GIST) showing moderate B-RAF expression (X200)

Wade et al., [30] and Li and Lozano [10] reported that *MDM2* oncogene played an important role in cancer progression and *MDM2* overexpression in tumour cells induced cell proliferation inhibited cell apoptosis. We found *MDM2* positivity in 31.4% of intestinal GC cases. Gunther et al., [31] found *MDM2* expression in 45% of intestinal GCs. However, Ye et al., [32] reported a much higher per cent, as they detected *MDM2* immunopositivity in 70.4% of their GC cases. Moreover, intestinal GC exhibited a significantly higher percentage of *MDM2* positive cells (8.51%) and higher intensity of expression compared to other groups. This matches the findings of Gunther et al., [31] and Nakajima [33] who detected *MDM2* positivity in 10% and 7.76% of gastric cancer cells respectively. Shen et al., [34] stated that *MDM2* expressed at higher levels in GC tissues than in non-cancerous gastric mucosa. On the contrary, Busuttil et al., [21] observed negligible levels of *MDM2* staining in GC samples. Variable results between studies may be attributed to different risk factors promoting to gastric cancer including *H. pylori*, obesity, tobacco smoking, red meat, a high-salt diet, alcohol, and low socioeconomic status, genetic polymorphisms, the age of cancer onset and gender.

On the other hand, *B-RAF* was expressed in

all GIST specimens that showed a significantly higher mean percentage of *B-RAF* positive cells (86.67%) and higher intensity of expression compared to other groups. This matches with findings of Holstein et al., [35] who observed *B-RAF* expression in all GIST cases in more than 80% of cells. On the contrary, several other studies reported a much smaller percentage of *B-RAF* positivity in GIST than ours [36] [37] [38] as they detected *B-RAF* mutation in 7%, 3.8% and 3.5% of GISTs respectively. Furthermore, intestinal GC cases showed significantly higher expression of *B-RAF* (higher number of positive cases, the percentage of positive cells and intensity of expression) compared to chronic gastritis and diffuse GC. Many previous studies reported the presence of a *B-RAF* mutation in patients with gastric adenocarcinoma [27] [39] [40].

Considering cases of intestinal type GC, no statistically significant difference was achieved when comparing fungating and ulcerating intestinal GC for parameters of *MDM2* and *B-RAF* expression (mean percentage of positive cells and intensity of expression). Tumours with diffuse location and at GEJ showed significantly higher mean percentage of *MDM2* positive cells and *MDM2* intensity of expression. On the other hand, tumours at GEJ and fundus showed non-significantly higher mean percentage of *B-RAF* positive cells and significantly higher *B-RAF* intensity of expression. To our knowledge, no other studies demonstrated *MDM2* or *B-RAF* expression about endoscopic appearance or anatomical site of intestinal GC.

In the present study, *MDM2* parameters were non-significantly higher in *H. pylori*-associated intestinal GC than in *H. pylori* non-associated ones. This goes with many previous studies reporting that *H. pylori* infection was associated with higher expression of *MDM2* in intestinal metaplasia and gastric carcinoma [33] [41] [42]. Furthermore, Kodama et al., [43] reported that successful eradication of *H. pylori* dramatically reduced *MDM2* levels. On the contrary, *B-RAF* parameters were non-significantly higher in *H. pylori*-non-associated intestinal GC than in *H. pylori*-associated ones; however, Sabry et al., [27] found a significant positive relationship between the qPCR of *H. pylori* and quantitative *B-RAF* in GC cases.

As regards different grades of differentiation in intestinal GC, we found a statistically significant higher percentage of *MDM2* positive cells and non-significant higher percentage of *B-RAF* positive cells in high-grade tumours compared to low-grade ones. This goes with findings of Sabry et al., [27] who detected a significant positive correlation between grades of GC and qPCR of *B-RAF*.

Our current results showed an increase in *MDM2* expression parameters with increasing depth of invasion, the presence of distant metastases and lymph node metastases. This matches with Ye et al., [32] results which reported that *MDM2* expression was associated with depth of invasion, lymph node metastases and distant metastases. Sepideh et al., [44] found a direct correlation between lymph node metastases and *MDM2* staining intensity; meanwhile, they did not find a remarkable correlation between *MDM2* expression and nodal involvement.

As regards *B-RAF* expression parameters in intestinal GC, no significant differences were achieved with different tumour stages, different stages of lymph node metastasis and state of distant metastases. These findings match results of other previous studies which did not find a relationship between *B-RAF* expression and histopathological variables of GC [45] [46] [47].

In conclusion, we found that: (1) *MDM2* up-regulation was more frequent in intestinal GC compared to other groups, while *B-RAF* up-regulation was more frequent in GIST compared to other groups; (2) *H. pylori* induces *MDM2* up-regulation in intestinal GC; (3) In intestinal GC cases, *MDM2* expression was correlated with high grade of differentiation, deep invasion, nodal and distant metastases, meanwhile, *B-RAF* expression was correlated with high-grade tumours but had no association with TNM stage. The possible role of both *MDM2 and B-RAF* in predicting progression of gastric tumours and prognosis deserves further investigations.

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Basic Science

The Effect of Treadmill Treatment on Oxidative Stress Markers and Endogenous Antioxidant Status in Obesity Mice

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Keywords: Obesity; Treadmill; MDA; GSH; MnSOD; Catalase

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Abstract

AIM: This study aimed to determine the effect of treadmill treatment on oxidative stress markers and endogenous antioxidant status seen from MDA, GSH, MnSOD enzyme specific activity and blood catalase of obese mice.

MATERIALS AND METHODS: This research is experimental laboratory research using post-test control design group only. The study lasted for 28 days and was divided into 4 groups of study, i.e., group K (normal control), KP (obesity control), P1 (obesity mice with 1 x 10-minute treadmill treatment a day), and P2 (obesity mice with 2 x 10-minute treadmill treatment a day).

RESULTS: The treadmill treatment had an effect on the improvement of the oxidative status of mice with a decrease of MDA level of obesity mice blood ($p \leq 0.05$) compared to KP control. An elevated endogenous antioxidant status of obesity mice was seen from elevated GSH levels, MnSOD specific activity and blood catalase of obesity mice ($p \leq 0.05$) compared with KP controls. Treatment of 1 x 10-minute treadmill per day decreased blood MDA level, increased GSH enzyme and increased specific activity of MnSOD enzyme and blood catalase of obese mice.

CONCLUSIONS: The 2 x 10-minute daily treadmill did not differ significantly in improving the oxidative status and endogenous antioxidant status compared with the treadmill 1 x 10 minutes a day ($p \geq 0.05$).

Introduction

Obesity is a complex disorder of appetite regulation and energy metabolism that is controlled by some specific biological factors. Physiologically, obesity is defined as a state with excessive fat accumulation [1]. Obesity increases because of a high-fat diet, lack of fibre, and lack of physical activity [2]. The prevalence of obesity around the world is always increasing year by year. The World Health Organization (WHO) in 2013 noted that around one billion people worldwide are overweight and at least 300 million are clinically obese. Obesity and overweight in Indonesia itself are also still high [2] [3].

The state of obesity can trigger the occurrence of oxidative stress conditions due to prooxidant and antioxidant imbalance in the body so that it will form Reactive Oxygen Species (ROS). Obesity occurs excessive lipogenesis and inhibition of lipolysis. Lipogenesis is stimulated by a diet high in carbohydrates. Obesity is closely related to oxidative stress, due to the role of the cyclic AMP (cAMP) in the balance of energy settings in obesity. Markers of oxidative stress include malondialdehyde (MDA) [4] [5].

MDA is a compound resulting from lipid peroxidation. Lipid peroxides are formed as a result of the reaction between free radicals with unsaturated fatty acids or polyunsaturated fatty acids (PUFAs) which are the major elements in cell membranes. Lipid peroxide levels in tissues and blood can be used as an indicator of oxidative stress. Current MDA measurements are often used to determine the extent of damage caused by peroxidation of lipid membranes or lipoproteins [4] [5].

The major antioxidant enzymes that neutralise Reactive Oxygen Species (ROS) are superoxide dismutase (SOD), catalase (CAT) and glutathione

peroxidase (GPx). SOD catalyses superoxide dismutase into H_2O_2 and oxygen, whereas CAT and GPx neutralize H_2O_2. The two major SODs in eukaryotes are MnSOD and Cu/ZnSOD found in the cytoplasm. After the increase of adipose tissue, antioxidant enzyme activity such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), were found to be significantly reduced. Finally, high ROS production and decreased antioxidant capacity lead to a variety of disorders, including endothelial dysfunction, characterised by decreased endothelial vasodilation and systemic RBC bioavailability. In the state of obesity can occur chronic inflammatory conditions of low levels with progressive infiltration of immune cells in obese adipose tissue. Cytokines secreted by immune cells and adipokines of adipose tissue promote tissue inflammation [6] [7].

The treadmill has been used as a model for studying aspects of behaviour, physiology, biochemistry and molecular response for acute or chronic physical exercise. The treadmill is one of the tools that can be used to see the physical activity of animal test. The treadmill apparatus in animal testing stamina test has the same working principle as the treadmill used by humans. The test animals ran against the direction of the treadmill at a standardised speed of about 10-18 m/min [8] [9].

Moderate physical exercise in animals with a high-fat diet can provide a protective effect in the development of obesity. Physical exercise triggers adaptation through the defence system against oxidative stress. Obesity has become a global health problem that can cause other health problems. The treadmill is used as a therapy to reduce obesity levels [9].

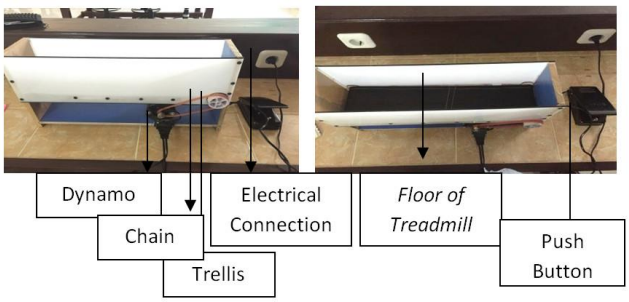

Figure 1: Mice treadmill tool

This study aimed to see the effect of treadmill treatment on the markers of oxidative stress in obese mice, by looking at cell damage markers such as MDA, as well as looking at endogenous antioxidant status by looking at MnSOD, and GSH, MnSOD and specific activity of blood catalase enzymes in obese mice. Expected by treatment of treadmill in obesity mice will be seen changes in oxidative status and endogenous antioxidants with improved oxidation markers and increased antioxidant activity, which has been damaged by the state of obesity.

Material and Methods

This research is experimental research. It was a type of experimental laboratory study using post-test control design group only. In this study, there were 4 study groups, i.e. normal control group (K), obesity control group (KP), treatment group (P1) obesity mice + treadmill treatment 1 time daily for 10 minutes, and treatment group (P2) obesity + mice treadmill 2 times a day every 10 minutes.

The population of this study was male mice (*Mus musculus* l.) aged 6-8 weeks with an average body weight of normal mice was 20-30 grams, and the average weight of obesity mice was more than 30 grams. The sample used was male mice. The sample size is determined based on the Federer formula - the sample determination formula for the full randomised complete experimental test (RAL). Based on the calculation results obtained the number of samples to be used in each group is 5 male mice (rounding $n \geq 4.75$). The number of samples to be used in the study was 20 male mice.

This study has inclusion criteria for normal mice and obese mice. The inclusion criteria for normal mice consist of this study were male mice, 6-8 weeks old, average weight 20-30 grams, obtained from the same breeding place and maintained at the same place and time. The inclusion criteria for obesity mice were obese male mice, 6-8 weeks old; mean weight > 30 grams were obtained from the same breeding place at the same place and time. Exclusion criteria in this study were weight loss during maintenance process of more than 10%, looked pain during maintenance process (limited motion, dull looking fur, bite wound, liquid faeces) and a dead mouse.

This study used a special treadmill that is connected with electricity. After the treadmill is turned on, the mouse is placed on it and allowed to run for 10 minutes for one treatment in the P1 group and 10 minutes a day twice for the P2 group.

Independent variable (independent variable) in this research is giving a treatment of treadmill and high fat and protein diet to male mouse (*Mus musculus l.*). The dependent variable in this research is MDA, GSH, MnSOD and catalase levels.

Measurement of MDA content is done by Wills method. The principle is that MDA with thiobarbituric acid will form a pink TBA pigment compound and provide maximum absorption at a wavelength of 530 nm. The absorption of the test solution is compared with the absorption of a standard set of standard solutions that have been known to measure. Measurement of GSH level is done by the Ellman method. The principle is that if glutathione is

reacted with the reactant ditiobisnitro benzoate (DTNB), it will produce yellow trinitrobenzene compounds whose absorption can be read by spectrophotometer at 412 nm wavelength. The absorption of the test solution is compared with the uptake of a series of standard GSH solutions that have been identified.

MnSOD activity is determined biochemically using a RanSOD® kit. The reagents in this kit consist of mixed substrates containing xanthine, phosphate buffers to dilute (standard as well as a sample), xanthine oxidase and standard solution to create standard curves. The principle of MnSOD examination using this kit is the measurement of the magnitude of inhibition of superoxide radical formation by MnSOD. Catalase is an enzymatic antioxidant that catalyses the decomposition of H_2O_2 into H_2O and O_2 molecules. H_2O_2 decomposition was observed spectrophotometrically based on decreasing absorption at the maximum wavelength. Measurements of the specific activity of the catalase enzyme are carried out at pH 7 because too acidic or alkaline atmosphere can cause loss of activity of specific catalase enzymes.

Results

This study has received ethical approval from the Medical Research Ethics Commission of the Medical Faculty, University of Lampung No. 2771/UN26/8/DT/2015 dated December 22, 2015. This study is part of another major study on obesity and treadmill.

Before and after treatment, mice were weighed, and body weight data before and after treatment were recorded.

Table 1: Changes in body weight of mice during the study

	Group K (gr)	Group KP (gr)	Group P1 (gr)	Group P2 (gr)
Average body weight of mice before treatment (D0)	28.2	45.6	45.4	45.7
Average body weight of mice after treatment (D28)	32.1	50.9	39.3	38.9
% weight change	+3.9	+5.3	-6.1	-6.8

It can be seen that in the treatment of treadmill 1 time a day for 10 minutes can lose weight obesity mice of 6.1 grams and in the treadmill treatment group 2 times a day for 10 minutes can lose weight by 6.8 g (Table 1).

The untreated control group (K) showed a blood MDA level of 4.69 ± 0.19 nmol/mL. In the KP group, there was an elevated blood MDA level of obese mice compared with MDA level of control group mouse blood (K) which was 5.97 ± 1.35 nmol/mL (p ≤ 0,05). In the treatment group P1 and P2, there was a decrease in blood MDA level of obese mice in the KP group, which was 4.82 ± 1.21 nmol/mL and 4.91 ± 0.29 nmol/mL (p ≤ 0.05). More data can be seen in Figure 2.

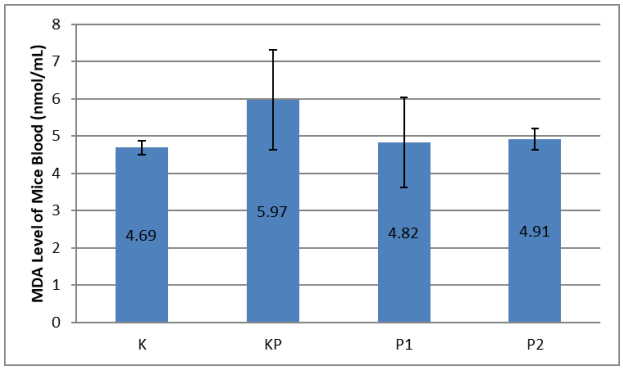

Figure 2: MDA blood levels of obese mice treated treadmill; K: normal control group; KP: obesity control group; P1: treatment group treadmill 1 x 10 minutes daily; P2: Treatment Group Treadmill 2 x 10 minutes daily

Of particular interest were the P1 and P2 groups treated with treadmill 1 x 10 minutes a day and 2 x 10 minutes a day, the examination data showed a significant reduction in mean MDA levels of 4.82 ± 1.21 nmol/mL compared with the control group K and KP p ≤ 0.05). The decrease in blood MDA levels in the treadmill treated group of 1x10 min was almost the same as the P2 group treated with treadmill 2x10 minutes a day. Statistically, they did not show any significant difference (p ≥ 0.05).

Examination of blood GSH levels showed the following results: The untreated control group (K) showed an average blood GSH level of 1.92 ± 0.08 µg/mL. In the positive control group KP, the blood GSH level of obesity mice decreased compared to the K group at 1.42 ± 0.39 µg/mL (p ≤ 0.05). In the P1 and P2 groups, the obese mice treated with treadmill 1 x 10 minutes a day and 2 x 10 minutes a day saw a rise in GSH levels of 1.72 ± 0.44 and 1.69 ± 0.25 µg/mL, which was significantly different (p ≤0.05) compared with the negative control group K and the KP group (p ≤ 0.05).

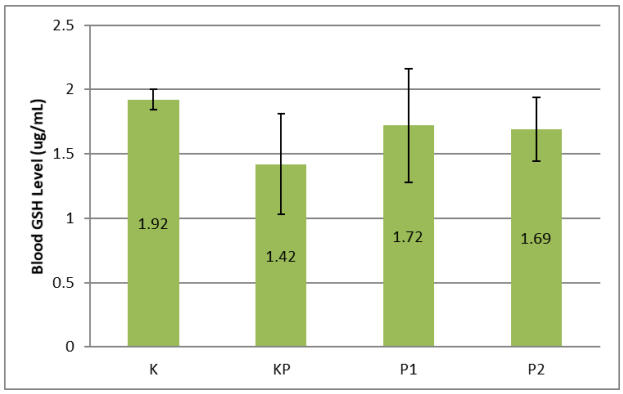

Figure 3: Blood GSH Levels of Obesity Mice; K: Normal Control Group; KP: Obesity Control Group; P1: Treatment Group of Treadmill 1 x 10 minute a day; P2: Treatment Group of Treadmill 2 x 10 minute a day

Interestingly, elevated blood levels of GSH obesity of mice in the P1 and P2 groups did not differ significantly from control group K. The increase in GSH levels in the P1 group was statistically, both of these values, showed significant differences ($p \leq 0.05$). Overall GSH levels of Obesity Mice group P1 and P2 were significantly different ($p \leq 0.05$) compared to the control group K and KP.

On the examination of specific activity of MnSOD plasma enzyme, it was found that plasma group mice control activity was 0.451 ± 0.321 U/ml, while in control group Obesity Control of MnSOD enzyme activity decreased, by 0.125 ± 0.126 U/ml, but in group P1 and P2 an increase in MnSOD enzyme activity is 0.391 ± 0.095 U/ml, and 0.316 ± 0.233 U/ml. The decrease of specific activity of MnSOD plasma enzyme in the KP group was statistically significant with p-value from 0.05 of 0.000. Increased activity of specific MnSOD enzyme in group P1 and P2 was also statistically significant, that is with p-value of 0.05.

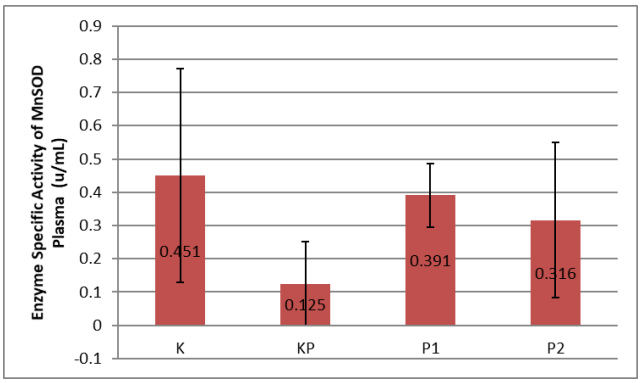

Figure 4: Enzyme Specific Activity of MnSOD Plasma of Obesity Mice who were treated Treadmill; K: Control Group; KP: Obesity Control Group; P1: Treatment Group of Treadmill 1 x 10 minute a day; P2: Treatment Group of Treadmill 2 x 10 minute a day

The specific activity of the MnSOD enzyme in the plasma of obese mice in the KP group decreased compared with control K. The decrease was statistically significant ($p \leq 0.05$). In the P1 and P2 groups, the specific activity of the MnSOD plasma enzyme increased compared with the KP group. This increase was also statistically significant compared with control K ($p \leq 0.05$) and against the KP group ($p \leq 0.05$).

Examination of the specific activity of the blood catalase enzyme of obese mice showed the following results: The untreated normal control group K showed the average activity of blood enzyme catalase 0.96 ± 0.22 U/mg. In the positive control group of KP, the specific activity of blood enzyme catalase enzyme obesity mice decreased compared to group K that is 0.54 ± 0.41 U/mg ($p \leq 0.05$). In the P1 and P2 groups, obesity mice treated with treadmill 1 x 10 minutes a day and 2 x 10 minutes a day saw an increase in the specific activity of catalase enzymes by 0.88 ± 0.29 and 0.75 ± 0.46 U/mg, which was significantly different ($p \leq 0.05$) compared with the negative control group K and the positive control group KP ($p \leq 0.05$).

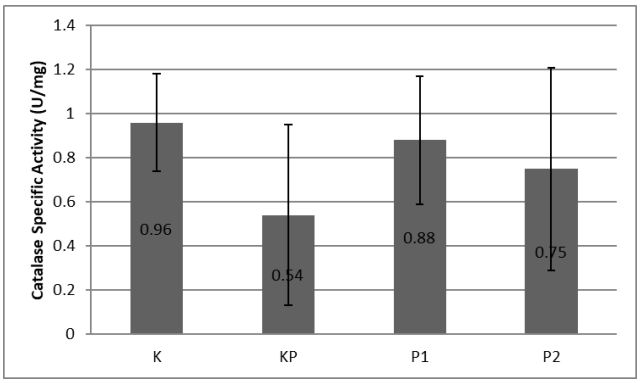

Figure 5: Activity of Catalase Enzyme Specific of Plasma Obesity Mice who were treated of Treadmill; K: Control Group; KP: Obesity Control Group; P1: Treatment Group of Treadmill 1 x 10 minute a day; P2: Treatment Group of Treadmill 2 x 10 minute a day

Discussion

Obesity is associated with a low-level chronic inflammatory condition with progressive infiltration of immune cells in adipose obesity tissue. Cytokines secreted by immune cells and adipokines of adipose tissue promote tissue inflammation. Obesity is a chronic disease with multifactorial causes, and this can be defined as an increase in body fat accumulation. Adipose tissue is not just a triglyceride storage organ, but studies have shown the role of white adipose tissue as a producer of certain bioactive substances called adipokines. These adipokines induce the production of reactive oxygen species (ROS), producing a process known as oxidative stress (OS). One marker of cellular damage due to oxidative stress is malondialdehyde (MDA) [6] [9].

When obesity persists for a long time, the source of endogenous antioxidants may decline, such as enzyme activity such as Glutathione (GSH), superoxide dismutase (MnSOD) and catalase (CAT). SOD and glutathione peroxidase (GPx) activity in individuals with obesity was significantly lower than in healthy people [10] [11].

Inflammation as a manifestation of increased oxidative stress, which increases in a person with obesity. The inflammatory mechanisms of obesity are related to the presence of adipose tissue that produces adipokine and acute phase proteins induced by hypoxia. Hypoxia will be generated during the overgrowth of adipose tissue during obesity. Adipose tissue produces 25% systemic IL-6, so this adipose tissue can cause low-grade systemic inflammation in people with excess body fat [12] [13] [14].

The mechanism of free radical formation in obesity is the increase of proinflammatory cytokines produced by adipocytes and preadipocytes such as

TNF-α, IL-1, and IL-6. This cytokine is a potent stimulator for the production of reactive oxygen and nitrogen by macrophages and monocytes. TNF-α increases the interaction of electrons with oxygen to produce superoxide anions. Adipose tissue also has an angiotensin II secretion capacity, which stimulates the oxidation activity of Nicotinamide adenine dinucleotide phosphate (NADPH). NADPH oxidation plays a major role in the production of ROS in adipocytes. Also, through the oxidation of fatty acids and increased oxygen consumption in obesity triggers increased mitochondrial respiration resulting in superoxide, radical peroxide and hydroxyl hydrogen [13] [14].

In obesity, the larger adipose tissue can lead to hypoxia (lack of O_2) and chronic inflammation. This can improve the state of oxidative stress by producing excessive ROS as well as decreasing the activity of endogenous antioxidant enzymes. This may increase lipid oxidation markers, such as MDA and carbonyl and decrease endogenous antioxidant enzyme activity such as GSH, MnSOD and catalase. Obesity results in increased inflammatory cytokines in the hypothalamus that increase and activate IL-β, TNF-α, and IL-6 which may affect metabolic processes [15] [16].

Physical exercise such as treadmill is widely used as one of the treatments in losing weight. Based on the results of statistical analysis there was a significant effect of the treadmill on decreasing MDA levels as well as increased endogenous antioxidant activity such as GSH, MnSOD and catalase when compared with control group of obesity (KP). Physical exercise (endurance) such as treadmill can decrease oxidative stress state through the mechanism of increasing mobilisation and oxidation of free fatty acids, affecting leptin signal pathways, hypothalamic inflammatory protection and AMPK activation pathway (AMP-activated protein kinase).

In this study, it is clear that the increase in MDA levels coupled with decreased activity of endogenous antioxidant enzymes such as GSH, MnSOD and catalase enzymes. Lipid peroxidation is a marker of oxidative damage (oxidative injury) presented as malondialdehyde (MDA). MDA levels in plasma in obesity mice can increase, higher than negative control mice. Malondialdehyde is a toxic compound that can disrupt the integrity of the cell membrane, so if the levels are not immediately lowered, it will interfere with the functioning of cells.

The state of oxidative stress can also decrease the activity of endogenous antioxidant enzymes. Several studies have shown that systemic oxidative stress is associated with increased NADPH oxidase 4 (NOX_4) enzyme, a member of nitrogen oxides (NOX) that plays an important role in the formation of H_2O_2 (strong oxidising agents). Therefore, the higher the NOX_4 and the formation of H_2O_2, so that the catalase enzyme work to catalyse the more severe, which eventually suppresses its activity [12] [13].

GSH enzyme activity is much lower than that of the positive control group. GSH enzyme works to convert H_2O_2 to H_2O and O_2, but with hypoxia, the availability of O_2 is reduced. This is probably because the H_2O_2 stack cannot be converted to O_2 perfectly, due to the low activity of the enzyme. The condition of obesity is independently correlated with high oxidative stress and inflammatory markers. Increased oxidative stress and inflammation in obesity play an important role in the initiation and progression of vascular disease, or it may also lead to the initiation of carcinogenesis in obesity [15] [16]. The mechanisms responsible for the high state of oxidative stress in obesity are not yet known, but clearly, adipose tissue is one of the important oxidative and inflammatory stress mediators as it contributes to the production of free radicals and proinflammatory cytokines, including IL-6, and TNF alpha.

Mitochondria provide the energy needed for virtually all cellular processes that ultimately allow performing physiological functions, besides that mitochondria play a central role in the death of the cell by apoptotic mechanisms. Obesity affects mitochondrial metabolism, which supports the formation of ROS and the development of oxidative stress. On the other hand, other mechanisms have been proposed involving the effects of high triglycerides (TG) on mitochondrial respiratory chain function, where intracellular TG, which is also high, inhibits the translocation of adenine nucleotides and results in superoxide formation. In the P1 and P2 groups, there was an increase in the specific activity of the MnSOD enzyme, presumably because the treadmill treatment could improve exogenous antioxidants. The low activity of MnSOD proves high oxidative stress in the body, so it is not able to eliminate the number of oxidants (free radicals). High oxidative stress is also associated with the condition of patients who are obese [17] [18].

The activity of lipolysis in obesity is impaired because of decreased mRNA expression that regulates lipoprotein lipase activity in adipose tissue and skeletal muscle. Aerobic physical exercise such as treadmill can lower cholesterol and triglyceride levels through the mechanism of improving the cholesterol-back transport process on the transfer of ester cholesterol. Physical exercise such as treadmill is one example of endurance exercise that induces increased AMPK. Improved AMPK stimulates the process of cellular glucose uptake, increased fatty acid oxidation, and decreases fat synthesis [18].

Physical exercise has been shown to increase the phosphorylation of JAK (Janus Kinase 2), activation of STAT3 signal Transducer and Activatortranscription 3 and SOCS3 which can affect appetite and inhibit more food intake. From the results of this study, it can be seen that in the treatment

group P1 and P2 there was no significant difference in treadmill 1 x 10 minutes a day and 2 x 10 minutes a day (p ≥ 0,05). Treatment of physical exercise treadmill 2 x 10 minutes a day did not show statistically significant differences in MDA, GSH, MnSOD and catalase levels [18].

Aerobic exercise 30-150 minutes per week is included in the moderate-intensity exercise category. The duration of the treadmill in the treadmill treatment group was both included in moderate intensity exercise and had similar effects at the interval. The intensity of extended physical exercise (150 minutes per week) is more potent than the intensity of 40 minutes of physical exercise performed over 3 times per week. In an extended physical exercise (exercise volume) it is more effective to decrease fatty tissue mass and lipid profile. A short period of physical exercise can be effective if exercise volume is also increased [14] [18] [19] [20]. The reduction in body fat will improve the oxidation marker and increase the antioxidant activity, which has been damaged by obesity. Therefore, weight loss through nutritional and pharmacological therapies, as well as supplementation with antioxidant nutrients such as vitamins E, A, and C, flavonoids, may be key to reducing the risk of developing pathologic conditions associated with OS and obesity such as high blood pressure and metabolic syndrome.

Lipid oxidation is one form of adaptation of hypercholesterolemic conditions to the frequency of physical exercise. This adaptation can occur in moderate physical exercise. This adaptation occurs because energy needs increase in the duration of the treadmill expansion resulting in the release of useful catabolic hormones in lipid degradation to be used as an energy source during physical exercise. Treadmills have protective effects on obesity through increased lipolysis activity, decreased free fatty acids, decreased blood MDA levels, increased GSH levels as well as the specific activity of MnSOD enzymes and catalase compared with positive control groups.

In conclusion, treadmill treatment affects the improvement of oxidative status and endogenous antioxidant status of obese mice. Treatment of 1 x 10-minute treadmill per day decreased blood MDA level, increased GSH enzyme and increased specific activity of MnSOD enzyme and blood catalase of obese mice. The 2 x 10-minute daily treadmill did not differ significantly in improving the oxidative status and endogenous antioxidant status compared with the treadmill 1 x 10 minutes a day (p ≥ 0.05).

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Basic Science

Assessment of Expression of Ki-67 in Benign and Malignant Prostatic Lesions among Sudanese Patients

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Keywords: Prostatic cancer; Ki-67; Sudanese patients; Prostatic lesions

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Abstract

BACKGROUND: Prostatic cancer is one of the most common cancers of males in a Sudanese population. The early detection is very important, as it is only curable at an early stage.

AIM: The objective of this study was to investigate the expression pattern of Ki-67 in benign and malignant prostatic lesions to improve the diagnosis that may help in better management and prevention of disease.

MATERIAL AND METHODS: Fifty-eight formalin fixed paraffin blocks from diagnosed cases of prostatic tumours with different grade, and stages were included in this study. Ki-67 expression was examined immunohistochemically by using monoclonal mouse anti-human Ki67 IS626. The results were correlated with Gleason score and tumour differentiation and stage.

RESULTS: The frequency of histological types was as follow: 11 cases of benign prostate,atic hyperplasia (19%) and 47 cases of prostatic cancer (81%). Our results stated that prostatic adenocarcinoma among Sudanese patients was of low grade which means tumours are less aggressive. Furthermore, the findings demonstrate that Ki-67 expression in prostatic carcinoma smears was correlated significantly with the degree of Gleason score ($P < 0.05$).

CONCLUSIONS: We found that the prostatic adenocarcinoma among Sudanese patients was less aggressive. Furthermore, Ki-67 expression was proportional to the grade of a tumour and it was a useful prognostic and diagnostic biomarker.

Introduction

Prostatic cancer is the second most common cancer of males in Western societies and those emulating Western lifestyles and diets. It was the fourth of the top 10 cancers found in Khartoum state – Sudan between 2009 -2010 (rate = 7.3 per 100,000) [1]. In the United States, the new cases were estimated to be 11,500; approximately one in seven American men diagnosed with prostate cancer during their lifetime [2]. Detection of this disease earlier, as a consequence of the introduction of the prostate-specific antigen (PSA) blood test, has been acknowledged by the National Cancer Institute (NCI) as one factor contributing to lowering the mortality rate over the past few years [3]. The factors that determine the risk of developing clinical prostate Cancer are not well known; however, a few have been identified; an important risk factor seems to be heredity (4). The most important risk factor such as food consumption, the pattern of sexual behaviour, alcohol consumption, exposure to ultraviolet radiation and occupational exposure have all been discussed as being of etiological importance [5]. Other factors increasing risk include low intakes of vitamin E, selenium, lignans and isoflavonoids [6]. The reviewed international trends in prostate cancer mortality and reported significant reductions in prostate-cancer mortality in the UK, USA, Austria, Canada, Italy, France, Germany, Australia and Spain with downward trends in the Netherlands, Ireland and Sweden [7]. Detection of this disease earlier, as a consequence of the introduction of the prostate-specific antigen (PSA) blood test, has been acknowledged by the National Cancer Institute (NCI) as one factor contributing to lowering the mortality rate over the past few years.

The use of PSA testing has been estimated to provide a diagnostic lead-time of up to 10 years [3] [8]. Ki-67 is a novel proliferative marker that can be readily detected by immunohistochemistry. The expression of Ki-67 is shown in all stages of the cell cycle, except G0, whereas resting cells entering from G0 lack Ki-67 in the early part of G1 [9]. The fact makes it an excellent marker for determining the so-called growth fraction of a given cell population. The usefulness of the Ki-67 labelling index has been well established for various types of malignant neoplasms [10]. Ki scoring is essential for diagnosis for tumour grade, based on proportional of tumour-positive cells has usually used as in indication for evaluation, and many reports have shown clinical significance in a variety of cancers regardless of whether the origins epithelial or non-epithelial [11]. The concordance rate of the Ki was determined by classifying the Ki 67 into less than 5% (< 5%) per 100/high power field (HPF), scored as zero (low), 5%-10% per 100/HPF, scored as 1 (intermediate), and higher than 10% (10% <) per 100/HPF, scored as 2 and 3(higher) [12]. Tissue microarrays (TMAs) are now widely accepted as a fast and cost-effective tool for use in almost any application requiring in situ tissue analysis [13]. According to its applications, TMAs was classified into predictive TMAs used to establish markers that predict response to therapy [14] and TMAs for validation of markers discovered by extracted protein, DNA- or RNA-based studies [15]. TMAs used to correlate staining results with clinical endpoints [16].

To the best of our knowledge, this is the first study to score ki-67 expression in prostatic cancer among Sudanese patients. The overall objective of this study was to improve the diagnostic and prognostic by investigating the expression of ki-67 this will provide data which may help in better management and control of this disease.

Material and Methods

This is a descriptive retrospective study, was conducted at the University of Science and Technology to Laboratories Administration and different laboratories in Khartoum State, Sudan.

A total of 58 patient, 11 cases of benign prostatic hyperplasia and 47 cases of prostate cancers were collected, and immunohistochemical staining was performed to evaluate the expression of Ki-67. The study was approved by the local Ethics Committee of the University of Science and Technology. Poorly fixed, overheated and too tiny specimen where excluded.

Formalin-fixed paraffin-embedded tissue blocks were sectioned by using Rotary microtome and low profile disposable knives by using 4 microns as the thickness of choice. Sections were then floated on a floating water bath adjusted to 45°C. Finally, clear-coated glass slides were used to pick up the floated sections and slides were left in a 60°C overnight in a hot air oven. Next day after oven drying sections were subjected to xylene and then to decrease graded of alcohols (100%, 90%, 70%, and 50%) for dehydration. The section was boiled in preheated retrieval buffer, performed in citrate buffer with pH 6.0 for 40 min at 95°C in a water bath followed by cooling at a refrigerator for 10 min. After cooling the slides, Phosphate Buffer Saline (PBS) was added to the slides for 5 min. Endogenous peroxidase activity was blocked by incubation with 3% hydrogen peroxide (Envision FLEX Peroxidase-Blocking reagent K8000/K8010) for 7 min and then washed with PBS for 7 min. The slides were then incubated with primary antibody (monoclonal mouse anti-human Ki67 IS626) for 30 min followed by phosphate buffer saline for 7 min. Then the slides were incubated with secondary antibody (EnVision FLEX/HRP K8000/K8010) for 30 min, and then washed with PBS for 7min, and also sections were incubated with streptavidin peroxidase. Substrate-DAB Chromogen (K8000/K8010 Substrate Working Mix) was added for 10 min. The DAB Chromogen was prepared as described by the manufacturer (Dako) by adding one drop from the chromogen to 1ml of the substrate buffer. Slides were then washed in distilled water for 20 min and counterstained with Mayer's hematoxylin. The slides were evaluated individually in a standard light microscope for immunohistochemical staining. Positive controls were included from the beginning.

A hundred fields were examined for Ki-67 expression. Expression of Ki-67 less than 5 per cent per 100 high power fields (HPF) was scored as zero (low). Expression from 5 to 10 per cent of Ki-67 per 100/(HPF) was scored as 1 (intermediate) and expression more than 30 per cent of ki-67 per 100/(HPF) was scored as 2 and 3 (high) [10].

Data were analysed by using the Statistical Package for the Social Sciences 20.0, SPSS, Inc., Chicago, IL) (SPSS).

Results

Fifty-eight cases of a prostate tumour were included in this study, patients' age ranged between 50-82 years with (mean 66 years). The frequency of histological types was as follow; 11 cases of benign prostatic hyperplasia (19%) and 47 cases of prostatic cancer (81%). 27 cases of low grade (57.4%), 20 cases of high grade (42.6%). Table 1, shows there was a significant correlation between age and prostatic cancer ($P < 0.05$).

Table 1: Association between age and diagnosis cross tabulation

Cross-tabulation		Diagnosis		Total
		B.PH	CA	
Age group (Years)	(50-60)	11	1	12
	(61-70)	0	20	20
	(71-82)	0	26	26
Total		11	47	58
Chi square p value = 0.000**				

Chi-square p-value less than 0.05 that's considered as statistically significant. ** Significant.

According to the Ki-67 expression among different tumour grades in the current study, the Gleason scoring was: twenty-seven cases of low-grade prostatic cancer were included, 74% cases were scored as score zero, while the remaining cases (16%) were scored as score 1 shown in Figure 1. On the other hand, Twenty cases of high-grade prostatic cancer were involved in this study; fourteen cases were scored as score 2, only two cases were scored as score 2 whereas the remaining three cases scored as score 3 as shown in Table 2.

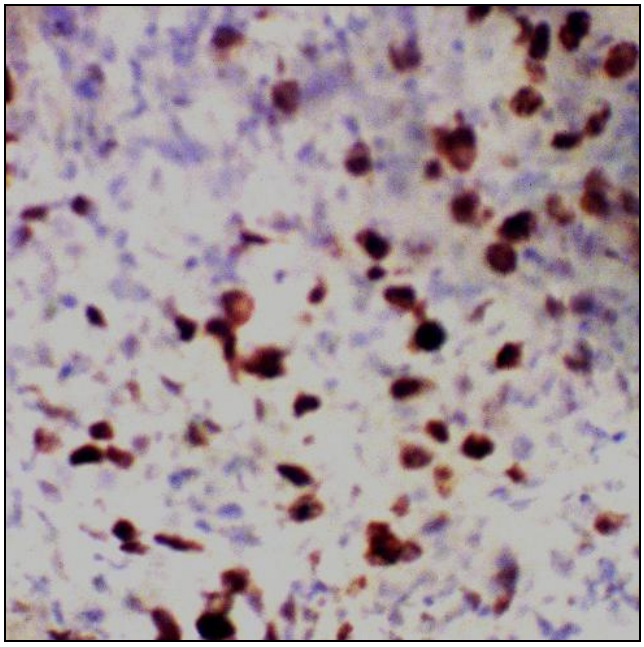

Figure 1: Expression of Ki-67 in malignant prostatic cancer

The result showed that there was a significant correlation between Ki-67 score and tumour grades ($P < 0.05$). This means that a high score is associated with increased Gleason grade as shown in Figure 2.

Table 2: Association between scoring (Gleason) Ki-67 and Grading cross tabulation

Cross-tabulation		Grading		Total	Percentage	Percentage
		Low grade	High grade		Of low grade	High grade
Scoring (Gleason) KI67	Low (<5% = Score 0)	20	1	21	95.5%	4.7%
	Intermediate (5-10% = Score1)	7	14	21	33.3%	66.6%
	High (score 2 =10-30% and score 3 = >30%)	0	5	5	00.0%	100%
Total		27	20	47		
Chi square p value = 0.000**						

Chi-square p-value less than 0.05 that's considered as statistically significant. ** Significant.

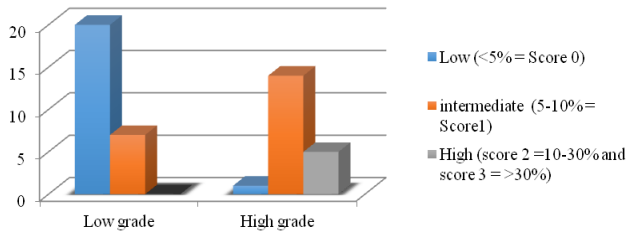

Figure 2: Distribution of scoring (Gleason) Ki-67 in low and high grade

Discussion

Prostatic cancer is one of the serious types of carcinomas that affect men all over the world. Although no strong data is available, a significant increase in the disease is noticed in Sudan. In this study, the expression of Ki-67 in 47 cases of prostatic adenocarcinoma was evaluated and scored according to KI Score system. In this study found that Ki-67 expression was significantly is low in benign prostatic hyperplasia (19%) as compared with prostatic carcinoma (81%), ($P < 0.05$). This finding is in agreement with Nornazirah et al., [17] and Renuka et al., [18] who also found that the ki67 marker is highly expressed in prostate carcinoma as compared with benign prostatic hyperplasia, ($P = 0.01$) and ($P = 0.05$) respectively. The results found in this study concerning the patient age is acceptable and in comparison to global age. Older ages who found the most vulnerable group in this study is agreement with Fantony *et al.,* [19] and Mohamed S A. Aziz *et al., [20]* who stated that with increasing age, men are significantly more likely to have high-risk prostate cancer. It is also in agreement with the American Cancer Society report (2016) that Prostate cancer is very rare in men younger than 40. About 60% of all prostate cancer cases are diagnosed in men 65 years of age and older, and 97% occur in men 50 and older [21].

In this study, there were significant differences between different prostatic Gleason scores. Since Ki-67 is a proliferative biomarker indicating proliferation of tumour cells expressing it is expected to be associated with the aggressiveness of the tumour proliferation index [22]. This explains the results that no Ki-67 expression was detected among benign prostatic tumours. In contrast, positive staining of Ki-67 was detected in all cases of prostatic malignancy regardless of tumour grade.

In the current studied group when the Gleason's score correlated with expression of Ki-67, there was an increased expression of Ki-67 with the increase in the grade of a tumour. This is strongly agreed with Sulik and Guzinska who there was a relationship between Gleason score and high

expression of Ki-67 in prostate cancers [23] it is also agreed with Bantis et al. their findings demonstrate that Ki-67 expression in prostatic carcinoma smears, correlated significantly with the degree of Gleason score [24]. Despite this agreement, Ojea *et al.* found that Ki-67 is less effective than classic factors such as PSA and Gleason score [25].

In conclusion, we found that the prostatic adenocarcinoma among Sudanese patients was of low grade (Gleason's score less than 4) which means tumours are less aggressive. Furthermore, Ki-67 expression was proportional to the grade of a tumour and it was a useful prognostic biomarker. We recommend to investigate for Ki-67 expression routinely for the diagnoses of prostatic cancer and to extend the investigation to involve more tumour biomarkers.

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Clinical Science

Impact of Maternal Obesity and Mobile Phone Use on Fetal Cardiotocography Pattern

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Abstract

BACKGROUND: The fetal heart rate (FHR) is a good marker of fetal well-being during labour. Cardiotocography is used to record the FHR and uterine contractions and can detect possible fetal hypoxia. Mobile phones use, and obesity is suggested to influence the FHR and cardiovascular development.

AIM: The present study aimed to study the differences in FHR pattern between fetuses of obese vs non-obese groups when using a mobile phone.

METHODS: We conducted a clinical trial to test the impact of mobile phone use on FHR using a single mobile phone with Specific Absorption Rate rating of 0.99 W/kg for 10 minutes. Data from this clinical trial were analysed to compare the FHR pattern between fetuses of obese women (exposed group) vs those of non-obese women (control group). The two study groups (obese vs non-obese) were compared regarding four FHR patterns: baseline FHR, variability, acceleration and deceleration scores. Data were analysed by SPSS software version 23.0 using the independent-samples t-tests.

RESULTS: Sixty-nine women were included in the final analysis (obese group: n = 22 and non-obese group: n = 47). Fetuses of the obese women had significantly higher baseline FHR and less FHR variability scores when compared with fetuses of the non-obese women (mean difference 2.9 and 3.18, respectively).

CONCLUSION: Fetuses of obese women had abnormal FHR pattern compared with fetuses of non-obese women. The use of mobile phone slightly influenced the FHR variability score. These results highlight the importance of proper management of obesity in women within the childbearing period.

Introduction

The prevalence of obesity is increasing worldwide. About one in two US women within the childbearing age are either overweight or obese [1]. Obesity of pregnant women has been linked to increased mortality, morbidity, and neonatal complications as prematurity, stillbirth, macrosomia, and large for gestational age infant. The literature suggests that maternal obesity might influence fetal development and well-being. Another risk factor that might influence fetal development is the mobile phone use.

Mobile phone use has become popular worldwide. Researchers found that the electromagnetic rays of mobile phones might interfere with the signalling process in the brain [2]. There have been concerns about the consequences of exposure to radiofrequency waves including infertility, stillbirths, congenital disabilities, and miscarriages [3] [4]. These poor reproductive outcomes can be explained by calcium efflux from the cell membranes under the effect of reactive oxygen species production causing DNA damage [2]. Elevated body temperature leads to cellular damage especially in organs like the brain, the testis, and the eye lenses which are more susceptible to heat-induced cellular damage [5]. In 2003, Goldstein *et al.,* [6] observed that biological damage to tissue occurs if the temperature rises above 10 °C above the baseline temperature for that tissue. In additions, the thermoregulatory mechanisms play an important role in disseminating the elevated temperature to minimise the damaging effect.

The fetal heart rate (FHR) is a prominent marker of fetal well-being in utero and during labour. FHR can be measured by cardiotocography (CTG) which records FHR and uterine contractions and therefore, helps obstetricians to detect the possible fetal hypoxia.

The impact of maternal obesity and mobile phone use on FHR pattern has not been established, yet. Therefore, we conducted this study to investigate the impact of maternal obesity and mobile phone exposure on FHR, an indicator for the general well-being of the fetus.

Methods

This study was approved by the ethics committee of the Sir Ganga Ram Hospital, Lahore Pakistan.

We conducted a clinical trial to assess the impact of mobile phone use of fetal heart rate. This study was conducted at the Department of Obstetrics and Gynaecology of Sir Ganga Ram Hospital, Lahore, Pakistan within the period one month May 2018.

Patients meeting the following inclusion criteria were included in the study:

(1) Pregnant women between 27-38 weeks of gestation; and

(2) Pregnant women are carrying a singleton pregnancy.

We excluded women with high-risk pregnancies and those with any accompanying disorders. Participants were classified into two groups: (1) *obese women group* defined as those with BMI > 30 Kg/m^2 and (2) *non-obese women group* defined as those with BMI < 30 Kg/m^2.

All patients gave a written informed consent before participation in the study. All participants were instructed not to use mobile phones one day before the start of the test. We used a single mobile phone with Specific Absorption Rate of 0.99 W/kg for 10 minutes in a room where no other mobile phone was placed. All participants had a CTG (BISTOS BT-300 Korea) for 20 minutes. CTG data were collected on a self-designed proforma, and they were blindly analysed. The variables measured were all the four types of FHR Pattern (baseline FHR, accelerations, decelerations, and beat to beat variability).

Cardiotocography, also known as electronic fetal monitoring, is used to record the changes in the FHR and their temporal relationship to uterine contractions. It aims to identify babies who may suffer from hypoxia and therefore, (1) subsequent well-being assessments can be done before delivery and (2) the baby is delivered by instrumental vaginal birth or cesarean section [7].

A baseline FHR of 110-160 beats/minute was considered normal. Acceleration is defined as a transient rise in FHR above the baseline more than 15 beats/minute and lasting at least 15 seconds. Decelerations are a transient slowing of FHR below the baseline, more than 15 beats/minute lasting more than 15 seconds. The baseline variability was defined as transient oscillations of FHR between 5-15 beats/minute [8].

The sample size was calculated to detect a difference in FHR between the fetuses of obese and to detect this difference with 90% statistical power and 5% margin of error, a minimum sample size of 69 was required for this study.

Categorical data were summarised as frequencies and percentages. Normality of continuous variables was tested by the Kolmogorov-Smirnov test. Continuous variables were summarised as means and standard deviations. Response parameters for participants' baseline FHR scores were measured on a 6-point interval scale (where 1 = 110-120, 2 = 121-130, 3 = 131-140, 4 = 141-150, 5 = 151-160, and 6 = 161 and above). Response parameters for participants' acceleration and deceleration scores were measured on a 3-point interval scale (where 1= absent, 2 = 1-3, and 3 = more than 3). However, all participants scored a value of 1 on deceleration, and the variable was removed from all analyses. Lastly, response parameters for participants' variability scores were measured on a 3-point interval scale where 1 = good (10 to 15), 2 = reduced (5 to 9), and 3 = absent. The comparisons between obese vs non-obese groups and with mobile phone use vs without mobile phone use were done using the independent-samples *t*-test. All analyses were conducted using the Statistical Package for the Social Sciences (SPSS) version 23. An alpha level below 0.05 was considered for statistical significance.

Results

Sixty-nine women were enrolled in the study. Of them, 22 pregnant women were classified in the obese women group (BMI > 30 kg/m^2) and 47 pregnant women were described as the non-obese group (BMI < 30 kg/m^2). Most of the participating women (62.3%) were younger than 30 years. The dominant parity was G2 and above (63.77%) and a minority of primigravida (36.23%). The majority of the patients were housewives (78.8%). A summary of the demographic characteristics of the study population is shown in Table 1.

Table 1: Demographic characteristics of the study population

		Obese Women N = 22		Non-Obese Women N = 47	
Variable	Level	n	%	n	%
Age	Below 25	10	45.40	7	14.89
	25-30	9	40.90	17	36.17
	Above 30	3	13.6	23	48.93
Education	Primary or below	5	22.72	16	34.04
	Secondary	10	45.40	27	57.44
	College and above	7	31.81	04	8.51
Parity	Primigravida	7	31.81	18	38.29
	G2 and above	15	68.18	29	61.70
Occupation	Housewife	18	81.81	35	74.46
	Professional	4	18.18	12	25.53
Gestational age (Weeks)	27-34	5	22.72	7	14.89
	35-38	17	77.27	40	85.10

BMI = Body mass index; Categorical variables are summarized as frequencies and percentages (n, %)

When comparing the FHR scores between the two conditions (with mobile phone use vs without mobile phone use), the differences were not statistically significant, except for the variability score in the subgroup of non-obese women (Figure 1 and Figure 2).

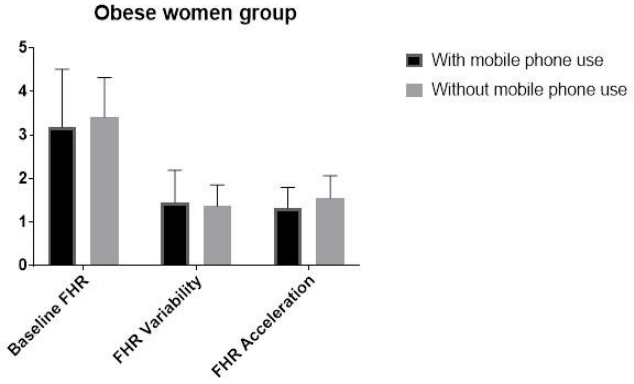

Figure 1: Bar chart of the FHR scores in the obese women group

In the non-obese women group, a significant increase in the variability score from 1.28 to 1.53 was observed (mean difference = 0.25, 95% CI from 0.04 to 0.46, P = 0.017, Figure 1). The comparison of FHR scores in the two conditions (with vs without mobile phone use) stratified by the BMI (obese women vs non-obese women) is shown in Table 2.

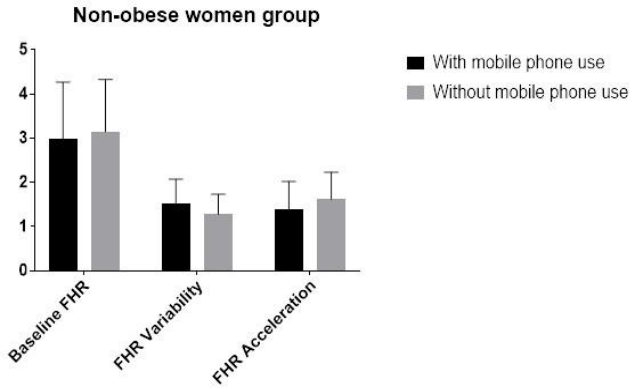

Figure 2: Bar chart of the FHR scores in the non-obese women group

Table 2: Comparison of the FHR scores between the two conditions (with vs without mobile phone use) stratified by BMI into obese and non-obese women

Variable		Without a mobile phone	With mobile phone	Mean Difference	95% CI	P value
Baseline FHR	Non-obese	3.15 (1.18)	2.98 (1.29)	-0.17	-0.68 to 0.34	0.507
	Obese	3.41 (0.91)	3.18 (1.33)	-0.27	-0.96 to 0.42	0.436
Variability FHR	Non-obese	1.28 (0.45)	1.53 (0.55)	0.25	0.04 to 0.46	0.017*
	Obese	1.36 (0.49)	1.45 (0.73)	0.09	-0.29 to 0.47	0.633
Acceleration FHR	Non-obese	1.62 (0.61)	1.38 (0.64)	-0.24	-0.50 to 0.02	0.065
	Obese	1.55 (0.51)	1.32 (0.48)	-0.23	-0.53 to 0.07	0.131

**Statistically significant; BMI = Body Mass Index; CI=Confidence Interval; FHR = Fatal Heart Rate.*

There were statistically significant differences between the obese and non-obese groups in dependent FHR variables: (1) the baseline FHR score with a mobile phone and (2) the FHR variability score with a mobile phone. Lower baseline FHR scores were found in the fetuses of non-obese women compared to the fetuses of obese women (MD -0.690, 95% CI -1.353 to -0.028, P = 0.041, Figure 3).

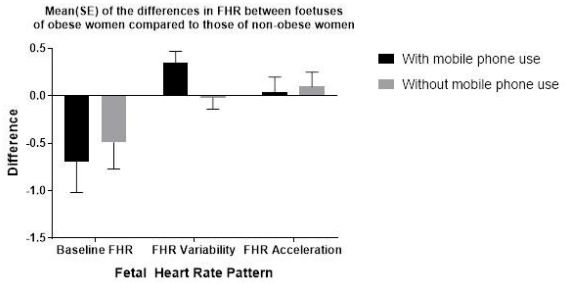

Figure 3: Bar chart of the mean (SE) of differences in the FHR between fetuses of obese women compared to those of non-obese women

Higher FHR variability score was found in the fetuses of non-obese women compared to the fetuses of obese women (MD 0.345, 95% CI 0.105 to 0.586, P = 0.006, Figure 3). The results of the independent-sample t-test are shown in Table 3.

Table 3: Mean difference between obese vs non-obese women groups regarding FHR scores

Variable		Mean Difference	SE	95% C.I.		P value
				Lower	Upper	
Baseline FHR	With mobile phone use	-0.69	0.33	-1.35	-0.03	0.041*
	Without mobile phone use	-0.49	0.28	-1.05	0.08	0.090
FHR Variability	With mobile phone use	0.35	0.12	0.11	0.59	0.006*
	Without mobile phone use	-0.02	0.12	-0.25	0.22	0.901
FHR Acceleration	With mobile phone use	0.04	0.16	-0.27	0.35	0.791
	Without mobile phone use	0.10	0.15	-0.20	0.40	0.507

**Statistically significant; N = 69; Independent variable = Body mass index; FHR = Fetal Heart Rate.*

Discussion

Our study showed that maternal obesity influences the FHR pattern. Fetuses of obese women

had significantly higher baseline FHR scores and lowered FHR variability scores when compared to fetuses of non-obese women. Additionally, mobile phone use significantly changed the FHR variability scores in the control group but not in the obese women group.

FHR pattern is controlled by the sympathetic activity of the fetus. It was found that maternal BMI positively correlates with fetal sympathetic activity and therefore, might explain the alteration in FHR pattern [9]. Also, it was found that the nutritional status of pregnant women and their pre-pregnancy BMI contributes to fetal blood pressure programming [10].

The literature suggests that high maternal BMI influences the fetal cardiac development. However, the impact of mobile phone use on fetal cardiac development has not been established yet. FHR has been used as an indicator of the fetal well-being in pregnancy and during labour. Therefore, for studies assessing the impact of mobile phone use on fetal well-being, FHR is considered a reliable outcome measure. Our study showed that mobile phone use significantly changes the FHR variability scores in the control group (BMI < 30). In 2004, Celik *et al.,* [11] investigated the effects of electromagnetic fields of cellular phones on baseline FHR, and they found that these electromagnetic fields do not influence the FHR scores highlighting that mobile phone use in pregnancy is safe. Another study was conducted by Rezk *et al.,* [12] who studied the FHR and cardiac output following acute maternal exposure to electromagnetic fields of mobile phones. The study group included 90 women with uncomplicated pregnancies enrolled from Benha University Hospital and El-Shorouq hospital in Egypt. In this study, the exposure to electromagnetic fields resulted in a significant increase in FHR and significant decreases in stroke volume and cardiac output [12].

Regarding maternal BMI, a longitudinal study of 610 pregnant women showed that maternal obesity affects FHR and alters the normal trajectory of cardiac and motor development [13]. Avci *et al.,* [14] studied a group of 931 pregnant females between March 2012 and March 2013. They found significantly more abnormal FHR pattern in the obese group than the control group. A Norwegian case-control study was conducted on 52 obese pregnant women and 25 normal weight pregnant women. Fetuses of obese women had more fetal myocardial dysfunctions with reduced left ventricular and right ventricular global strain rate compared with fetuses of women with normal weight [15]. The impact of maternal obesity on cardiac functions and development extends to the development of congenital heart defects [16]. It was found that infants with congenital heart disease are more likely to have obese mothers (OR 1.22, 95% CI 1.15-1.30) [16]. Also, the strength of association increased with increasing the maternal BMI [16]. The relationship between maternal BMI and fetal cardiac functions extends beyond the pregnancy period. An observational study of Gademan *et al.,* [17] showed that high maternal BMI was associated with high diastolic (β = 0.11 mm Hg; 95% confidence interval, 0.05-0.17) and systolic blood pressure (β = 0.14 mm Hg; 95% confidence interval, 0.07-0.21) in their children aged 4-5 years. These results highlight the importance of proper management of obesity in women within the childbearing period.

Our study has several strong points: (1) the exposure to mobile phone use was standardised in all participants, and (2) our study had a control group unlike some of the previous reports. However, our study is limited by the use of only one type of mobile phones with specific magnetic field properties.

Fetuses of obese women had abnormal FHR pattern compared to fetuses of non-obese women. The use of mobile phone slightly influenced the FHR variability score. These results highlight the importance of proper management of obesity in women within the childbearing period.

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Determinants of Exclusive Breastfeeding in a Sample of Egyptian Infants

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Abstract

BACKGROUND: Breastfeeding is an optimum, healthy, and economical mode of feeding an infant. However, many preventable obstacles hinder exclusive breastfeeding in the first six months of life.

AIM: We aimed to assess the social-, maternal- and infant-related factors disturbing exclusive breastfeeding in the first six months of life.

METHODS: It is a retrospective study included 827 dyads of mothers and infants older than 6 months (411 exclusively breastfed, 311 artificially-fed and 105 mixed feds). Mothers were interviewed to obtain sociodemographic information, maternal medical history and perinatal history and a detailed history of infant feeding.

RSULTS: Many factors were found to support the decision for artificial feeding rather than exclusive breastfeeding, including maternal age < 25 years (OR = 2.252), child birth order > 3rd (OR = 2.436), being a primi-para (OR = 1.878), single marital status (OR = 2.762), preterm infant (OR = 3.287) and complicated labor (OR = 1.841). Factors in favor of mixed feeding included cesarean section (OR = 2.004) and admission to the Neonatal Intensive Care Unit (OR = 1.925).

CONCLUSIONS: Although it isn't a community-based study and its results can't be generalised, plans to improve health and development of children are preferable to include the following: health education and awareness programs about the importance of exclusive breastfeeding should be directed for young and first-time mothers. Improved antenatal care to reduce perinatal and neonatal problems; and training, monitoring, and supervising community health care workers to recognise labour complications and provide support and knowledge to lactating mothers.

Introduction

Infant feeding practices directly affect the nutritional status of children under two years of age and ultimately influence child survival [1]. Breastfeeding is an unequalled way of providing ideal food for the healthy growth and development of infants; it also affects the reproductive process, with important implications for maternal health [2]. The global public health recommendation is that infants should be exclusively breastfed for the first six months of life, starting in the first half hour after delivery [3] [4]. Exclusive breastfeeding is defined as providing infants with only breast milk without the addition of water, herbal preparations or food in the first six months of life, except for vitamins, mineral supplements and medicine [5]. Non–exclusive breastfeeding can increase the risk of dying due to diarrhoea or pneumonia by more than two-fold among infants aged 0–5 months [6]. It is estimated that every day, as many as 4,000 infants and young children die

worldwide because they are not breastfed [7]. Moreover, recent studies indicated that increases the rate of non-communicable diseases such as diabetes, obesity, autoimmune disorders, and cardiovascular disease (CVD) is likely associated with a decrease in the practice of breastfeeding [8] [9].

There are various factors that affect the decision regarding the initiation and duration of exclusive breastfeeding, including sociodemographic factors (education level, monthly household income, and parity), residence and cultural beliefs, employment policies, health-related factors and biosocial factors (breastfeeding support) [10] [11]. Breastfeeding initiation and maintenance require the collaborative efforts of different medical and social subspecialties [12]. Unfortunately, health care providers may lack the skills and knowledge needed to help mothers improve their infant feeding practices [13].

In Egypt, there are gaps in the understanding why many mothers have difficulties in initiating and maintaining exclusive breastfeeding in the first six months of life and instead introducing artificial feeding. Therefore, exploring these difficulties is important for directing governmental intervention efforts to decrease infant morbidity and mortality.

The present study aimed to assess the social, maternal, and infant-related factors disturbing exclusive breastfeeding in the first six months of life.

Material and Methods

This study was a retrospective comparative study of exclusive breastfed, mixed fed and artificial-fed infants. It was a part of a project supported by National Research Centre of Egypt under the title of: “Infant Feeding Practices: Assessing Influence on general health, nutritional status, growth and development”. The project ethical approval Number is 11020.

Mothers and their infants were recruited from the pediatric out-patient clinics of Ain Shams University (ASU), different hospitals in the Cairo metropolitan area and the well-baby clinic at the Medical Research Centre of Excellence of the National Research Centre (NRC) in the period from September 2014 to February 2015.

Inclusion criteria: Only mothers who consented to participate in the study were enrolled if their infants were over 6 months and less than 24 months old.

Exclusion criteria: Infants with specific health problems who needed special feeding programs were excluded. Infants with any obvious congenital anomalies or features of genetic diseases or if they had a medical history of any metabolic or physical problems were also excluded.

In Egypt, the EDHS (Egypt Demographic and Health Survey) 2014 [14] shows that exclusive breastfeeding is common but not universal in very early infancy. Among infants under two months of age, 71% receiving only breast milk. However, the proportion exclusively breastfed drops off rapidly among older infants. By age 4-5 months, only 13% of children were exclusively breastfed.

By referring to tables for estimating population proportion with specified relative precision [15], it was found that: with a prevalence (P) of 13% and precision of 0.05, the table indicated that the sample size required is 196 and with a prevalence (P) of 71% and precision of 0.05, the sample size required is 316.

A total of 827 infants were recruited and classified according to the mode of feeding in the first six months into - 411 (49.7%) exclusively breastfed infants; - 311 (37.6%) artificially-fed infants; - 105 (12.7%) mixed fed infants (artificial milk and breastmilk).

A special questionnaire was designed for this study to obtain information on maternal age, maternal and paternal education and occupation, marital status, family income and childbirth order. According to the Egyptian economic scale of the family [16], the socioeconomic standard was determined.

The information obtained included parity; history of chronic diseases such as hypertension, diabetes and hypothyroidism; gestational age of the infant; mode of delivery; complications during delivery such as premature rupture of membranes, fetal distress and umbilical cord prolapse; postnatal problems such as cyanosis, jaundice and convulsions; and whether the infant was admitted to the Neonatal Intensive Care Unit (NICU).

Infant feeding practices in the first six months of life

This was the fundamental question addressed in this study; infants were classified as exclusively breastfed, artificial-fed and mixed fed.

Ethical considerations

The study, which was approved by the Medical Research Ethical Committee of NRC, complies with the International Ethical Guidelines for Biomedical Research Involving Human Subjects [17]. Informed consent was obtained from the mothers enrolled in the study.

Data were analysed with Statistical Package for the Social Sciences (SPSS) version 18. Data were summarised using descriptive statistics such as the frequency and percentage. Data were analysed using

the χ2-test and odds ratio [OR = (*a*/*c*)/(*b*/*d*)] [18] Multivariate logistic regression analysis was performed to predict significant factors affecting the decisions and choices regarding infant feeding in the first six months of life. Differences were considered statistically significant at P < 0.05 and highly statistically significant at P < 0.001.

Results

Table 1 shows the background variables suspected to affect exclusive breastfeeding: maternal social variables (maternal age, parity, education and occupation), maternal medical variables, prenatal and natal variables and gender of the newborn. There are 2 or 3 categories for each variable according to the characteristics of the recruited sample. The number and percentage of exclusive breastfed, artificially fed and mixed-fed infants were statistically significantly different among these categories. The majority of exclusively breastfed were males (52.5%), full-term infants (96.1%) and those who were delivered vaginally (51.3%), while the majority of artificially fed were female infants (71.1%) and those who were delivered by cesarean section (63.3%).

Table 1: General characteristics of the studied groups

Variable	Exclusive Breastfed N % 411 49.7%	Artificially Fed N % 311 37.6%	Mixed Feeding N % 105 12.7%	P Value
Sex				
Male (N = 366)	216 52.5%	90 28.9%	60 57.1%	<0.001
Female (N = 461)	195 47.5%	221 71.1%	45 42.9%	
Gestational age				
Full term (N = 768)	395 96.1%	274 88.1%	99 94.3%	0.001
Premature (N = 59)	16 3.9%	37 11.9%	6 5.7%	
Type of delivery				
Vaginal (N = 358)	211 51.3%	114 36.7%	33 31.4%	0.003
CS (N = 469)	200 48.7%	197 63.3%	72 68.6%	
Child birth order among siblings				
>3 (N = 595)	270 65.7%	253 81.4%	72 68.6%	<0.001
≤3 (N = 232)	141 34.3%	58 18.6%	33 31.4%	
Maternal age when pregnant				
≤25 (N = 322)	134 32.6%	159 51.1%	29 27.6%	<0.001
>25 (N = 505)	277 67.4%	152 48.9%	76 72.4%	
Parity				
Primipara (N = 218)	96 23.4%	92 29.6%	30 28.6%	<0.001
Multipara (N = 609)	315 76.6%	219 70.4%	75 71.4%	
Maternal education				
Illiterate (N = 196)	100 24.3%	61 19.6%	35 33.3%	<0.001
Educated (N = 631)	311 75.7%	250 80.4%	70 66.7%	
Maternal occupation				
Housewife (N = 664)	335 81.5%	259 83.3%	70 66.7%	0.001
Working (N = 163)	76 18.5%	52 16.7%	35 33.3%	
Mothers with a chronic disease (N = 163)	35 8.5%	112 36%	16 15.2%	<0.001
Mothers without a chronic disease (N = 664)	376 91.5%	199 64%	89 84.8%	

Table 2 illustrates how each social variable affected exclusive breastfeeding decision. The likelihood of artificial or mixed feeding versus exclusive breastfeeding was examined. The data indicated that infants of young (25 years or younger), primipara and single mothers, whether divorced or widowed, were more likely to be artificially fed than exclusively breastfed (OR = 2.252, P < 0.001; OR = 1.878, P < 0.001; and OR = 2.762, P = 0.008, respectively). Also, infants of higher birth order (3rd or higher) were more likely to be artificially fed (OR = 2.436, P < 0.001). On the other hand, infants of educated and working mothers were more likely to be mixed fed than exclusively breastfed (OR = 0.435, P < 0.005; and OR = 0.453, P < 0.001). Family income had no significant effect on the probability of artificial or mixed feeding.

Table 2: Infant feeding practices about social variables

Variable	Exclusive Breastfeeding N % 411 49.7	Artificial Feeding N % 311 37.6%	Mixed Feeding N % 105 12.7
Maternal Age			
≤ 25 (N = 322)	132 32.1%	161 51.8%	29 27.6%
>25 (N = 505)	279 67.9%	150 48.2%	76 72.4%
OR (95% CI)		* 2.252 (1.662-3.053)	0.773 (0.478-1.25)
P		<0.001	0.293
Maternal Education			
Illiterate (N = 196)	120 29.2%	61 19.6%	15 14.3%
Educated (N = 631)	291 70.8%	250 80.4%	90 85.7%
OR (95% CI)		1.204 (0.779-1.86)	**0.435 (0.24-0.789)
P		0.403	0.005
Maternal Occupation			
Housewife (N = 664)	337 82%	256 82.3%	71 67.6%
Working (N = 163)	74 18%	55 17.7%	34 32.4%
OR (95% CI)		1.058 (0.711-1.576)	**0.453 (0.28-0.733)
P		0.87	0.001
Family Income			
Lower Middle (N = 377)	189 46%	145 46.6%	43 41%
Upper Middle (N = 450)	222 54%	166 53.4%	62 59%
OR (95% CI)		1.03 (0.763-1.39)	0.841 (0.545-1.297)
P		0.845	0.433
Child Birth Order			
> 3 (N = 595)	270 65.7%	251 80.7%	74 70.5%
≤ 3 (N = 232)	141 34.3%	60 19.3%	31 29.5%
OR (95% CI)		*2.436 (1.685-3.52)	1.189 (0.746-1.897)
P		<0.001	0.466
Parity			
Primipara (N = 218)	90 21.9%	105 33.8%	23 22%
Multipara (N = 609)	321 78.1%	206 66.2%	82 78%
OR (95% CI)		*1.878 (1.337-2.637)	1.197 (0.724-1.978)
P		<0.001	0.484
Marital Status			
Divorced or Widowed (N = 39)	13 3.4%	25 8%	1 1%
Married (N = 788)	398 96.8%	286 92%	104 99%
OR (95% CI)		*2.762 (1.273-5.991)	0.363 (0.046-2.864)
P		0.008	0.316

*Artificial feeding vs Exclusive Breastfeeding; ** Mixed feeding vs Exclusive Breastfeeding.

Table 3 shows that many perinatal factors affected exclusive breastfeeding decision. The likelihood of artificial or mixed feeding versus exclusive breastfeeding was examined based on these variables. Infants of mothers with a chronic disease (DM or hypertension), preterm infants and infants who experienced a complicated labor were more likely to be artificially fed than exclusively breastfed (OR = 1.721, P < 0.005; OR = 3.287, P < 0.001; and OR = 1.841, P < 0.013, respectively).

Infants born by cesarean section (CS) were 2.004-times more likely to be mixed fed (P = 0.002) and 1.429-times more likely to be artificially fed (P = 0.02) than to be exclusively breastfed. Infants admitted to the NICU for jaundice were more likely to be mixed fed than exclusively breastfed (OR = 1.925, P = 0.048), while infants admitted to the NICU for other causes were 4.073-times more likely to be artificially fed (P < 0.001) and 3.926-times more likely to be mixed fed (P < 0.001) than to be exclusively breastfed.

Table 3: Infant feeding practices about perinatal variables

Variable	Exclusive Breast Feeding N % 411 49.7	Artificial Feeding N % 311 37.6%	Mixed Feeding N % 105 12.7
Maternal chronic disease before pregnancy			
Yes (N = 163)	71 17.3%	71 22.8%	21 20%
No (N = 664)	340 82.7%	240 77.2%	84 80%
OR (95% CI)		* 1.721 (1.176-2.518)	1.345 (0.777-2.239)
P		0.005	0.288
Gestational age			
Preterm (N = 59)	19 4.6%	35 11.3%	5 4.8%
Full term (N = 768)	392 95.4%	276 88.7%	100 95.2%
OR (95% CI)		*3.287 (1.684-6.418)	1.747 (0.648-4.708)
P		<0.001	0.265
Type of delivery			
CS (N = 469)	211 51.3%	185 59.5%	73 69.5%
Vaginal (N = 358)	200 48.7%	126 40.5%	32 30.5%
OR (95% CI)		*1.429 (1.056-1.934)	**2.004 (1.28-3.137)
P		0.02	0.002
Complicated labor			
Yes (N = 104)	39 9.5%	50 16.1%	15 14.3%
No (N = 723)	372 90.5%	261 83.9%	90 85.7%
OR (95% CI)		*1.841 (1.129-3.001)	1.808 (0.942-3.47)
P		0.013	0.072
Admission to neonatal intensive care unit for jaundice			
Yes (N = 96)	41 10%	40 12.9%	15 14.3%
No (N = 731)	370 90%	271 87.1%	90 85.7%
OR (CI %)		1.411 (0.833-2.39)	**1.925 (0.997-3.715)
P		0.199	0.048
Admission to neonatal intensive care unit for other causes			
Yes (N = 93)	28 6.8%	50 16.1%	15 14.3%
No (N = 734)	383 93.2%	261 83.9%	90 85.7%
OR (CI %)		*4.073 (2.344-7.077)	**3.926 (1.98-7.785)
P		<0.001	<0.001

*Artificial feeding vs exclusive Breastfeeding; ** Mixed feeding vs exclusive Breastfeeding.

Logistic regression analysis identified the most significant factors affecting exclusive breastfeeding of mothers (Table 4).

Table 4: Logistic regression indicating factors that favour artificial feeding

	B	S.E.	Wald.	df	Sig.	Exp(B)
Maternal age	0.603	0.206	8.609	1	0.003	1.828
Maternal chronic disease	0.734	0.244	9.003	1	0.003	2.082
Gestational age	1.450	0.472	9.458	1	0.002	4.263
Type of labor	0.675	0.195	11.985	1	0.001	1.963
Marital status	1.459	0.444	10.796	1	0.001	4.301
NICU admission for causes other than jaundice	1.139	0.349	10.648	1	0.001	3.125
Constant	-6.602	1.563	17.833	1	< 0.001	0.001

Variables entered on step 1: Mother age, Mother education, child order, Maternal chronic disease, complicated labour, Gestational age, type of labour, parity, marital status, perinatal insult, NICU admission for causes other than jaundice.

Factors that influenced the preference of artificial feeding over exclusive breastfeeding included maternal age ≤ 25 years, chronic maternal disease before pregnancy, preterm delivery, CS delivery, single marital status and neonatal admission to the NICU for reasons other than jaundice.

Factors that led to the choice of mixed feeding instead of exclusive breastfeeding included maternal employment outside the home and neonatal admission to the NICU for jaundice. Non-working mothers were more likely to breastfeed their infants, as shown in Table 5.

Table 5: Logistic regression indicating factors that favour mixed feeding

	B	S.E.	Wald.	df	Sig.	Exp(B)
Complicated labour	0.623	0.349	3.176	1	0.075	1.864
Type of labour	0.507	0.240	4.459	1	0.035	1.659
Maternal occupation	-0.807	0.259	9.735	1	0.002	0.446
NICU admission for jaundice	1.391	0.376	13.718	1	<0,001	4.020
Constant	-2.242	1.036	0.031	1	0.031	0.106

Variables entered on step 1: mother education, complicated labour, delivery problems, type of labour, Maternal occupation, NICU admission for jaundice.

Discussion

Breast milk is the best gift a mother can give her baby. Currently, there is solid evidence that exclusive breastfeeding had short-term and long-term health benefits for infants and mothers [19]. Breastfeeding rates vary by region, country and culture. In this study, 52.5% of exclusively breastfed were male infants, but artificially fed were predominantly female infants (Table 1). This might be due to prevailing cultural beliefs and social standards in oriental communities, which have a bias toward males. This finding is in agreement with those of studies conducted in Ethiopia and India but not with those of studies performed in Singapore [20] [21] [22] [23]. Many factors affect exclusive breastfeeding in the first six months of life, including maternal sociodemographic traits and medical factors. The findings of this study indicate that maternal social characteristics have a significant influence on the decision of exclusive breastfeeding. **Young mothers** (25 years or younger) and first-time mothers had a higher tendency to choose artificial feeding rather than exclusive breastfeeding (OR = 2.252, P < 0.001; and OR = 1.878, P < 0.001) (Table 2). These mothers appeared to lack knowledge of the benefits of breastfeeding, or they may have misbeliefs about the effects of breastfeeding on body shape. Even after logistic regression, younger maternal age was an influential factor for artificial feeding. This finding is in agreement with the results of the study by Chudasama et al., [24] who found that young maternal age and primiparity were factors that favoured artificial feeding. The study by Girish and Gandhimathi [25] found that the exclusive breastfeeding rate among primiparous mothers was greatly affected by ignorance of the importance of breastfeeding.

In contrast to our results, Labib and El Shafei [26] reported a significantly higher percentage of exclusive breastfeeding among women in the ≤ 25 age group compared with those in an older group; they explained this finding by the fact that these younger women are eager to engage in all acts of motherhood. We found that childbirth order > 3rd influenced the mode of feeding toward artificial feeding rather than exclusive breastfeeding (OR = 2.436, P < 0.001) (table 2). We assume that mothers

preoccupied with caring for many children will find it is easier to bottle-feed their infant. Also, we found that single mothers tend to choose artificial feeding (OR = 2.762, P = 0.008) (Table 2), which strongly suggests that mothers are in need of continuous social support to be able to breastfeed. Illiterate mothers and homemakers preferred exclusive breastfeeding rather than mixed feeding. This finding is consistent with the results of Samayam and Krishna [27], who showed that lower socioeconomic status, which is determined by maternal education and occupation, favours exclusive breastfeeding; Maternal instinct will always push a mother toward breastfeeding.

Concerning medical factors that affect exclusive breastfeeding, we found that mothers with a preterm newborn had a higher tendency toward artificial feeding than exclusive breastfeeding (OR = 3.287, P < 0.001) (Table 3). In our community, there is a fixed belief that preterm babies must have the most valuable nutrition, which comes from an artificial fortified source; breastfeeding is not the ideal choice for such infants. Moreover, some preterm infants are not physically or developmentally able to suckle, swallow and breathe in a coordinated manner, and the duration of the mother's stay in the hospital with them varies from one hospital to another [28].

The most common misconception that undermines successful lactation for mothers of preterm infants is that the initiation of milk expression can be delayed until an infant is stable.

To overcome this problem, collaborative efforts are needed so that neonatal physicians and nurses provide additional counselling and support to mothers of preterm newborns to ensure the early establishment of frequent milk expression [29] [30]. Gianni et al., [31] found that the percentage of exclusive breastfeeding is higher among term infants than among preterm infants. This finding was explained by an increased risk of morbidity and a longer stay in the NICU among preterm infants.

What infants admitted to the NICU for jaundice were more likely to be mixed fed than exclusively breastfed (OR = 1.925, P = 0.048), while infants admitted to the NICU for other causes were more likely to be artificially fed (P < 0.001) than to be exclusively breastfed. These findings signify the ultimate importance of direct breastfeeding in NICU as Briere et al., [32] proved in their study how this process makes the continuation of breastfeeding at home after discharge is an easy one.

We recorded that Infants born by cesarean section (CS) were more likely to be mixed fed (P = 0.002) and artificially fed (P = 0.02) than to be exclusively breastfed those results goes with Hobbs et al., [33] study which found women who delivered by CS had no intention to breastfeed or did not initiate breastfeeding. We also found that women who had complicated labour preferred artificial feeding to exclusive breastfeeding (OR = 1.841, P = 0.013) (Table 3). A mother who suffers during labour may find it difficult to breastfeed her infant, and this provides an opportunity for the introduction of prelacteal to this infant. This finding was consistent with that of Onah et al., [34], who reported that mothers who experienced delivery complications were more likely to not exclusively breastfeed their infants. Yes, from our study we could clearly call for more and more Baby Friendly Initiative (BFI) accredited hospitals in our country to support breastfeeding and to give more support to those women who delivered via CS or even suffering from complicated labour.

Unfortunately, our study had the limitation of not being a community-based study and hence cannot be generalised for the entire population of Egypt. Further Research is needed on a larger scale to display the actual prevalence and duration of exclusive breastfeeding among Egyptian mothers, and to explore all possible reasons behind discontinuation of exclusive breastfeeding before the sixth month of age.

In conclusion, plans to improve the health and development of children are preferable to include the following: health education and awareness programs about the importance of exclusive breastfeeding should be directed for young and first-time mothers. Improved antenatal care to reduce perinatal and neonatal problems; and training, monitoring, and supervising community health care workers to recognise labour complications and provide support and knowledge to lactating mothers.

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Tertiary Lymphoid Structures in Colorectal Cancers and Their Prognostic Value

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Abstract

BACKGROUND: Tumor-infiltrating lymphocytes (TIL) in tumour stroma are considered to be involved in the elimination of malignant cells and prevention of metastasis formation. TIL consist of T lymphocytes including cytotoxic lymphocytes that are a constituent part of the effector mechanism of anti-tumour immunity and B lymphocytes that can form tertiary lymphoid structures (TLS). TLS has been described in several solid tumours and colorectal carcinoma (CRC), and the influence on the local and systemic anti-cancer response.

AIM: This study aimed to quantify the presence of TLS in CRC patients and to determine their role in tumour progression.

PATIENTS AND METHODS: The study included 103 patients with CRC who underwent surgery at the University Clinic of Digestive Surgery in Skopje, whose operative material was analysed at the Institute of Pathology, Medical Faculty in Skopje. The density of TLS was determined and correlated with the neoplasm status of local growth (T), positive lymph nodes, lymphatic invasion, and stage of the disease and tumour grade.

RESULTS: The density of TLS was significantly higher in patients with higher stage, lower T status, and negative lymph nodes, in patients with no lymphatic invasion and with better-differentiated tumours.

CONCLUSION: The density of TLS plays an important role in controlling the tumour growth, and it can be a parameter for neoplasm progression in CRC patients. The density of TLS influences the control of tumour progression.

Introduction

Tumour stroma (cancer-associated stroma), as opposed to normal-tissue stroma, is a suitable environment for spreading of cancer and plays a key role in the growth and development of malignant neoplasms [1] [2]. It is considered that cellular-stromal interactions in malignant tumours play a significant role in the process of their progression. During this process, tumour cells are influenced by signals coming from stromal, endothelial, inflammatory and immune cells [3]. Inflammatory cells in tumour stroma have a double role; they are involved in tumour progression, and tumour-infiltrating lymphocytes or so-called tumour-associated lymphocytes (TAL), which are also present in the stroma of solid malignant tumours, are involved in the elimination of malignant cells and prevention of metastasis [4] [5]. TALs consist of T lymphocytes including cytotoxic lymphocytes, which are a constituent part of the effector mechanism of anti-tumour- immunity, and B-

lymphocytes that can form tertiary lymphoid structures [5] [7].

Formation of tertiary lymphoid structures (follicles) (TLS) has been described in several solid tumours and colorectal carcinoma (CRC) [5]. They are transient accumulations of lymphoid cells that develop in non-lymphoid tissue in case of chronic inflammation of this tissue. They are built identical to lymph follicles of lymphoid organs, that is, lymph nodes. They contain B cell zone, which can form germinal centres, T cell zone, mature dendritic cells and high endothelial venules [7].

It is assumed that, in addition to other inflammatory cells, B lymphocytes play a significant role in the formation of inflammatory infiltrate during the onset of colorectal cancer (CRC) [5] [8], and TLS detected in tumours influence the local and systemic anti-cancer response [8].

This study aimed to quantify the presence of TLS in CRC patients and to determine their role in tumour progression.

Material and Methods

The analysed patients in this study have clearly defined a cohort of patients with CRC, whose a tumour infiltrating lymphocytes along with B CD20+ cells were previously analysed and those results have already been published [9].

A total of 103 patients with CRC were included in the study. Sixty-eight (66.2%) were men and 35 (33.98%) women, with a mean age of 64.57 ± 11.5 years; they all underwent surgery at the University Clinic of Digestive Surgery in the period from 2013 to 2017. The operative material was analysed at the Institute of Pathology in Skopje, where the diagnosis was confirmed, and the pTNM stage was determined.

In this study we analyzed the elements of the TNM classification: T status (local growth of the tumor), the presence of positive lymph nodes (LN), the disease stage (according to TNM classification 2010) [10] as well as the grade of tumor differentiation (G), and the density of TLS.

TLS was detected microscopically on routine prepared slides. Areas of an invasive tumour front with a high density of TLS were chosen, and additional sections for immunohistochemistry were made.

The TLS was defined by immunohistochemical staining with antibodies against CD4 (Dako Monoclonal Mouse Anti-Human CD4, Clone 4B12); CD8 (Dako Monoclonal Mouse Anti-Human CD8, Clone C8/144B); CD20 (Dako Monoclonal Mouse Anti-Human CD20, Clone L26); and CD21 (Dako Monoclonal Mouse Anti-Human CD21, Clone 1F8) with a standard procedure using Immunoperoxidase LSAB + system.

The number of TLS was counted in 10 consecutive low-power fields (10 h 4) in the invasive front of the neoplasm. They were quantified as: 0 – no TLS found in 10 low-power field; + - 1 to 5 TLS found; ++ - 6 to 10 found; +++ - > 10 TLS found in10 consecutive low-power fields.

To confirm the consistency of grading, the cases were scored independently by two investigators. Examples of TAL and immune cell staining are shown in Figure 1.

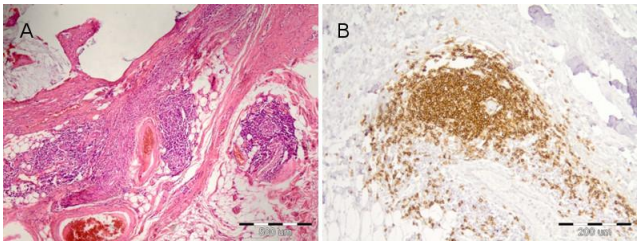

Figure 1: TLS in the invasive front of CRC; A) Well-formed TLS in the invasive front of CRC at the right side of the microphone (10 x 4); B) The same LS immunostained with an antibody against CD20. The cells around the venue are CD20+ (10 x 10)

Descriptive statistical methods were used for statistical analysis of the data. Categorical variables are presented with absolute and relative numbers (%).

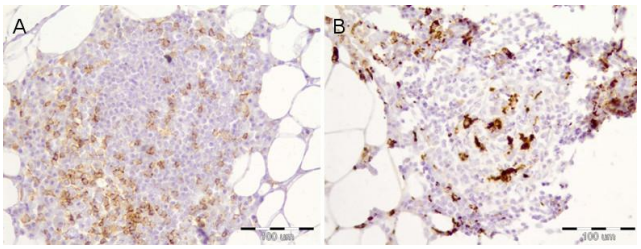

Figure 2: TLS in the invasive front of CRC; A) CD4+ T lymphocytes in the periphery of the follicle (10 x 20); B) CD21+ dendritic cells in the centre of B zone (10 x 20)

Fisher's exact test was used for comparison of categorical variables. Spearman's correlation coefficient was used to determine the degree of correlation between analysed parameters. The statistical program SPSS for Windows, version 19.0 was used.

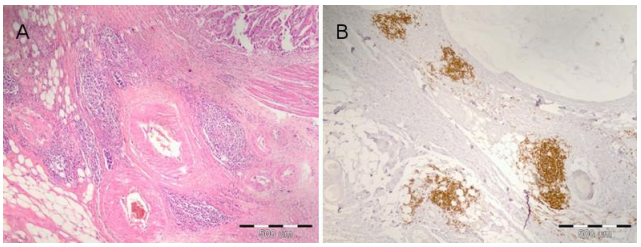

Figure 3: TLS in the invasive front of CRC; A) Five TLS in one low-power field (H.E. 10 x 40); b) CD20+ areas of TLS (CD20 10 x 4)

Results

The presence of TLS was found in 85 (82.52%) cases of the total of 103, whereas in 18 (17.47%) cases TLS was not found, that is the inflammatory infiltrate which contained a different number of CD20+ lymphocytes confirmed with immunostainings showed no TLS organisation.

Table 1 shows the distribution of TLS in the tumour of the examined patients.

Table 1: TLS distribution in invasive tumour front in analysed series of 103 patients

TLS	No of patients	Per cent (%)
0	18	17.47
+ (1-5)	43	41.74
++ (6-10)	27	26.21
+++ (> 10)	15	14.56

For the value of p<0.0001, the statistical analysis showed a significant influence of a tumour local growth on the quantity of TLS in the infiltrative front of the neoplasm. Their quantity was significantly larger in tumours of lower T status.

Table 2: TLS distribution about the examined parameters

Parameter		Tertiary Lymphoid Structures - TLS				
T status		0	+ (1-5)	++ (6-10)	+++ (>10)	P value
	N	N = 18	N = 43	N = 27	N = 15	
T1	5	0	0	0	5 (100%)	<0.0001
T2	7	0	1 (14.29%)	1 (14.29%)	5 (71.43%)	p (Kruskal-
T3	60	7 (11.67%)	25 (41.67%)	23 (38.33%)	5 (8.33%)	Wallis test)
T4	31	11 (35.48%)	17 (54.84%)	3 (9.68%)	0	
Lymph node involvement						
No	56	4 (7.14%)	17 (30.36%)	22 (39.29%)	13 (23.21%)	P =
Yes	47	14 (29.79%	26 (55.32%)	5 (10.64%)	2 (4.26%)	0.000011** p (Chi-square test)
Lymphatic invasion – L1						
No	54	5 (9.26%)	13 (24.07%)	22 (40.74%)	14 (25.93%)	P =
Yes	49	13 (26.53)	30 (61.22%)	5 (10.2%)	1 (2.04%)	0.000001** p (Chi-square test)
Stage						
I	11	0	0	1 (9.09%)	10 (90.91%)	< 0.0001
II	42	2 (4.765)	16 (38.1%)	21 (50%)	3 (7.14%)	p (Kruskal-
III	44	12 (27.27%)	26 (59.09%)	4 (9.09%)	2 (4.55%)	Wallis test)
IV	6	4 (66.67%)	1 (16.67%)	1 (16.67%)	0	
Grade of differentiation - G						
G1	5	0	1 (20%)	1 (20%)	3 (60%)	0.028*
G2	83	15 (18.07%)	33 (39.76%)	23 (27.71%)	12 (14.46%)	p (Kruskal-
G3	15	3 (20%)	9 (60%)	3 (20%)	0	Wallis test)

*p < 0.05; **p < 0.01.

TLS quantity was significantly different among patients with or without disease spread in the regional lymph nodes (p = 0.000011). In patients with involved regional lymph nodes more often than in patients without lymph node metastasis, no tertiary follicles were found (29.79% *vs* 7.14%). They were also found to have 1 to 5 tertiary follicles (55.32% *vs* 30.36%).

Patients without or with lymphatic invasion also showed a significant difference regarding the finding of tertiary lymphatic structures (p = 0 .000001).

Patients with lymphatic invasion more often than those without invasion had no tertiary follicles in the infiltrative front of the neoplasm (26.53% *vs* 9.26%). They were also more frequently found to have a small number of tertiary follicles, from 1 to 5 when compared to patients without lymphatic invasion (61.22% *vs* 24.07%).

The finding of TLS in the infiltrative front of the neoplasm was significantly dependent on the spread of the disease (p < 0.0001). A smaller quantity of TLS was found in patients with colorectal carcinoma in the more advanced stage.

Patients with good, moderate and poor tumour differentiation had a significantly different quantity of tertiary lymphoid structures (p = 0.028).

The largest quantity of tertiary follicles was found in the group of well-differentiated tumours, that is, in 3/5 (60%) of patients, more than 10 tertiary follicles were found. In the group of moderate and poorly differentiated tumours, the largest number/percentage had from 1 to 5 tertiary follicles - 33/83 (39.76%) and 9/15 (60%) of patients, respectively.

The correlations between TLS and stage of the disease and tumour grade showed that the number of tertiary lymphoid structures had a negative, indirect correlation with the stage of the disease (R = -0.635), and with the degree of differentiation (R = -0.243). Therefore, the number of tertiary follicles was larger in tumours with lower stage and better differentiation, and vice versa. The two correlations were confirmed to be statistically significant: the association of the number of tertiary follicles with the staging was significant for the value of p < 0.0001, and with the degree of differentiation for the value of p < 0.05.

Discussion

Colorectal cancer (CRC) being the third most common malignant disease in the humans and the second most common cause for the lethal outcome of malignancies in the West European countries and eighth in the developing countries is a major public health problem [6] [9] [10] [11] [12]. Prognosis of colorectal cancer mostly depends on the stage of disease, and the TNM staging (AJCC/UICC) remains the most reliable prognostic indicator for patients with CRC [6]. It also depends on many other factors such as a tumour and surgery-related factors, histological, genetic, loss of heterozygosity at 18q, microsatellite instability status and molecular, protein biomarkers ant others factors [6] [13] [14] [15] [16].

During the phases of CRC progression, a different amount of inflammatory cells infiltrate a tumour, among them T and B lymphocytes, macrophages and mast cells [3] [6] [8]. It is considered that inflammatory infiltrate promotes tumour growth, but the immune cells may control cancer progression and outcome. The control of

immune system over tumors is based on the theory of 3E rules i.e. 3-phase interaction between the tumor and host immune system: elimination (immune system eliminates tumour cells), equilibrium (immune system controls a tumour) and escape (tumour cells develop resistance to the immune system) [3] [17].

There are evidences that high amount of tumour-infiltrating cells are more common in CRC with lower stage, in tumours with lower T status-local tumour growth, without nodal involvement and metastases and that CRC progression is influenced by inter-reaction between cancer cells and tumour microenvironment belonging to the patient [3] [18] [19] [20]. Human B cells develop in the bone marrow and after activation by Ag, enter primary follicles of lymph nodes or other lymphoid tissues where they undergo extensive proliferation and differentiation in plasma cells producing antibodies. After tumour antigens are recognised, it is discovered that the majority of patients with cancer develop tumour-specific antibodies [21] [22]. B cells present in a tumour infiltrating lymphocytes (TIL) might directly kill tumour cells through Ab-independent mechanisms or could mediate TIL effects by regulating other immune cells. They can promote the differentiation of Th1 and Th2 cells, facilitate the formation of CD4+ T cells memory, and promote the survival and proliferation of activated $CD8^+$ T cells [23].

TLS are described to occur in tumour stroma, and invasive tumour front in different types of cancer and a correlation was found between high densities of TLS and prolonged patient's survival in breast cancer, high grade serous ovarian cancer, non-small cell lung cancer, and CRC [5] [23] [24]. Change in the immune response to host aimed at strengthening or rejection of tumour cells can be a solid ground for cancer immunotherapy and can offer an optimal anti-tumour response in patients with malignant tumours including CRC [25]. Also, published data confirm that chemotherapy might stimulate the immune system against a tumour by increasing the density of intra-tumour lymphocytes, which is associated with a reduction of neoplasm and prolonged patient's survival [26].

This study analysed the quantity of TLS regarding elements of the TNM system (T, N) as well as regarding lymph vessels invasion and tumour differentiation. We found a statistically significant difference in the density of TLS in patients with different stage, with or without lymphatic invasion and with different grade. The density of TLS was higher in patients with lower stage, lower T status, without lymph node metastasis and in cancer patients with better differentiation. This finding in our series of analysed patients has shown that the density of TLS plays an important role in the control of tumour growth in the phase of elimination and equilibrium and that it can be a parameter for the progression of a neoplasm in CRC patients. The density of TLS influences the control of tumour progression.

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Clinical Science

EBV Positive Gastric Carcinomas and Their Clinicopathological Characteristics

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Keywords: EBV; Gastric carcinoma; Immunohistochemistry; Clinicopathological characteristics

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Abstract

BACKGROUND: The understanding of the etiopathogenesis of gastric carcinoma (GC) can be a base for development of new therapeutic methods to reduce mortality and to increase survival in patients with GC. The percentage of Epstein - Barr virus (EBV) positive gastric carcinomas is uncertain, and the etiologic importance of EBV in the pathogenesis of GC has still not been elucidated.

AIM: This study aimed to determine the percentage of EBV associated GC as well as to determine their clinicopathological characteristics.

MATERIAL AND METHODS: The study included 80 patients with GC who were analysed for ethnicity, local growth of a tumour (T status), the presence of nodal metastases (N), the presence of distant metastases (M), stage of the disease and degree of carcinoma differentiation. For detection of EBV, immunostainings were performed on tumour tissue and the peripheral non-tumour gastric mucosa.

RESULTS: Positive immunostaining with an antibody against EBV was found in 19 (23.75%) of the 80 patients with gastric carcinomas. EBV immunostainings were significantly different in patients with or without metastasis and between patients of Macedonian and Albanian ethnicity ($p < 0.0001$, $p < 0.009$, respectively). EBV immunoexpression was significantly associated with the presence of distant metastases and with patients of Albanian ethnicity.

CONCLUSION: Association of EBV immunostainings with distant metastasis in patients with GC suggests the influence of EBV infection on the progression of gastric carcinoma. Due to scarce and doubtful literature data on EBV associated GC, further studies are necessary to determine the role of EBV regarding aetiology, treatment and prognosis in patients with EBV associated gastric carcinoma.

Introduction

The survival rate of patients with gastric carcinoma (GC) is still low in spite of the numerous surgical techniques and development of supplementary preoperative, neoadjuvant and adjuvant protocols for chemotherapy [1] [2] [3] [4]. Therefore, medical treatment of gastric carcinoma urgently requires new therapeutic options. The understanding of the etiopathogenesis of this carcinoma can be a solid base for the development of new therapeutic methods for decreasing the mortality and increasing the survival rate in GC patients.

Over the last decade, a large number of data have been published on the association of gastric carcinoma with Epstein - Barr virus (EBV) that is

believed to play a role in the carcinogenesis of this neoplasm. The percentage of EBV positive gastric carcinoma is uncertain, and the etiological importance has still not been elucidated [5] [6]. The conducted meta-analysis of 70 studies that included a total of 15,952 cases of GC revealed that EBV positive gastric carcinomas differed from the other gastric carcinoma by gender distribution, anatomic localisation and surgically different anatomy, indicating that EBV-associated gastric carcinoma is a particular etiological entity [5]. Epidemiological studies from different regions and studies that contribute to defining the role of EBV in the carcinogenesis and progression of GC are useful for the development of new therapeutic modalities [6].

This study aimed to determine the association of EBV with gastric carcinoma as well as to correlate it with different clinicopathological parameters.

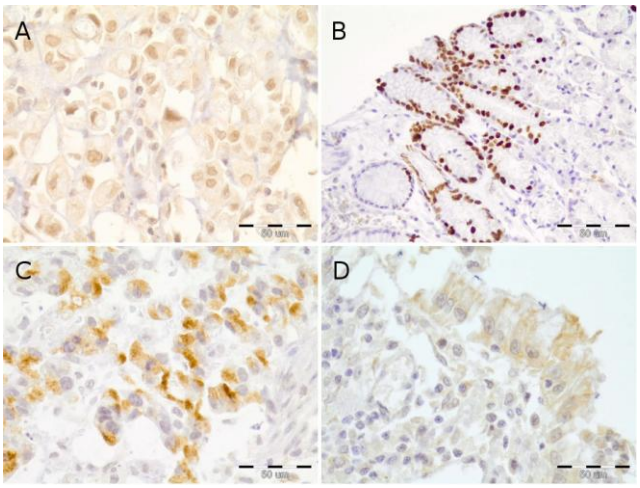

Figure 1: A) Nuclear positivity for EBV in signet cell gastric carcinoma (10 x 20); B) Nuclear EBV positivity in a cluster of glands in peri-tumour mucosa with intestinal metaplasia (10 x 10); C) Cytoplasmic positivity for EBV in gastric cancer (10 x 20); D) Cytoplasmic EBV positivity in peri-tumor cylindrical cells and in plasma cells present in submucosa of the gaster (10 x 20)

Material and Methods

This study included 80 patients with gastric carcinoma surgically treated at the University Clinic for Abdominal Surgery in Skopje, whose operative material was analysed at the Institute of Pathology, Medical Faculty in Skopje.

The following parameters were analysed in the study: ethnicity of patients, local tumour growth (T status), the presence of nodal metastasis (N), the presence of distant metastasis (M), stage of the disease and degree of carcinoma differentiation.

The data for the parameters of the TNM classification (AJCC Cancer Staging 2017) were obtained from the archival histopathological reports of the Institute of Pathology in Skopje, and for the clinical stage we used patient's files from the University Clinic for Abdominal Surgery in Skopje, including ultrasound and computed tomography files.

For detection of EBV, immunostainings were performed on tumour tissue and the peripheral non-tumour gastric mucosa.

A standard commercial control was used for immunostaining control.

Immunohistochemical stainings for EBV were made with a standard procedure using Immunoperoxidase LSAB + system and specific primary monoclonal EBV-antibody (DAKO – Monoclonal Mouse. Anti-Epstein Barr Virus, LMP. Clones CS. 1-4. Code IR753).

EBV expression was defined in 2 histological patterns, nuclear and cytoplasmic (Figure 1).

Descriptive statistical methods were used for statistical analysis of the data. Categorical variables are presented with absolute and relative numbers (%). Fisher's exact test, Student's t-test and Chi-square test were used for comparison of categorical variables. Spearman's correlation coefficient was used to determine the degree of correlation between analysed parameters. The statistical program SPSS for Windows, version 19.0 was used.

Results

Expression of EBV in cells was nuclear and cytoplasmic. Nuclear expression was found in 15 (18.75%) cases and cytoplasmic in 4 (5%) cases. In 10 (66.66%) of positively stained cases, nuclear EBV expression showed the patchy distribution in clusters of cells and the other 5 (33.33%) cases EBV positivity was diffuse in the tumour cells. The expression of EBV in the peri-tumour gastric mucosa showed patchy distribution.

Positive expression of EBV protein was also detected in plasma cells present in the tumour stroma, or gastric submucosa of the patients with GC (Figure 1).

Positive immunostaining with the antibody against EBV was found in 19 (23.75%) of the total of 80 gastric carcinomas.

The mean age of patients with negative EBV expression was 66.05 ± 10.6 years, and of those with positive EBV expression was 63.2 ± 9.5 years ($p > 0.05$).

The immunoexpression of EBV about the analysed clinicopathologic parameters is shown in Table 1.

Table 1: Immunoexpression of EBV about the analysed clinicopathologic parameters

Parameter		EBV expression		
Gender	N	EBV -	EBV+	P-value
Male	25	21(84%)	4 (16)	0.27 ns
Female	55	40 (72.73)	15 (27.27)	P (Chi-square test)
Intragastric localisation				
Cardia	31	23 (74.19)	8 (25.81)	0.55 ns
Body	20	14 (70)	6 (30)	P (Fisher exact
Pylorus	29	24 (82.76)	5 (17.24)	test)
T				
T1	4	4 (100%)	0	0.25 ns
T2	13	11 (84.62)	2 (15.38)	P (Fisher exact
T3	14	8 (57.14)	6 (42.86)	test)
T4	49	38 (77.55)	11 (22.45)	
Lymph node metastasis				
No	16	12 (75%)	4 (25%)	1.0 Ns
Yes	64	49(76.56%)	15 (23.44%)	P (Fisher exact test)
M				
No	72	53 (73.61)	19 (26.39)	<0.0001 sig
Yes	8	0	8 (100)	P (Fisher exact test)
Stage				
I	7	7 (100%)	0	0.076 ns
II	17	11 (64.71)	6 (35.29)	P (Fisher exact
III	47	34 (72.34)	13 (27.66)	test)
IV	9	9 (100)	0	
Grade				
G1	1	0	1 (100%)	0.12 ns
G2	36	30 (83.33)	6 (16.67)	P (Fisher exact
G3	43	31 (72.09)	12 (27.91)	test)
Ethnicity				
Macedonian	62	52 (83.87)	10 (16.13)	0.009 sig
Albanian	18	9 (50)	9 (50)	P (Fisher exact test)

The results obtained in this study regarding positivity of EBV between patients with gastric carcinoma with different N stage showed no significant difference ($p > 0.05$).

A significant difference regarding immunoexpression of EBV was found in GC patients with or without distant metastases ($p < 0.0001$). In patients with gastric carcinoma, the presence of infection along with EBV was significantly associated with distant metastases.

Infection with Epstein-Bar virus was significantly more common among patients of Albanian nationality than in Macedonian patients ($p = 0.009$). EBV immunoexpression was detected in 50% (9) of Albanian patients against 16.13% (10) of Macedonian patients.

Discussion

EBV is a herpes virus that is widely spread among the human population. The infection with EBV is commonly acquired during early childhood by salivary transmission [7]. The virus causes long-term infection of B lymphocytes in about 90% of adults, who are asymptomatic. A small percentage of infected people develop hematopoietic, epithelial and mesenchymal tumours. The EBV infection is a cause for the development of Burkitt lymphoma, lymphoma associated with immunosuppression, Hodgkin's lymphoma, sinonasal angiocentric T lymphoma, nasopharyngeal carcinoma and leiomyosarcoma in immunocompromised patients [5] [7]. It is believed that the oncogenic effect of the virus is carried out by expression of an EBV nuclear antigen and latent membranous proteins that interact with some suppressor genes and signal pathways [7].

According to literature data, the EBV infection is associated with 2-16% of gastric carcinoma, but the published data, in general, refer to the role of EBV in carcinogenesis. Few data on the association of EBV with gastric carcinoma and its characteristics are available in the literature [7] [8] [9] [10]. The prevalence of gastric carcinoma associated with EBV infection shows geographic variations [11] and is related to the lifestyle of patients. Thus, studies about EBV associated gastric carcinomas are necessary and very actual [5] [6].

Gastric carcinomas of the antrum show a low percentage of EBV-associated infection compared to the carcinoma of the cardia [5], and it is found in the highest percentage (90.5%) in lymphoepithelioma-like gastric carcinoma [12].

Some studies have demonstrated the association of EBV gastric carcinoma with the age of patients [13].

The meta-analysis conducted by Lee JH *et al.,* of 48 studies on EBV-associated gastric carcinoma (EBVaGC) showed a significant association with the nationality. The analysis also found that EBVaGC was more frequent in men, young individuals, Caucasians and Latin Americans, in cardia as a localisation of the carcinoma, and in the diffuse histological type [14].

In our study, we detected EBV presence in 23.75% (19) of our patients, which is a high percentage of EBV-associated GC in comparison with the results in the literature [11] [13]. EBV immunoexpression was significantly different in patients with or without metastasis and patients of Macedonian and Albanian nationality.

EBV immunosuppression was significantly associated with the presence of distant metastases and with Albanian ethnicity.

The significant correlation of EBV immunosuppression with distant metastasis in GC patients suggests the influence of EBV infection on the progression of gastric carcinoma.

Due to relatively limited and doubtful literature data about EBVaGC, further studies are necessary to determine the role of EBV regarding the aetiology, treatment and prognosis in patients with EBV associated carcinoma.

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A Comparison of Metoclopramide and Ondansetron Efficacy for the Prevention of Nausea and Vomiting In Patients Suffered From Renal Colic

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Abstract

BACKGROUND: Renal stones are the third common disease of the urinary system after infections and diseases of the prostate. One of the most common manifestations of this disease after acute pain is nausea and vomiting.

AIM: To compare the efficacy of metoclopramide and ondansetron in improving nausea and vomiting in patients referred to the emergency department with a chief complaint of nausea and vomiting.

METHODS: This randomised double-blind clinical trial was conducted on patients referred to the emergency department of Vali-e Asr Hospital. Mg5 intravenous morphine and ketorolac ampoule were injected to control renal colic. Then, patients were randomly divided into two groups. Group 1 consisted of 90 subjects receiving 10 mg intravenous metoclopramide and group 2 including 90 subjects receiving 4 mg intravenous ondansetron. Vital signs were also measured and recorded.

RESULTS: The mean and standard deviation of nausea in 0, 15, 30, 45, 60 and 120 minutes showed no significant difference between the two groups. Mean and standard deviation of vomiting at 0 minutes showed no significant difference between the two groups, but the remaining minutes, 15, 30, 45, 60 and 120, exhibited significant difference as a comparison of two groups, indicating that vomiting in the metoclopramide group was higher than ondansetron group.

CONCLUSION: Our findings indicated that ondansetron was more effective than metoclopramide in preventing and improving vomiting in patients referred to emergency renal colic, where can be used with more efficacy and more acceptable side effects to improve nausea and vomiting.

Introduction

Nausea and vomiting are among the most common complaints of patients referred to the emergency department, and kidney stones are the third most commonly reported urinary tract infection after infections and prostate diseases [1]. One of the most common manifestations of this disease after acute pain is nausea and vomiting [2].

Currently, Treatments such as venous morphine and NSAIDs (Ketorolac) are currently used for the treatment of renal colic [3] [4] [5]. Although these drugs are widely used in the treatment of renal colic, however, these drugs are applied to treat acute pain. There is little evidence for the treatment of nausea and vomiting in renal colic patients (in particular) referred to the emergency department. Treatment of nausea and vomiting in patients, in addition to facilitating the patient's well-being and better collaboration, prevents complications such as dehydration, hypokalemia, aspiration [6]. Treatment of nausea and vomiting in patients, in addition to facilitating the patient's well-being and better collaboration, prevents complications such as dehydration, hypokalemia, aspiration [7]. Although evidence is available on the use of antiemetic drugs in oncology, post-operative nausea and vomiting [8], and

other conditions associated with nausea and vomiting [9], however, little research has been done to apply these medications for the treatment of nausea and vomiting in patients referred to an emergency such as patients with renal colic [6]. Although metoclopramide is one of the most widely employed drugs for the improvement of nausea and vomiting in patients [6]; however, the occurrence of extrapyramidal side effects due to the use of this drug has always been a factor in the cautious use of the drug [10] [11].

Nevertheless, the increasing trend of the use of ondansetron in improving nausea and vomiting in emergency rooms is seen in comparison with metoclopramide [6] [10] [11]. Further evidence has focused on the Preventive or therapeutic roles of these drugs in patients undergoing chemotherapy and surgery [6] [7] [8], where there is little evidence for comparing these two drugs in the treatment of patients presenting to the emergency department [6]. It has recently been reported that the use of serotonin receptor antagonist (Granisetron) can be significantly more effective than metoclopramide in preventing nausea and vomiting in patients undergoing cataract surgery [12].

To prevent postoperative nausea and vomiting after laparoscopic cholecystectomy surgery, it has been shown that patients receiving ondansetron with dexamethasone before surgery had a lower prevalence of postoperative nausea and vomiting than the dexamethasone group alone [13]. The importance of improving nausea and vomiting in patients referred to an emergency such as patients with renal colic is well known. The prevention of complications from continuous nausea and vomiting and the lack of evidence for drug therapies in the emergency department, such as metoclopramide and serotonin receptor antagonists (ondansetron), suggest that comparative studies are required about these drugs.

Therefore, the present study was aimed to compare the efficacy of metoclopramide and ondansetron in the improvement of nausea and vomiting in patients referred to the emergency department.

Material and Methods

A randomised, double-blind, clinical trial study was conducted among patients referring to the emergency ward of Vali-e-Asr Hospital in Arak, Iran with renal colic and nausea and vomiting. The occurrence of renal colic was determined based on the medical history of the subjects and the clinical manifestations of these patients (type and severity of pain and blood in the urine) (3 and 14). The sampling method was carried out using available samples based on inclusion and exclusion criteria.

The inclusion criteria were considered as follow: 1) patients with renal colic and complained of nausea and vomiting; 2) patients classified as ASA I and II; 3) patients 18 to 80 years; 4) obtaining informed consent from patients.

The exclusion criteria included: 1) hypotension (systolic blood pressure < 90) and unstable hemodynamics in general; 2) drug addiction; 3) uncontrolled underlying disease (Parkinson's disease, restless leg syndrome, epilepsy, gastrointestinal bleeding, Pheochromocytoma, etc.) [2]; 4) recent use of central nervous system depressants; 5) recent use of anti-nausea and vomiting drugs (at least the past 8 hours); 6) a previous known allergic reaction to metoclopramide or ondansetron.

Patients willing to participate in the study were evaluated regarding the inclusion and exclusion criteria, and basic information (age and sex) was then obtained. For all patients, 5 mg intravenous morphine was injected with distilled water to a final volume of 10 cc, and patients received Ketorolac ampules for controlling renal colic [4] [5]. Then, the patients were randomly divided into 2 groups: group 1 (90 subjects receiving 10 mg intravenous metoclopramide) and group 2 (90 subjects receiving 4 mg intravenous ondansetron). It should be noted that the patients of the two groups were matched regarding age and sex. Patients were evaluated for the response variables at the 0 and 30 minutes after drug injection, that were considered as follow: 1) the severity of nausea; 2) the number of vomiting; 3) vital signs (systolic, diastolic and patient temperature); 4) the need for additional drug therapy after 30 minutes to improve nausea and vomiting; 5) drug side effects. It is worth noting that the patients were matched in two groups according to their age, sex and severity of nausea (5-10 cm based on VAS).

The severity of nausea in the two groups was evaluated before the injection of drugs (0), and 30 minutes after taking the drugs by VAS (Visual Analogue Scale). This criterion consists of a 10 cm ruler extending longitudinally between zero and 10, in which the zero number indicates no pain, and the number 10 indicates an unbearable pain. Patients were asked to mark their pain in this ruler the patient's markup distance from point 0 indicates the patient's pain level [15].

The frequency of vomiting was also evaluated from patients 30 minutes before injection and up to 30 minutes after medication administration based on patient information and medical history. The vital signs of the patients were measured at 0 and 30 minutes. Drug complications were also documented by the presence of the following variables or other symptoms (based on the diagnosis of an emergency physician) after the injection of drugs based on the patient's history and doctor's visit.

Criteria for metoclopramide included

akathisia, dyskinesia, and other extrapyramidal side-effects, dizziness, restlessness, muscle spasm, sweating, etc. The ondansetron criteria were as follows: dizziness, abdominal pain, rash, diarrhoea, etc. It is noteworthy that the task of selecting patients and prescribing drugs for patients was to the emergency medicine specialist. The resident researcher is responsible for reviewing and recording the clinical response variables of patients. In this study, assistant specialist and patients are blind to the type of injectable treatment. For this reason, the drug content in the 10 cc syringes was first prepared by a nurse and administered by the assistant specialist who was blind to the type of treatment.

Because in the treatment of patients referred to the emergency department the first exposure to anaesthetics and vomiting drugs (such as metoclopramide or endonestrone) is used, the placebo group was not considered to be due to the deprivation of these patients. Regarding the treatment of patients referred to the emergency department in the first step with anti-nausea and vomiting drugs (such as metoclopramide or ondansetron), the placebo group was not considered in the study due to the deprivation of these patients from treatment.

Data analysis was performed using SPSS 21 software. To analyse the results, we used mean indexes, standard deviation, standard error, the percentage of frequency. Moreover, the covariance analysis test, Chi-square and Independent T-test or its nonparametric equation or its nonparametric equation were used to compare the mean.

Results

In this double-blind clinical trial, 180 patients were enrolled, and 90 patients were randomly assigned to receive metoclopramide and 90 patients to the ondansetron group.

The mean and standard deviation in the metoclopramide group and ondansetron group determined to be 34.56 ± 8.98 and 33.2 ± 8.5, respectively. There was no statistically significant difference between the two groups regarding age (P = 23.88). Mean, and standard deviation of metoclopramide group was calculated as 171.48 ± 6.26 while this level was determined to be 172.77 ± 5.32 for ondansetron group, no statistically significant difference was found between the two groups regarding height (P = 307). As shown in Table 1, the mean and standard deviation of pain score at 0, 30, and 60 minutes did not show a significant difference. This suggests that the severity of pain in the ondansetron group was less than the metoclopramide group at these moments.

Table 1: Mean and standard deviation of pain score in control and case groups; T indicates the time

	Group	Mean	*Standard Deviation*	P value
T 0	Metoclopramide	97.8	83.0	0.408
	Ondansetron	07.9	78.0	
T 30	Metoclopramide	97.4	46.1	0.001
	Ondansetron	13.4	90.0	
T 60	Metoclopramide	07.3	54.0	0.001
	Ondansetron	58.2	49.0	

The mean and standard deviation of systolic blood pressure in 0 minutes were determined in the metoclopramide and ondansetron groups as 136.74 ± 10.15 and 137.03 ± 7.54, respectively, where there was no statistically significant difference between the two groups for systolic blood pressure (P = 0.829).

The mean and standard deviation of systolic blood pressure of 30 minutes in metoclopramide and ondansetron groups were 130.62 ± 7.81 and 128.75 ± 8.22, respectively. There was no significant difference between the two groups regarding systolic blood pressure at 30 min (P = 0.120). The mean and standard deviation of systolic blood pressure during 60 minutes in the metoclopramide group were determined as 124.91 ± 9.05, while these values for the ondansetron group was calculated to be 124.73 ± 6.24. However, there was no significant difference between the two groups regarding systolic blood pressure (P = 0.088).

On the other hand, the mean and standard deviation of diastolic blood pressure *in 0 minutes* was determined in metoclopramide and ondansetron groups as 81.68 ± 6.61 and 81.33 ± 6.78, respectively, which demonstrated no significant difference between the two groups regarding diastolic blood pressure (p = 722).

Furthermore, the mean and standard deviation of diastolic blood pressure were determined in the metoclopramide group during the 30 and 60 minutes as 77.51 ± 7.63 and 73.58 ± 7.44, respectively, while these values in the ondansetron group were 76.22 ± 7.10 and 73.33 ± 6.75 were calculated. According to the P value, there was no significant difference between the two groups regarding diastolic blood pressure in the mentioned minutes (P = 0.243; p = 0. 810).

Table 2: Mean and heart rate deviation in case and control groups; T shows time

	Group	Mean	*Standard Deviation*	P value
T 0	Metoclopramide	14.96	41.3	0.686
	Ondansetron	90.95	59.4	
T 30	Metoclopramide	64.82	19.4	0.001
	Ondansetron	33.79	51.3	
T 60	Metoclopramide	31.77	77.5	0.001
	Ondansetron	78.73	99.2	

The mean and standard deviation of heart rate at 0, 30, and 60 minutes after receiving metoclopramide and ondansetron by patients are summarised in Table 2. The results of this study revealed that there was a significant difference in

heart rate between the two groups receiving the drug 30 and 60 minutes after the intervention, indicating that the heart rate in the metoclopramide group was higher as compared to the ondansetron group (p = 0.001; p = 0.001).

Based on the results presented in Table 3, the mean and standard deviation of body temperature in 0, 30 and 60 minutes after intervention were calculated in metoclopramide and ondansetron groups. There was a statistically significant difference between the two groups regarding body temperature after 0 and 30 minutes (p = 0.001; p = 0.002). This finding suggests that body temperature was lower in the metoclopramide group when comparing with the ondansetron group in times above. Nevertheless, the mean and standard deviation of body temperature after 60 minutes in two groups did not exhibit significant difference (p = 1.000).

Table 3: Mean and deviation of morphine in both case and control groups

	Group	Mean	Standard Deviation	P value
Morphine at 0 min	Metoclopramide	96.6	92.0	0.653
	Ondansetron	91.6	71.0	
Morphine at 30min	Metoclopramide	76.0	31.1	0.484
	Ondansetron	0/63	1/23	

The mean and standard deviation of the need for morphine at 0 and 30 minutes in both groups were shown in Table 4, where no significant difference was observed between the two groups at both times.

Table 4: Mean and standard deviation of morphine in both case and control groups; T indicates the time

	Group	Mean	Standard deviation	P value
Morphine T0	Metoclopramide	96.6	92.0	0.653
	Ondansetron	91.6	71.0	
Morphine T30	Metoclopramide	76.0	31.1	0.484
	Ondansetron	63.0	23.1	

As indicated in Table 5, the mean and standard deviation of nausea in 0, 15, 30, 45, 60 and 120 minutes after intervention in the two groups did not show a significant difference.

Table 5: Mean and standard deviation of nausea rate in case and control groups; T indicates the time

Group		Mean	Standard deviation	P value
Vomiting at 0 min	Metoclopramide	02.3	62.0	1.000
	Ondansetron	03.3	73.0	
Vomiting at 15 min	Metoclopramide	51.1	50.0	0.001
	Ondansetron	35.1	52.0	
Vomiting at 30 min	Metoclopramide	65.0	47.0	0.001
	Ondansetron	36.0	48.0	
Vomiting at 45 min	Metoclopramide	38.0	49.0	0.001
	Ondansetron	04.0	20.0	
Vomiting at 60 min	Metoclopramide	12.0	32.0	0.001
	Ondansetron	08.0	36.0	
Vomiting at 120 min	Metoclopramide	07.0	26.0	0.001
	Ondansetron	02.0	31.0	

Furthermore, the results of the present study showed that the mean and standard deviation of vomiting in both groups were not statistically significant at 0 minutes, while after intervention, there was a significant difference between the two groups, where vomiting in minutes after intervention (Time: 0, 16, 30, 45, 60, 120) was significantly higher in the metoclopramide group than the ondansetron group.

Discussion

Renal colic is one of the most common urological emergencies that is very painful for the patient. The renal colic annually affects 1.2 million people, accounting for about 1% of hospital admissions [16]. The incidence of kidney stones for men and women is about 12% and 4% throughout life, respectively, which the disease is affected by age, family history, race, place of residence, occupation [17].

This double-blind clinical trial was conducted to compare the efficacy of Metoclopramide and ondansetron in the treatment of nausea and vomiting in patients with renal colic. The results of our study showed that there was no statistically significant difference between the two groups regarding age, height and weight, where the two groups were matched. There was no statistically significant difference between the mean score of pain in 0 minutes in the intervention and control groups. However, there was a significant difference between the two groups regarding mean pain score in the 30th and 60th minutes, which indicates the severity of pain in the ondansetron group was less than the metoclopramide group.

Moreover, there was no significant difference between systolic and diastolic blood pressure in the two groups at 0, 30 and 60 minutes. Furthermore, no significant difference was found between the two groups in terms of heart rate at 0 minutes; however the mean difference was statistically significant between the two groups in terms of heart rate at 30 and 60 minutes, indicating that the heart rate was lower in the metoclopramide group at 30 and 60 minutes as compared to the ondansetron group.

Based on the data presented here, the mean and standard deviation of body temperature at 0 and 30 minutes exhibited a significant difference between the two groups, indicating that the body temperature in the metoclopramide group was lower than the ondansetron group. The mean and standard deviation of initial morphine and morphine levels of 30th minutes did not reveal any significant difference between the two groups. Also, the mean and standard deviation of nausea in minutes 0, 15, 30, 45, 60 and 120 were not significantly different between the two groups. Mean, and standard deviation of vomiting was not significantly different between the two groups at 0 minutes, while the remaining minutes, 15, 30, 45, 60 and 120, demonstrated a significant difference

between the two groups, indicating that the vomiting rate in the metoclopramide group was higher when comparing with ondansetron group.

As other previous study indicated the decreased rate of nausea severity for nausea and vomiting were determined to be similar for 20 mg intravenous metoclopramide, and 4 mg intravenous ondansetron, as well as placebo. However, this was not significant, and the changes were not significant in the two drug groups compared to the placebo group [6], while, the rate of nausea was similar in both groups in our study, but the vomiting rate in the ondansetron group was lower than the metoclopramide group [6].

Zahedi study has shown that both metoclopramide and ondansetron have been significantly and prominently effective in preventing nausea and vomiting in these patients during spinal anaesthesia for cesarean section, and their effect on reducing nausea and vomiting is significantly greater compared with placebo group. While our findings revealed that the effect of ondansetron on nausea and vomiting was more than metoclopramide, which our results were not consistent with the findings of the study above [18]. It has been reported that droperidol was more effective than metoclopramide or prochlorperazine in patients with moderate to severe nausea, but extrapyramidal symptoms could be increased, where metoclopramide and prochlorperazine have had a proportional effect on the improvement of nausea and vomiting of patients referred to the emergency department, as well as we're not seen to be more effective compared to the saline placebo [19]. Our study on nausea recovery was consistent with the study above. Contrary, the results of vomiting improvement were not similar to the present study.

Another study showed that ondansetron and metoclopramide, plus dexamethasone, did not show a significant effect on postoperative nausea and vomiting after laparoscopic cholecystectomy surgery and was not consistent with our results [20].

The findings of our study revealed that ondansetron was more effective than metoclopramide in preventing and improving vomiting in patients referred to emergency suffering from renal colic. Therefore, ondansetron can be used with more efficacy and more acceptable side effects to improve nausea and vomiting.

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Clinical Science

Tolerability of Omalizumab in Asthma as a Major Compliance Factor: 10-Year Follow Up

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Abstract

BACKGROUND: There is a lack of data related to real life, long-term safety, tolerability and compliance of omalizumab treatment in asthma patients beyond 6 years.

AIM: Study aimed to assess safety, tolerability, compliance and all reasons for treatment discontinuation during 10 years on omalizumab.

SUBJECT AND METHODS: This is a retrospective, observational study of uncontrolled asthma patients receiving omalizumab for the last 10 years. All data were collected from patients' files (demographics, adverse events, comorbidities, compliance index, reasons for discontinuation of omalizumab). Reactions to omalizumab were classified as local and systemic, and their severity as mild, moderate or severe. Reactions were either immediate (minutes to hours after drug administration) or delayed (after days). Compliance to omalizumab, defined as Compliance index (CI), was calculated by comparing milligrams of given to milligrams of prescribed dose/ per year.

RESULTS: Out of 35 patients receiving omalizumab, 15 drop out at different time points mostly due to treatment efficacy or appearance of new comorbidities. Patients who continue for the next ten years had mild to moderate adverse events related to omalizumab. There was no increased risk of severe adverse events during 10 years on omalizumab. Patient's treatment tolerability, despite mild to moderate adverse events, is in favour of compliance.

CONCLUSION: Compliance with omalizumab mildly decreased over 10 years but was not affected by severe adverse events of treatment or new comorbidities. Although, omalizumab is safe medicine appearance of new comorbidities has to be closely followed up.

Introduction

Asthma is a common, chronic respiratory disease affecting 15% of adults and 18% of children in Kuwait [1]. Worldwide, approximately 20% of asthma patients have severe asthma, of which 20% is inadequately controlled [2]. The Global Initiative for Asthma (GINA) guidelines recommends a stepwise treatment until control is achieved and maintained. GINA recommends adding oral corticosteroids (OCS) or anti-Immunoglobulin E (IgE) treatment with omalizumab in uncontrolled asthma patients [3]. Due to well-known severe side effects of oral corticosteroids, omalizumab represented promising, safer, approach to difficult to control allergic asthma [4].

Omalizumab was first approved in 2003 to treat adults and children 12 years of age and older with moderate to severe persistent allergic asthma not controlled by inhaled corticosteroids (ICS) and is approved lately for children aged ≥ 6 years [5].

Based on current data it is still unclear when omalizumab treatment should be stopped after asthma control is achieved [6]. This statement raised many issues regarding the long-term safety, tolerability, compliance, and possible correlation of same during omalizumab treatment.

There are noted side effects of omalizumab recognised by the manufacturer [7] or FDA (The Food and Drug Administration) [8]. However, omalizumab was found to be in general a well-tolerated therapy with frequency and severity of adverse events (AE) similar to patients receiving placebo or best available

therapy [9]. The study aimed to assess safety, tolerability, compliance and all reasons for treatment discontinuation during 10 years on omalizumab.

Patients and Methods

This was real life, retrospective, observational study, conducted at Al Rashed Allergy Centre, the first Medical Institution that applied omalizumab for uncontrolled, moderate to severe allergic Asthma, since 2008 in Kuwait. Inclusion and exclusion criteria applied for 35 patients after treatment is stepped up on level 5 GINA [10]. All data were collected from patients' files. Patients who stopped with omalizumab for different reasons at different time points till the last assessment in 2017, were defined as drop out (15 patients) and a patient who continued (20 patients) as an ongoing group. Details of any adverse events (whether reported or not in literature) occurred during treatment were recorded, as well as details of any newly diagnosed comorbidities. Omalizumab was administered every 2 or 4 weeks, subcutaneously, at the dose calculated based on patients pre-treatment total IgE serum level and body weight [11]. Adverse events to omalizumab were classified as systemic and local reactions, and their severity was classified by both physician and patient (mild, moderate, severe) to assess eligibility for continuation of treatment. Reactions were divided into immediate (few minutes to hours after administration drug) and delayed (after days). Compliance to omalizumab (Compliance index-CI) was calculated by comparing milligrams of given dose to milligrams of prescribed dose/per year and defined as CI ≤ 50% not compliant, 50-75% poor, 76-89% good and ≥ 90% as high compliance [12].

Results

A total of 35 patients started omalizumab during 2008. All patients fulfilled GINA stepping up criteria [13]. Only one patient required daily use of oral corticosteroids prior omalizumab but stopped gradually after 6 months of treatment. Thirteen patients were receiving omalizumab every 2 while resting every 4 weeks. Till assessment in 2017, 15 patients (11 females) discontinued treatment for different reasons at different time points. Demographic data of drop out and ongoing treatment group (20 patients) are presented in Table 1.

The ongoing group was younger ($p < 0.05$), while gender, BMI and monthly doses of omalizumab showed the similar distribution in both groups ($p > 0.05$).

Table 1: Characteristics of patients in ongoing and dropouts group

	On going N = 20	Drop outs N = 15	p value
Age* in years, mean±SD	41.4 ± 8.95	51.87 ± 16.37	0.0210**
Female (n; %)	15 (75.0%)	11 (73.3%)	0.7802
BMI*	30.13 ± 6.78	30.58 ± 4.29	0.8224
Duration of treatment in years, mean±SD			
-Any reason	-	3 ± 1,65	ND
-Treatment-related AE	-	4	
Comorbidities at baseline (n, %)			
Nasal polyps	9 (45.0%)	0 (0.0%)	0.0043**
Diabetes mellitus type 2	3 (15.0%)	2 (13.3%)	1.000
Hypertension	3 (15.0%)	0 (0.0%)	0.24
Gastroesophageal reflux disease	6 (30.0%)	2 (13.3%)	0.42
Chronis rhinosinusitis	4 (20.0%)	3 (20.0%)	1.00
Seasonal allergic rhinitis	2 (10.0%)	1 (6.67%)	1.00
Hypothyroidism	3 (15.0%)	1 (6.67%)	0.62
Eczema	1 (5.0%)	0 (0.0%)	1.00
Osteoporosis	4 (20.0%)	1 (6.67%)	0.36
Psoriasis	1 (5.0%)	0 (0.0%)	1.00
Obesity (BMI≥30 kg/m^2)	10 (50.0%)	6 (40.0%)	0.29
Comorbidities diagnosed during treatment (n, %)			
Diabetes mellitus type 2	2 (10.0%)	0 (0.0%)	0.5
Hypertension	2 (10.0%)	0 (0.0%)	0.5
Psoriasis	1 (5.0%)	1 (6.67%)	1.00
Obesity (BMI≥30 kg/m^2)	3 (15.0%)	0 (0.0%)	0.24
Thyroiditis	1 (5.0%)	0 (0.0%)	1.00
Gastroesophageal reflux disease	1 (5.0%)	0 (0.0%)	1.00
Ischaemic heart disease	1 (5.0%)	0 (0.0%)	1.00
Megaloblastic anaemia	2 (10.0%)	0 (0.0%)	0.5
Alzheimer disease	0 (0.0%)	1 (6.67%)	0.429
Cervical tuberculose adenitis	0 (0.0%)	1 (6.67%)	0.43
Liver cirrhosis	0 (0.0%)	1 (6.67%)	0.43
Hypogonadism	0 (0.0%)	1 (6.67%)	0.43

Index: BMI-body mass index; SD-standard deviation; AE-adverse events; ND-not did; (*)-Mean age and BMI before the start of omalizumab; (**)-difference was significant.

Among an equal number of presented comorbidities in both groups ($p > 0.05$), nasal polyposis was more frequent in the ongoing group ($p < 0.01$), number of new comorbidities diagnosed while on omalizumab were similarly noticed in both groups ($p > 0.05$).

Anaphylaxis related to omalizumab has been described as a combination of any of the following: angioedema of the throat or tongue, bronchospasm, hypotension, syncope, and urticaria [14] which defines severe, systemic, treatment stopping reaction.

On treatment with omalizumab, no immediate systemic reaction (anaphylaxis or generalised urticaria) was observed in our patients at the beginning or during the next ten years. From 35 patients, 6 had mild to moderate local, and 6 had a moderate systemic reaction during the first week after omalizumab injection, and all of them continue treatment for the next 10 years (Table 2).

The majority of patients reported adverse events from the start of omalizumab while others after more than 5 years (e.g. back pain), and few patients had occasional (> 3 times per year) occurrence of symptoms. All patients who reported any of these side effects were evaluated fully for organic and non-organic causes of symptoms. However, no cause was found.

One 33-year-old female patient stopped omalizumab after 4 years (CI = 75%) due to pain in the arms and legs up to 5 days after each injection, and this has been increasing in intensity with years of treatment.

Table 2: Treatment-related and other adverse events (AE) in patients on omalizumab

	Patients N = 35 (100%)
Discontinuation for any reason	15 (42.8%)
Treatment related AEs	12 (34.3%)
Immediate systemic reaction	0 (0%)
Immediate local reaction	6 (17.1%)
Patient with AE non-causing discontinuation	12 (34.3%)
Other than treatment-related AEs causing discontinuation	
a) poor or very good response on Omalizumab	8 (22.8%)
b) Psoriasis, newly diagnosed	1 (2.8%)
c) Alzheimer disease	1 (2.8%)
d) Liver cirrhosis	1 (2.8%)
e) Cervical tuberculose adenitis	1 (2.8%)
f) Hypogonadism	1 (2.8%)
g) Death during an asthma attack	1 (2.8%)
Type of treatment-related AE causing discontinuation	
a) pain in arms and legs	1 (2.8%)
Type of AE non causing discontinuation	
a) pain at the site of injection	5 (14.3%)
b) pain in arms and legs	1 (2.8%)
c) pain in legs	2 (5.1%)
d) back pain	3 (8.6%)
e) nervousness, fatigue and insomnia	6 (17.1%)
f) swelling at the site of injection	2 (5.1%)
g) subjectively perceived increase in hair loss	6 (17.1%)
h) venous thromalizumabosis	1 (2.8%)
Dropouts according to treatment years	
a) after 1 year	1 (2.8%)
b) after 2 years	7 (20%)
c) after 3 years	3 (8.5%)
d) after 4 years	1 (2.8%)
e) after 6 years	3 (8.5%)

Other 14 patients stopped omalizumab due to other than treatment-related adverse events. Reason for discontinuation of omalizumab by a physician, for five patients after 2 years, was poor compliance and poor effectiveness estimated by asthma control parameters [15]. During the first 3 years of treatment, three patients showed significant clinical improvement and subjectively felt very well, so they decided to stop omalizumab. In 5 from 15 patients reason for omalizumab discontinuation was the appearance of new comorbidity and one female patient died during severe asthma attack during the second year on omalizumab (deep depressive state after a family tragedy, history of near-fatal asthma attacks, CI = 60%).

Median CI for drop out group was 72% for all years on omalizumab, and for ongoing group significantly decreased over 10 years to 80% (Table 3). Annual Compliance index was higher in period from 2008 till 2012, compared to 2013 till 2017 ($p < 0.05$, $p < 0.0001$, $p < 0.05$, $p < 0.001$ and $p < 0.0001$). There is no significant difference in CI between patients with and without AE in ongoing group ($p > 0.05$).

Table 3: Annual Compliance Index for the ongoing group (n = 20)

Year	Compliance index
2008.	1
2009.	1
2010.	1
2011.	0.9
2012.	0.9
2013.	0.8
2014.	0.8
2015.	0.8
2016.	0.8
2017.	0.8
p-value	< 0.0001*

*difference was significant.

Discussion

If omalizumab considers years-long treatment for moderate to severe uncontrolled asthma, there are some concerns regarding tolerability that requires close follow up.

As concluded by Di Bona et al., long-term treatment with omalizumab appears remarkably safe and well tolerated in a real-life setting. Prolonged omalizumab treatment for many consecutive years did not increase the risk of side effects, particularly anaphylaxis [16]. Data from Randomized Controlled Trials (RTC) and post-marketing surveillance showed that hypersensitivity reaction to omalizumab are not that frequent and anaphylaxis is rare, occurring in about 0.09% of patients [17]. Safety data from real life observational studies are consistent with the results of RCT mostly for short-term studies [18]. Based on our data, even the 10-year long treatment with omalizumab does not increase the rate of anaphylaxis. These results confirm that omalizumab has a good safety profile, both in the experimental and real-life setting [19].

Three studies reported adverse events as the main cause of treatment discontinuation, without any significant differences regarding drop-out rate [20] [21] [22].

Only one female patient in our study stopped omalizumab due to increased, post injection pain in arms and legs, lasting up to 5 days. She was satisfied with the effectiveness of treatment and tolerated pain for 4 years with CI 75%.

This finding suggests that tolerability is an important issue and consequently it has to be carefully considered; as evidenced with other treatments, it can significantly affect compliance [23]. Most observational studies reported a low discontinuation rate due to AE over a mean treatment period of 1-2 years [24] [25] [26] [27] [28] [29] [30], same applied for period of 3 and 4 years respectively [31] [32] and 9 years study reported a 6.6% drop out over a mean treatment period of 3.8 years [16]. In our study, local reaction at the injection site was the commonest adverse event. Pain in arms and legs or legs only reported 7.9 % (3 out of 35 patients) and immediate local reactions (pain/swelling at the site of injection) 17.1% patients, but that was not reason enough to stop with the treatment during the next 10 years. Di Bona et al. reported only one patient with immediate local reaction (injection site swelling) [16]. Subjectively perceived increase in hair loss in 17.1% of our patients is also recognised in different reports [33], but it has to be properly assessed and evaluated to be labelled as omalizumab induced. Nervousness, fatigue and insomnia are reported by 17.1% of patients in our study. There is no data about nervousness and its correlation with asthma or asthma treatment. It is known that chronic diseases

such as asthma can cause depression [34]. Fatigue and insomnia can be part of depression symptoms spectrum [35]. Fatigue and insomnia are also reported as mild side effects of omalizumab, and our patients reported that it lasted 2 days after injection [36].

In real-life studies, the drop-out rate ranged from 0 to 45.5 %, and in most cases, lack of efficacy was responsible for treatment discontinuation [37].

Majority of our patients (n = 8) who stopped with omalizumab did so because of the poor or excellent effect of treatment after the first 2 years, and the others due to newly diagnosed comorbidities. In individuals with severe asthma, comorbidities are common, with the most prevalent being gastroesophageal reflux disease (GERD), sinusitis, allergic rhinitis and nasal polyposis [38]. Same comorbidities were present in our patients before the start of omalizumab, but they didn't affect later treatment tolerability and compliance. Although there is no confirmed correlation with omalizumab treatment, it's notable that 5 patients in drop out group developed new comorbidities over the years on omalizumab. A 70-year-old female patient, otherwise healthy, stopped omalizumab when diagnosed with Alzheimer disease during the sixth year on treatment (CI = 90%). There is no data about Alzheimer disease in patients on omalizumab, but there are data about the increased incidence of Alzheimer in Arab countries [39]. A 73-year-old male patient, who had no history of smoking, alcohol intake or chronic disease, developed liver cirrhosis in full clinical feature during the 6th year on omalizumab (CI = 75%) and died few months after diagnosis. Male patient (38-year-old) with mild improvement on omalizumab stopped the treatment when diagnosed with hypogonadism during the first year of treatment (CI = 80%), and one female patient (39-year-old) was diagnosed with thyroiditis (normal hormonal status) after 7 years on omalizumab, and she continues with omalizumab treatment. We couldn't find any published reports of the liver, thyroid or gonadal hormones issue in omalizumab patients. A 34 year old female decided to stop omalizumab after 2 years (CI = 90%) when diagnosed with cervical tuberculous adenitis.

There are no studies supporting the correlation between omalizumab and tuberculosis, but an extra-pulmonary tuberculosis infection rate of 30% in Saudi Arabia remains above the global rate [40]. Regarding infectious disease, the only low risk of parasitic infestation while on omalizumab is reported by a specific study carried out in Brazil [41]. A male (53 years old) patient stoped omalizumab when diagnosed with psoriasis during the first year of treatment (CI = 90%). After 7 years of omalizumab one (51-year-old), the female patient is also diagnosed with psoriasis and continue with treatment. Al-Mazeedi et al. conducted a descriptive study to determine the extent of psoriasis in Kuwait and the risk factors associated with it. The incidence and prevalence of psoriasis in Kuwait were calculated to be 0.11% and 0.45%, respectively and usual age of onset is between 15 and 30 years, although it can present at any age [42]. The appearance of psoriasis doesn't seem to be affected by omalizumab or even treatment duration.

Newly diagnosed comorbidities in the ongoing group seem not to affect tolerability and compliance. We noted 2 patients with newly diagnosed type 2 diabetes mellitus (after 8 years on omalizumab, older than 60 year of age with positive family history for diabetes mellitus), two cases of hypertension (patients with positive family history for hypertension, both older than 60 year), and one gastroesophageal reflux disease-GERD (after 4 years of omalizumab treatment, history of treated Helicobacter pylori infection). In our study, one female patient has ischemic heart disease-IHD (49-year-old, history of hypertension and transient ischemic brain attack - TIA, after 3 years on omalizumab). Although EXCELS study's interim safety data showed an excess of cardiovascular and cerebrovascular events in the patients on omalizumab compared with the asthma control group [8] [43], FDA did not recommend any changes to the prescribing information (i.e., package insert) but did recommend increased awareness [44]. There is a question of possible adjustment for asthma treatment, omalizumab dosing and parameters for follow up, for these high-risk patients.

In our study, there is also, no newly diagnosed malignancies over the 10 year which is also consistent with EXCEL study [45].

Two patients had megaloblastic anaemia (females, after 6 years on omalizumab) and one (female, 46-year-old, after 9 years on omalizumab) had elevated specific liver enzymes with negative assessment for infective, autoimmune and malignant diseases.

For noted comorbidities, the bigger cohort with long-term follows up is needed, with a closer observation on all details that can help in selecting patients for omalizumab. Some studies reported that about 50% of asthma patients are not compliant with the given treatment. The issue becomes even more relevant in specific age groups such as children, adolescent and elderly [46]. A univocal and standardised tool for evaluation of adherence is lacking [47]. Another controversial aspect concerns the definition of "acceptable adherence". In some large studies, an adherence rate greater than 80 % has been considered satisfactory, but a consensus about this issue has not been reached. Patients requiring treatment with injected drugs, like omalizumab, are more easily monitored, as treatment administration requires medical supervision [48]. Treatment discontinuation can be easily detected and considered as a consistent marker of compliance.

Harjinder et al., the study reported that visit compliance does not statistically impact the response rate to omalizumab and higher compliance does not

correspond to the high response rate [12]. In our study, there is a significant decrease in compliance expressed as drop-in compliance index from high to good for 10 years. In an ongoing group, 12 patients had mild to moderate adverse events that should be noted as possible reasons for compliance decrease. Although there is no significant difference in CI between patients with or without reported AE in an ongoing group. Efficacy seems to be a more significant factor affecting omalizumab treatment discontinuation than, other than severe, AE of the same medicine. Tolerability of mild to moderate AE in favour of treatment efficacy points out an acceptable range of CI from 76% and more.

That emphasises better patient selection and devoted follow up by medical stuff during treatment of moderate to severe uncontrolled Bronchial Asthma. More tool is still required to lead physician, and patient as well, from the predicted effect of omalizumab to real beneficial one.

As limitation of our work it can be noted that in real-life observational studies is difficult to avoid or properly assess bias and conclusions are not easily applicable across a generalised population. Furthermore, often only a descriptive analysis has been provided.

Nevertheless, to our knowledge, this is first 10 years study of tolerability, safety and compliance which may help in finalising some practical suggestions to improve compliance in routine clinical practice.

In conclusion, the most important benefit of our study is a long observational period for omalizumab treatment. Our results indicate that the drug can be administered for many years without increased risk of severe adverse events. Continuation of treatment despite mild to moderate adverse events is due to the patient's perception of omalizumab effectiveness. Therefore, clinicians should discuss tolerability issues with their patients as part of a strategy aiming at improving compliance. To our knowledge, newly diagnosed conditions such as liver cirrhosis, thyroiditis and megaloblastic anaemia documented after more than 6 years of treatment in our patients, are not described in available studies and demand closer further observation regarding the possible causative role of omalizumab.

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Clinical Science

Probiotics Improve Urogenital Health in Women

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Abstract

BACKGROUND: Urogenital recurrent infections represent a global medical issue in the world, affecting millions of women because of dramatic shifts in bacterial composition and concentrations in response to numerous endogenous and exogenous factors. Urogenital microbiota forms a mutually beneficial relationship with their host and has a major impact on health and disease.

AIM: This study aimed to compare probiotic therapy versus placebo in Oxidative Stress Values (OSVs) and histological features in urogenital infections in female patients.

METHODS: Forty (n = 40) patients diagnosed with recurrent urogenital infections were recruited to be treated as test group (n = 20), receiving Probiotics, and a control group (n = 20), receiving looking similar placebo, both for 90 days. Both the groups were assessed for total oxidant capacity (d-ROMs test) and biological antioxidant potential as iron-reducing activity (BAP test) at baseline, after 1 and 3 months. Histological changes on inner vaginal mucosa were also investigated, during the entire study.

RESULTS: d-ROM assay clearly showed that the values of the test group were significantly different, thus leading the general health conditions from a state of high oxidative stress to low oxidative stress levels. Increasing of BAP values were more significant, and clinically relevant, in probiotic test group over time.

CONCLUSION: Our pilot study gave interesting and promising elements to confirm the safety and effectiveness of oral probiotics in preventing/reducing the recurrent urogenital infections by an overall modification of inner vaginal microbiota.

Introduction

Urogenital infections are among the most common worldwide human infectious diseases [1]. Annually it is estimated that one billion women around the world suffer from non-sexually transmitted urogenital infections, including bacterial vaginosis, yeast vaginitis, and urinary tract infection (UTI). Although most patients respond to antimicrobial treatment, the recurrence rate is high and associated with side effects [2], with costs to health care providers amounting to over $6 billion annually worldwide. [3] UTI can lead to pelvic inflammatory disease, infertility, ectopic pregnancy, premature labour, low birth weight babies, chronic pain, and increased vulnerability to human immunodeficiency virus (HIV) and other sexually transmitted infections (STIs). The urogenital system is an amalgamation of the urinary tract and reproductive system. Because both systems are open to the external environment, they are susceptible to infections. Some infections are introduced from outside, whereas others result from imbalances in the microbiota of the urogenital tract.

Urinary Tract Infections are typically caused by bacteria that normally live in the colon and rectum. Once bacteria are introduced into the urethra, they multiply and travel up to the bladder. *E. coli* is usually the most prevalent organism responsible for UTIs and accounts for 80–85% of the total isolates, with *Staphylococcus saprophyticus* being the cause in 5–

10% [4] [5]. Other bacterial causing UTIs include *Klebsiella*, *Proteus*, *Pseudomonas*, *Enterococcus*, *Enterobacter spp*. Etc. Women are especially more prone to developing UTIs due to anatomical factors that allow quick bacterial access to the bladder, poor hygiene; sexual intercourse and use of contraceptive are also contributory factors. Other complications caused by UTIs are a bladder infection (cystitis), urethra infection (urethritis), kidney infection (pyelonephritis) and ureter (ureteritis) [6] [7] The main clinical and biological aspects reported in UTIs are briefly listed in Table 1.

Table 1: UTIs Common Features

- A strong, persistent urge to urinate.
- A burning sensation when urinating.
- Passing frequent, small amounts of urine.
- Urine that appears cloudy.
- Urine that appears red, a sign of blood in the urine.
- Strong-smelling urine.
- Pelvic pain.

Recent studies have emphasised the importance of a healthy, *lactobacilli* dominated microbiota not only to prevent sexually transmitted diseases and preterm labour [8] but also to maintain the quality of life of women [9] [10]. The depletion of *lactobacilli* organisms in women susceptible to urinary and vaginal infections raised the question of whether artificial supplementation of *lactobacilli* could lower infection rates [8] [9] [10]. Nevertheless, drug resistance to commonly used antibiotics (e.g., trimethoprim/sulfamethoxazole) is increasing among uro-pathogens and patients are trying continuous alternative natural remedies such as cranberry juice, which appears to contain antiadhesive compounds that are active against uro-pathogens and can help prevent UTIs. Preventive therapies for UTIs are currently almost completely dependent on the use of antibiotics. In real terms, no true prophylaxis exists: current therapy involves long-term, low-dose antibiotic treatment, which involves the active killing of bacteria that enter the bladder. To develop a non-chemotherapeutic means to restore and maintain a healthy urogenital tract, probiotic therapy using *lactobacilli* has been considered, and there is evidence to indicate that certain strains can be effective when inserted directly into the vagina or when ascending from the rectum after oral ingestion [11] [12] [13]. Two strains, *Lactobacillus rhamnosus* and *Lactobacillus fermentum*, appear to be particularly adept at the latter [11] [12] [13] [14]. Repeated intake of probiotics could be important not only in women subjected to recurrent urogenital infections but also for all the healthy women to prevent severe infections and superinfections of their vaginal mucosa; in fact, the urogenital environment is often altered, and *lactobacilli* are decreased, thereby increasing the risk of locally acute infection [13]. Probiotics have been demonstrated to be able to protect against UTIs, maintaining the vaginal microbiome in proper balance. Researches in this field have shown that daily administration of *Lactobacillus rhamnosus* and *Lactobacillus fermentum,* specifically, can positively improve vaginal flora.

The aim of this pilot small-sized study is to investigate, in a 90days long clinical trial, whether daily administration of probiotics replacing *lactobacilli (*Hyperbiotics PRO-Women) can influence the vaginal microbiota.

Material and Methods

The present study was multicentric, entirely carried out in Italian clinics and healthcare buildings. All patients involved in this study were requested to read, understand and sign an informed consent. The study was conducted in compliance with the "Ethical principles for medical research involving human subjects" of Helsinki Declaration. Patients received a verbal description of the clinical protocol to be followed in this proposal of a clinical study.

Subjects recruited

A group of 51 patients were randomly recruited, after a careful preliminary screening for inclusion and exclusion criteria (Figure 1): patients receiving antibiotic therapy were excluded from the study.

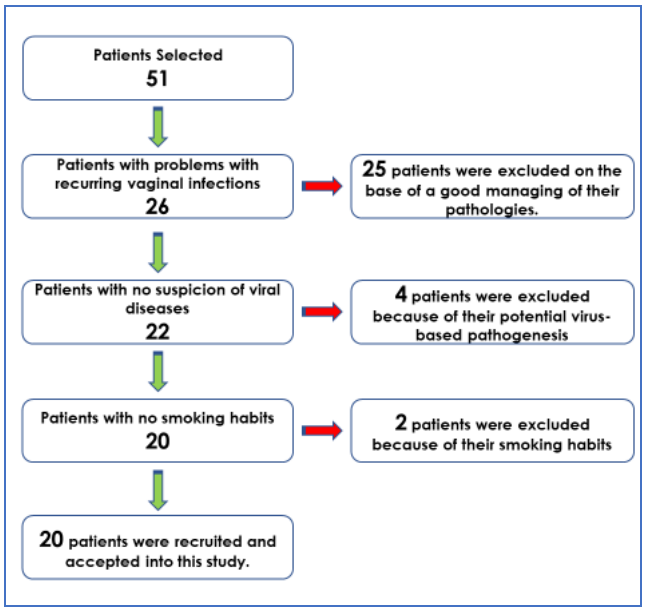

Figure 1: Patient recruitment and selection

Urine samples were analysed in private microbiological labs to assess the pathogens underlying the infections. A positive result was mainly found with specific pathogens, like *E Coli* (found in 44/51 patients) and *Candida spp* (found in 37/51 patients). Inclusion criteria were recurrent UTIs in

patients reporting dysuria, frequency, urgency, and abdominal/flank pain with or without fever. Forty (n = 40) patients diagnosed with recurrent urogenital infections were recruited in this study. Half of them has been treated as test group (n = 20), receiving Probiotics investigated in this study, and a a control group (n = 20), receiving tablets looking similar to probiotics: tablets were administered for 90 days.

Table 2: Subject Selection Criteria

Inclusion Criteria
a. Caucasian aged between 18-50 years.
b. Volunteer to participate in the study.
c. Diagnosed with recurrent urogenital infection.
Exclusion Criteria
a. Any systemic disease like liver, kidney and thyroid.
b. Recent surgical treatments.
c. Pregnant Women.
d. Smoking habits.
e. Patients with sexually transmitted viruses onset on vaginal mucosa.
f. Patients with frequently recurring complicated vaginitis with a high fever.
g. Diseases are leading to influence oxidative stress, like syndromic conditions & rheumatological diseases.
h. Subject received antibiotics for the last 3 months and/or during the study
i. Subjects received immunosuppressive drugs

Both the groups were assessed for total oxidant capacity (d-ROMs test) and biological antioxidant potential as iron-reducing activity (BAP test) at baseline, after 1 and 3 months. Histological changes on inner vaginal mucosa were also investigated, during the entire study. The investigated probiotics were administered as oral tablets, thrice a day for the first month, and one tablet per day for the remaining two months (a total of 90 days was considered enough to get significative results). Variations in tablets administration, in patients' compliance and other variations of protocol, were also recorded and reported in the final results.

Oxidative Stress Assay

The technical procedure for the evaluation of oxidative stress has been previously reported [15]. Oxidative stress assessment was performed using an integrated analytical system composed of a photometer and a mini-centrifuge (FRAS4, H&D S.R.L., Parma, Italy). Samples of whole capillary blood were taken by a finger puncture in a heparinized tube and immediately centrifuged; a small amount of samples plasma (10 µL) was thereafter tested for total oxidant capacity (d-ROMs test) and biological antioxidant potential as iron-reducing activity (BAP test) (Diacron International S.R.L., Grosseto, Italy). The d-ROMs test is based on the ability of a plasma sample to oxidise the N,N-Diethyl-p-phenylenediamine to its radical cation; the reaction is monitored photometrically at 37°C at 505 nm, and the results are expressed as Carratelli Units (CARR U, ΔAbs5050 nm/min). The oxidant capacity of plasma against N,N-Diethyl-p-phenylenediamine is mainly due to hydroperoxides, with the contribution of ferroxidase activity of ceruloplasmin and myeloperoxidase. Normal Values of d-ROMs Test range between 250 and 300 CARR Units. The BAP test is based on the ability of a plasma sample to reduce the iron of a coloured complex containing ferric ions to its ferrous derivative, not coloured; the reaction is monitored photometrically at 37°C at 505 nm, and the results are expressed in mol/L of reduced iron (using ascorbic acid as standard reference). Such biological antioxidant potential is mainly due to vitamin C, uric acid, bilirubin, albumin and tocopherols hydroperoxides. Normal values of BAP test are assessed over 2200 µM. BAP test values over 2200 µM was detected on the same machine, by using the same lot of kits; all tests were performed by the same operator in each of the sampling points, and the analytical instrumentation was calibrated before the analytical session using plasma with known values of d-ROMs and BAP test. To further check the reliability of the analysis mean, it was performed the same tests on samples of whole capillary blood taken from 5 healthy volunteers (recruited from the research staff) as a control. For each patient, a sample of capillary blood was taken up and subjected to BAP-test (normal values >2200 µM) and d-ROMs test (normal values between 250 and 300 U CARR). After 30 days (T1) and after 90 days (T2) the same assay was performed for Test-group and Control-group, as well as for healthy patients [16].

Microbiological assays

Patients of both groups were subjected to sampling in the inner part of their vaginal mucosa, for Gram staining examination. A Gram-staining system, proven to be effective in assessing the physiology of healthy vaginal flora was performed [15] [16]. Sealed packages containing the swabs, slides and transport medium for each subject were labelled and distributed by physicians to patients of each group. During the examination with a speculum, three cotton-tipped swabs of upper vaginal secretions were taken up. A first swab was rolled against a glass slide, which was sprayed immediately with cytological fixative. A second swab was placed in a modified Amies clear transport medium, and the third swab was rolled onto a glass slide which was air-dried. The air-dried slide was delivered to the laboratory within 8 hours. All slides were heat-fixed and then Gram-stained. The Gram-stained slides were prepared by expert laboratory technicians. Each of two technicians independently assessed the quality (clarity and preservation of Gram-negative and positive organisms) of each slide. The same examinations were carried out at intervals T1 and T2.

Probiotics

The probiotics used in this study are made with 6 targeted probiotic strains (*L. plantarum*, *L. fermentum*, *L. acidophilus*, *L. reuteri*, *L. rhanmnosus*, *B. bifidum*). Each probiotic tablet contains more than 5

billion Colony Forming Units. The probiotics used in this study have been created and produced to support digestive, urinary, and immune function, by combining the efficacy of cranberry extract with the clinically studied effects of naturally occurring D-Mannose. D-Mannose actively works to prevent undesirable recurrent pathologies, like bacterial infections, located in the urinary tract.

Statistical Analysis

All data were collected and statistically evaluated. T0 data were statistically compared with T1 and T2 data, using “paired t-test”.

Results

All recruited patients were compliant to this pilot study: no patients reported adverse reactions, and none dropped out of the study before the conclusion of the experimental stages. Free radical production occurs continuously in the cell during metabolism. These radicals (hydroxyl, superoxide anion, nitric oxide, etc.) are in part toxic to cell and cell membranes; however, they are normally controlled by countervailing biologic mechanisms. Severe oxidative stress produces ROS and induces uncontrolled lipid peroxidation [17]. Urinary tract infection causes oxidative stress, increases lipid peroxidation level, and leads to insufficiency of antioxidant enzymes [18]. Oxidative stress in test and control groups showed significant differences among baseline (T0) and at the end of the study, i.e. 90 Days (T2). The results showed significantly higher levels of oxidative stress at baseline in the test group compared to the control group (Table 3).

Table 3: Test group: oxidative stress values

	BAP Test (mmol/L)					d-ROM Test (U CARR)				
Group	T_0	T_1	T_2	T_0-T_1 % Variation	T_0-T_2% Variation	T_0	T_1	T_2	T_0-T_1 % Variation	T_0-T_2% Variation
Test	1629	2199	2365	34.99%	45.18%	432	358	322	-17.12%	-25.46%
Control	1645	1905	1805	15.80%	9.72%	440	450	457	2.27%	3.86%

The oxidative stress reduction was significantly lower in the test group, compared to the control group. On recruitment (T0), the mean value of d-ROMs in test and control group was 432 and 440 respectively. The first follow up (T1) result in -17.12 % decreases in d-Rom values in the test group as compared to an increase of 2.27% increase in the control group. The second follow (T2) there was further decrease in d-ROM value in the test group, i.e. total reduction of 25.46% from baseline value as compared to control group where d-ROM values further increased and reached to 3.86% enhancement from baseline to second follow up. On recruitment (T0), the mean value of BAP in test and control group was 1629 and 1645 respectively. The first follow up (T1) result in 34.99 % increases in BAP values in the test group as compared to an increase of 15.80% increase in the control group. The second follow (T2) there was further increase in BAP value in the test group, i.e. total improvement of 45.18% from baseline value as compared to control group where BAP values further decreased and hence reached to only 9.72% enhancement from baseline to second follow up. The ANOVA test showed high statistical significance ($p < 0.001$) between compared data (Figure 2 and 3). This statistically significant difference in test and control group in both d-ROM and BAP values highlights the importance of oral probiotics in improving oxidative stress level in cases of urogenital infections.

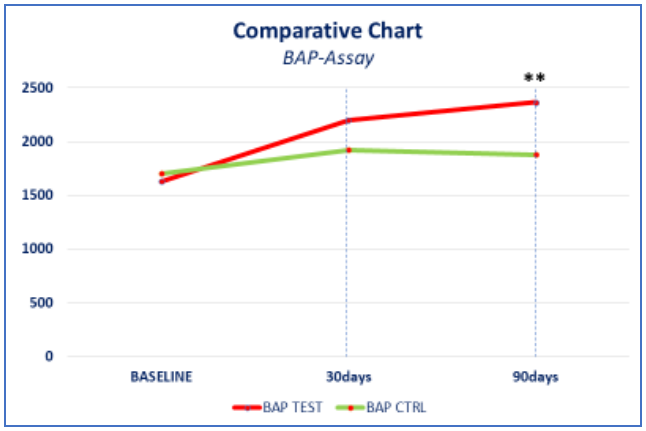

Figure 2: Comparative chart: bap-assay

Histological examinations also confirmed the positive feedback from vaginal mucosa in test-group. The mature epithelial cells of the surface are returned to be largely flattened and fused, and they show small dense pyknotic nuclei (< 4 μ). The elimination of glycogen by lactobacilli causes an acidic pH that protects against bacterial and fungal infections such as from candida albicans.

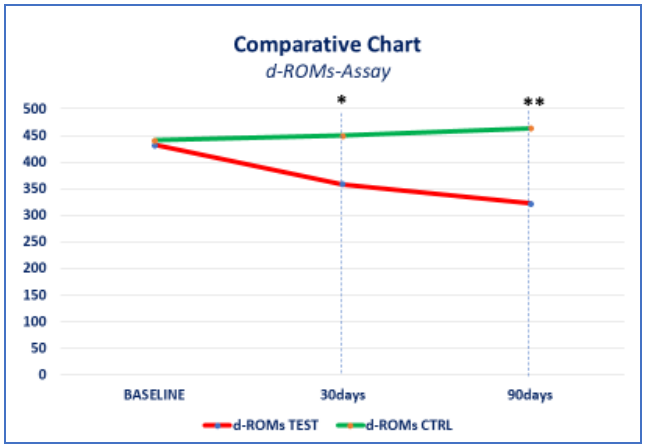

Figure 3: Comparative chart: d-ROM-Assay

Discussion

The vaginal environment has an acidic pH; this condition helps prevent the proliferation of harmful bacteria and maintains lactobacilli levels (beneficial bacteria that protect the environment). Vaginal pH balance can be influenced by some factors such as menstrual flow, antibiotic intake, excessive hygiene, and use of an IUD (intrauterine device), sperm. If the pH changes, there may be an excessive development of anaerobic organisms that replace the normal lactobacilli, causing bacterial vaginosis. Women of reproductive age, between 15 and 44 years, are the category most exposed to risk. In many cases the infection is asymptomatic, but symptoms such as light-coloured vaginal secretions, a strong odour, especially after sexual intercourse, more rarely pain, itching and burning during urination can occur. The causes of bacterial vaginosis are not fully known as their link to sexual activity has not been fully understood. However, an increased incidence of this infection was found in women with many partners. Bacterial vaginosis can be relapsing, and if it is not treated, it can give rise to complications. For example, if it arises during pregnancy, it may increase the risk of spontaneous abortion or premature birth [19].

The application of exogenous organisms, such as *lactobacilli*, to the host, is termed probiotics, which is broadly defined as a living microorganism administered to promote the health of the host by treating or preventing disease [20]. The results of this study are inconsistent with the previous studies that also advocated the beneficial effects of supplementing oral probiotics for prevention of urogenital infection [12] [20]. Study by *Anukam et al.* reported in their study an effective 90% improvement of bacterial vaginosis using probiotic *lactobacilli* [21]. In vivo study concluded by *Pascual et al* indicated that the probiotic produced both preventative and curative effects on *E. coli* growth [22]. Studies affirmed that healthy epithelial and mucosal tissues, without any oncological onset, equally in younger and older patients, are the first step towards the protective barrier made by probiotics, that has widely demonstrated to prevent colonisation by pathogenic microorganisms [23] [24]. There is good clinical evidence to show that the intestinal and urogenital microbiota have a central role in maintaining both the health and wellbeing of humans. Furthermore, the use of good bacteria to replace or augment bacterial populations is increasingly achieving scientific acceptance.

In conclusion, the role of the human microbiota in urogenital women's health has been demonstrated to influence the duration, the relapses and the severity of infective episodes. Microbiota has also been related to changes in women's physiology during pregnancy and some dysmetabolic diseases. The oxidative stress seems to have the role of a biological marker, highlighting such conditions of inflammatory stress that may lead to superinfections or histological changes. Some studies have demonstrated the positive effects of many nutraceuticals and probiotics in regulating the commensal microbiota composition and distribution: these effects reflect biology and physiology of tissues involved, thus preventing and reducing the severity of infections and related inflammatory phenomena. In our pilot study, significant improvements have been reported in test-Group, related to systemic oxidative stress (BAP/d-ROMs), confirming the positive influence of probiotics on preventing/reducing UTIs. Histological findings describing the gram-staining of the vaginal inner mucosa, before and after probiotics treatment, in test-group reported a healthy vaginal microbiota. Our results, in conclusion, in this preliminary study, limited by the small size of involved patients, suggest that a daily probiotic administration should represent a useful tool for improving the women's overall health, with a specific benefit on UTIs, with no adverse effects. This approach may provide a valuable addition to the current therapeutic options for UTIs in women also by regulating the local mucosal microbiota.

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Clinical Science

The Impact of Pursed-lips Breathing Maneuver on Cardiac, Respiratory, and Oxygenation Parameters in COPD Patients

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Abstract

BACKGROUND: Respiratory system, together with the cardiovascular and central nervous system, is responsible for all processes related to oxygenation and hemodynamics and the defect in the functioning of each of these systems, along with ageing, can have mutual effects on their performance and physiological symptoms. The use of Pursed-lips Breathing (PLB) training is an essential part of the treatment of patients with the obstructive pulmonary disease, PLB stimulates the autonomic nervous system and causes relaxation and improvement of physiological parameters.

AIM: This study was conducted to evaluate the effect of PLB on cardiac, pulmonary and oxygenation level in patients with Chronic Obstructive Pulmonary Disease (COPD).

METHODS: A three-group clinical trial study with experimental and control which was purposefully conducted with the participation of patients with COPD and healthy individuals referring to Madani hospital Khoy, in 2017. The sample size was selected to be 60 subjects. The patients were randomly allocated to two groups of intervention and control with 20 patients, and 20 healthy subjects were assigned to the healthy intervention group. The demographic, anthropometric information form and checklist recording changes in levels of oxygenation, respiration, temperature, heart rate and blood pressure with cardiopulmonary follow up in three stages before, during and after PLB were used for data collection. Data were analysed using descriptive statistics, repeated measure test, ANOVA, and Chi-square.

RESULTS: On evaluation within the COPD patient intervention group in Saturation of Peripheral Oxygen (SPO_2) index with the mean difference of 2.05 percent, Respiratory Rate(RR)-0.65 minute and Pulse Rate(PR)-1.6 bpm was significant ($p \leq 0.05$), and systolic blood pressure index in healthy subjects was increased (3.35 mmHg).

CONCLUSION: The results of this study indicated that using effective PLB as an easy, inexpensive, non- invasive and non-pharmacological method is considered as an important factor in improving the status of oxygenation and physiological indicators in patients with COPD and should be considered as an important part of rehabilitation programs for these patients.

Introduction

Chronic illness is a multidimensional health challenge with various manifestations and disabilities that the patients are in need of long-term care and education to adapt to their physiological changes [1]. COPD is a collection of physiological disorders, in which the airflow restriction is their most important characteristic. Emphysema and chronic bronchitis are included in this complex [2]. These diseases cause a wide range of pathological changes in the respiratory system, and with a gradual decrease in the air flow of exhalation, increasing dyspnea, coughing, and confusion [3]. COPD is a common progressive, preventable, therapeutic disease [4] and spirometry is the most important test for the diagnosis and determination the stage of disease, where the Forced Expiratory Volume in the second first (FEV1) is a good marker for determining the severity of the disease and the function of the lung.

According to the results of spirometry, the patients are divided into 4 types of mild, moderate, severe and very severe [5]. The importance of this disease in public health is increasing worldwide, and the increasing prevalence of chronic obstructive pulmonary disease as one of the priorities of the WHO has a significant impact on health care system [6] [7]. This illness is the fourth leading cause of death and the fifth cause of disability in the United States, and

according to Global Initiative for Chronic Obstructive Lung Disease (GOLD) estimates, it will move from the world's sixth most common cause of death to the third rank in 2020. It is estimated that about 64 million people in the world will get COPD by 2030 [5].

To assess signs and predisposing factors, consideration of the index of vital signs is the most important physiological criteria for assessing hemodynamic status [8]. The early prediction of the patient's physiological conditions based on vital signs is an important and valuable issue, the regular and continuous monitoring of it results in proper decisions and provision of necessary care to patients [9]. Along with vital signs, pulse oximetry as the sixth sign of vitality is a standard measure and reliable tool for monitoring cardiac and respiratory conditions [10]. Lung rehabilitation as part of COPD treatment aimed at relieving uncomfortable symptoms, preventing cardiovascular and respiratory complications and improving quality of life [11].

In the study conducted by Emtner, Herala, Stalenheim [12], results indicated that after the implementation of lung rehabilitation programs, the clinical status and functional tests of the lung have improved in COPD patients. The study conducted by Solanes et al., [13] showed the importance of using lung rehabilitation programs to increase activity tolerance, improving quality of life, and reducing the clinical symptoms of COPD. Although breathing exercises in the form of pursed-lip breathing may be useful to reduce the symptoms of dyspnea and improve pulmonary function and quality of life, objective evaluation based on pulse oximetry, respirogram, and arterial blood gas analysis indicates contradictory results and pursed-lip breath exercises is not considered as a major component of the lung rehabilitation program, because their usefulness is still uncertain [14] [15].

Therefore, the role and efficacy of respiration with the pursed lips has remained unclear in the rehabilitation of people with COPD. Since all patients cannot access the formal and regulated lung rehabilitation program, the nurse can play an effective role in educating and following-up of a rehabilitation program such as self-care, pursed-lips breathing, exercise, and energy conservation techniques in daily activities [1] [4]. Based on observations and clinical experience, nurses do not consider this technique as part of a complementary clinical treatment program and to improve the health of the patient.

Studies conducted in Iran regarding the lung rehabilitation program, especially the pursed-lip breathing, do not appear to be adequate in the field of nursing, this study was conducted to evaluate the effect of pursed-lips breathing on cardiac, pulmonary and oxygenation index in patients with COPD.

Material and Methods

This study was a three-group clinical trial, randomised controlled and interventional which was purposefully conducted with the participation of patients with COPD and healthy individuals referring to the spirometric unit of Madani hospital Khoy, in 2017. The content and methods of this study were approved by the Ethics Committee of the Deputy of Technology and Research of Urmia University of Medical Sciences (Approval no. 1395.438).

Informed written consent was obtained from all participants before they took part in the study. Participants were informed that they could leave the study at any time without penalty, and all personal information was kept confidential. The required sample size was selected to be 60 subjects based on the study conducted by Rossi et al., [16] with $\alpha = 0.05$, $\beta = 0.2$, the effect size of 0.17, in the three groups by using the G* Power software. Participants were randomly allocated to one of two treatment groups: posterolateral fusion with pedicle fixation or cognitive intervention and exercises. Each eligible patient was assigned an identification number by the randomisation central at the University of Bergen. The concealed random allocation was conducted by a computer-generated the random list. Blocks of 10 patients were used to ensure fairly even-numbered treatment groups.

The samples were selected purposively with the participation of 40 COPD patients. Participants were randomly allocated in two groups of 20 subjects: PLB intervention and the control group. The control group received just routine cares and drug treatments. In PLB intervention group, the patients with mild to moderate disease were selected. For data gathering, first, demographic and anthropometric information was recorded then the pulmonary function parameters, vital signs and spo2 were measured by the Italian SpiroLab MIR Maggiotiro 125 Spirometric device. The vital signs were measured and recorded in three stages, before PLB with rest and normal breathing, during PLB and after PLB with rest, within 30 minutes in two groups of COPD patients and healthy subjects. Recording the measurements in the control group was carried out in just three 10-minute periods.

To perform the PLB, the subjects were trained to breathe by relaxing the neck and shoulder muscles and breathe in the tidal volume range through the nose and count up to number 2, then close the mouth. In exhalation, she should almost press her lips and be constricting the abdominal muscles; she should slowly exhale the air in her lungs through her mouth by extending the exhalation time through the pursed lips counting from 1 to 5. The inclusion criteria for the study include the age over 40, diagnosis of COPD, stability in clinical condition, unused rehabilitation programs other than PLB, the

absence of underlying chronic illnesses (hypertension, cardiomyopathy, or diabetes) and the patient's willingness to participate in the study. SPSS version 22.0 was used for statistical data analysis. All analyses were two-tailed, and the significance level was set a 0.05. General characteristics were analysed with descriptive statistics. The difference of between groups to general characteristics and Cardiac, Respiratory, and Oxygenation Indicators were analysed with Chi-square and ANOVA or Kruskal Wallis. Repeated measure test was used to examine the influence of PLB Maneuver on Cardiac, Respiratory, and Oxygenation Indicators within groups.

Table 1: Demographic and Anthropometric Characteristics of the Studied Groups

Groups Frequency Variables		Intervention (Patient) n%	Intervention (Health) n%	Control (Patient) n%	P
Gender	Male	9 (45)	7(35)	10 (50)	0.621
	Female	11 (55)	13(65)	10 (50)	
History of Smoking	Yes	14 (70)	20(100)	16 (80)	0.035
	No	6 (30)	0(0)	4 (20)	
History of Drug use	Yes	8 (40)	20(100)	9 (45)	0.001
	No	12 (60)	0(0)	11 (55)	
History of Hospitalization	No	15 (75)	20(100)	17 (85)	0.168
	Once	1 (5)	0(0)	0 (0)	
	Twice and more	4 (20)	0(0)	3 (15)	
Groups Statistical Indicator Variables		Intervention (COPD) M ± SD	Intervention (Healthy) M ± SD	Control (COPD) M ± SD	F	P
Age (Year)		60.65 ± 12.80	38.80 ± 10.85	61.85 ± 13.38	21.93	*0.001*
Duration of COPD (Month)		48.27 ± 52.44	0.0 ± 0.0	205.71 ± 83.84	29.33	*0.001*
Weight (Kg)		72.37 ± 17.82	73.35 ± 11.73	73.55 ± 16.50	0.03	*0.968*
Height (cm)		159.45 ± 10.90	164.70 ± 10.27	163.95 ± 10.31	1.46	*0.240*
BMI (kg/m2)		28.45 ± 7.20	27.36 ± 4.34	27.34 ± 5.65	0.23	*0.792*
BSA (m2)		1.75 ± 0.203	1.80 ± 0.17	1.79 ± 0.224	0.32	*0.731*

Results

Based on the pulmonary function indexes, the highest mean of Forced Volume Capacity (FVC) (4.41 ± 1.31 L), FEV1 (3.39 ± 0.97 L), FEV (70.36 ± 15.3%), FEV/FVC (85.3 ± 6.8 %) were in the control group of healthy subjects (Table 2).

Table 2: Mean, Standard Deviation and Variance Analysis within Group of Pulmonary Function Indexes in Three Group of COPD Patients and Healthy Subjects

Groups Statistical Indicator Variables	Intervention (COPD) M ± SD	Intervention (Healthy) M ± SD	Control (COPD) M ± SD	F	P
FVC (L)	2.64 ± 1.025	4.41 ± 1.31	3.11 ± 1.35	11.09	*0.001*
FEV1 (L)	1.94 ± 0.85	3.39 ± 0.97	2.02 ± 0.94	15.71	*0.001*
FEV (%)	65.10 ± 21.92	70.36 ± 15.45	59.98 ± 13.15	1.81	*0.173*
FEV/ FVC (%) Predicted	71.57 ± 16.27	85.32 ± 6.89	64.16 ± 13.19	5.37	*0.007*
VC (L)	2.79 ± 1.53	3.87 ± 1.43	2.80 ± 1.16	4.01	*0.023*
PEF (L/s)	3.52 ± 1.55	6.73 ± 2.18	3.85 ± 2.07	16.27	*0.001*
PEF2575 (L/s)	1.70 ± 0.10	3.08 ± 1.095	1.38 ± 0.77	17.57	*0.001*

Comparing between the groups in the SPO2 index the highest mean with 96.9 ± 1.2 per cent was increased in the healthy group after the intervention of the pursed lips breathing. Pulse Rate at the time of pursed-lip breathing was decreased than before in intervention groups. There was a significant difference within groups in the three stages before, during and after PLB in comparison with Blood Pressure Systole (BPS), Blood Pressure Diastole (BPD) and Arterial Mean Pressure (AMP) that were decreased in three stages.

Table 3: Mean, Standard Deviation and Variance Analysis between Group of Oxygenation and Cardiopulmonary Parameters, Before, During and After PLB in the Studied Groups

Groups Statistical Indicator Variables		Intervention (COPD)	Intervention (Healthy)	Control (COPD)	F	P
SPO2 %	Pre	92.10 ± 3.76	94.05 ± 2.41	91.75 ± 4.98	2.05	*0.138*
	Inter	94.15 ± 5.23	95.75 ± 1.68	92.5 ± 4.92	2.91	*0.043*
	Post	93.25 ± 4.81	96.90 ± 1.20	93,05 ± 5.53	5.10	*0.009*
RR min	Pre	20.15 ± 1.92	20.45 ± 1.76	21.90 ± 1.71	5.08	*0.093*
	Inter	19.50 ± 1.87	19.20 ± 1.03	20.85 ± 1.75	3.35	*0.042*
	Post	19.65 ± 1.53	19.75 ± 1.10	20.60 ± 1.67	7.98	*0.001*
T °C	Pre	37.06 ± 0.23	37.13 ± 0.23	37.07 ± 0.16	0.65	*0.528*
	Inter	36.96 ± 0.18	37.11 ± 0.23	37.08 ± 0.23	2.68	*0.077*
	Post	36.97 ± 0.16	37.13 ± 0.23	37.08 ± 0.20	3.09	*0.053*
PR	Pre	90.75 ± 14.70	84.10 ± 11.23	82.50 ± 12.37	2.32	*0.108*
BPM	Inter	89.15 ± 14.34	80.55 ± 12.75	80.55 ± 12.13	2.87	*0.053*
	Post	90.25 ± 15.32	85.35 ± 14.16	84.70 ± 13.47	0.89	*0.414*
BPD	Pre	121.50 ± 12.89	121.50 ± 14.34	134.75 ± 11.18	7.07	*0.002*
mmHg	Inter	119.12 ± 12.70	124.85 ± 14.79	133.65 ± 9.69	6.78	*0.002*
	Post	117.75 ± 12.62	121.15 ± 12.61	133.07 ± 10.14	9.23	*0.001*
BPD	Pre	78.75 ± 10.87	78.50 ± 13.09	89.75 ± 7.16	7.27	*0.002*
mmHg	Inter	76.50 ± 8.90	78.25 ± 12.28	88.62 ± 6.75	9.35	*0.001*
	Post	76.75 ± 9.77	77.75 ± 10.94	87.40 ± 7.75	7.62	*0.001*
AMP	Pre	92.99 ± 11.33	92.83 ± 13.38	104.75 ± 7.75	7.62	*0.001*
mmHg	Inter	90.70 ± 9.99	93.78 ± 12.74	103.68 ± 7.44	8.69	*0.001*
	Post	90.91 ± 9.98	92.21 ± 11.33	102.62 ± 8.07	8.43	*0.001*
RPP	Pre	10960.25 ± 2292.15	9771.75 ± 1825.98	10788 ± 1300.15	2.41	*0.099*
mmHg/min	Inter	10729.40 ± 2155.53	10505.55 ± 1916.30	11014.85 ± 1472.089	0.37	*0.690*
	Post	10744.50 ± 2445.67	15620.90 ± 27033.66	11241.70 ± 1852.87	0.58	*0.561*

Saturation of Peripheral Oxygen (SPO2); Respiratory Rate (RR); Temperature (T); Pulse Rate (PR); Blood Pressure Systole (BPS); Blood Pressure Diastole (BPD); Arterial Mean Pressure (AMP); Rate Pressure Product (RPP).

In evaluation within COPD patient intervention groups with repeated measure test in spo2 with a mean difference of 2.05 RR -0.65 and PR 1.6, there was a significant statistical difference (Table 4).

Table 4: Mean, standard deviation and variance analysis within the group of oxygenation and cardiopulmonary parameters, before, during and after PLB in the intervention COPD group

Stage Statistical Indicator Variables	Pre (PLB) M ± SD	Inter(PLB) M ± SD	Post (PLB) M ± SD	Repeat Measure	*P*
SPO2%	92.10 ± 3.77	94.15 ± 5.23	93.25 ± 4.81	F(2) 4.47	*0.018*
RR min	20.15 ± 1.92	19.50 ± 1.87	19.65 ± 1.53	F(2) 0.91	*0.049*
T °C	37.07 ± 0.23	36.96 ± 0.18	36.97 ± 0.16	F(1.26) 0.28	*0.099*
PR bpm	90.75 ± 14.70	89.15 ± 14.34	90.25 ± 15.32	F(2) 0.37	*0.054*
BPS mmHg	121.5 ± 12.88	119.12 ± 12.70	117.75 ± 12.61	F(2) 2.36	*0.108*
BPD mmHg	78.75 ± 10.87	76.50 ± 8.90	76.75 ± 9.77	F(2) 1.56	*0.310*
AMP mmHg	92.99 ± 11.33	90.70 ± 9.99	90.90 ± 9.98	F(1.17) 1.40	*0.259*
RPP mmHg/min	10960.25 ± 2292.15	10729.40 ± 2155.35	10744.50 ± 2445.67	F (2) 0.37	*0.691*

Saturation of Peripheral Oxygen (SPO2); Respiratory Rate (RR); Pulse Rate (PR); Temperature (T); Blood Pressure Systole (BPS); Blood Pressure Diastole (BPD); Arterial Mean Pressure (AMP); Rate Pressure Product (RPP).

In the intervention group of healthy subjects, there is a significant difference within the group in evaluation spo2 1.7, respiratory rate -1.20, heart rate 3.55, and systolic blood pressure 3.35 (Table 5) ($p \leq 0.05$).

Discussion

The respiratory system plays a crucial and determining role in maintaining and sustaining vital human processes. This system, together with the

cardiovascular and central nervous system, is responsible for all processes related to oxygenation and hemodynamics and the defect in the functioning of each of these systems, along with ageing, can have mutual effects on their performance and physiological symptoms [5, 17, 18].

Table 5: Mean, Standard Deviation and Variance Analysis within Group of Oxygenation and Cardio- Pulmonary Parameters, Before, During and After PLB in the Healthy Intervention Group

Stage Statistical Indicator Variables	Pre (PLB) M ± SD	Inter (PLB) M ± SD	Post (PLB) M ± SD	Repeat Measure	*P*
SPO2%	94.05 ± 2.41	95.75 ± 1.68	96.90 ± 1.20	F(2) 6.09	*0.001*
RR min	20.45 ± 1.76	19.20 ± 1.03	19.70 ± 1.10	F(2) 0.85	*0.342*
T c0	37.135 ± 0.23	37.115 ± 0.23	37.130 ± 0.23	F(2) 677	*0.677*
PR bpm	84.10 ± 11.23	80.55 ± 12.75	85.35 ± 14.16	F(2) 3.40	*0.044*
BPS mmHg	121.50 ± 14.33	124.85 ± 14.79	121.15 ± 12.61	F(2) 3.47	*0.041*
BPD mmHg	78.50 ± 13.09	78.25 ± 12.27	77.75 ± 10.94	F(2) 0.16	*0.856*
MAP mmHg	92.83 ± 13.38	93.78 ± 12.74	92.21 ± 11.33	F(2) 0.80	*0.458*
RPP mmHg/min	9771.75 ± 1825.98	10505.55 ± 1916.30	15620.90 ± 27033.66	F(1.01) 0.87	*0.363*

Saturation of Peripheral Oxygen (SPO2); Respiratory Rate (RR); Pulse Rate (PR); Temperature (T); Blood Pressure Systole (BPS); Blood Pressure Diastole (BPD); Arterial Mean Pressure (AMP); Rate Pressure Product (RPP).

In this study, smoking history in the intervention and control group was 30% and 20% respectively, and the healthy group did not have a history of smoking. In the study conducted by Izadi, Afshar, Adib-Hajbaghery [19], 56.3% of COPD patients were smokers. In the study of Wade [4], cigarette smoking was the main cause of disease and quitting was regarded as an essential step in controlling COPD. Comparing the mean FVC index in the patient intervention group with an average of 2.64 litres in comparison to the control group (3.11 litres) and healthy subjects (4.41 litres), the results were indicating a high intensity of shortness of breath in the intervention group. In the patient intervention group, the mean FEV1 was 65 ± 10%, and FEV1 was 1.94 ± 0.85 litres, which was matched with the study conducted by Ramos (FEV1 60 ± 25%, FEV1 1.53 ± 0.60 litres) [20]. In the FEV1/FVC index in the COPD intervention group with a mean of 71.57 ± 16.27%, the severity of the disease was less than the control group (64.16 ± 13.19 %) that indicating the presence of patients with stable status in this study.

In the study conducted by Wade [4], before pursed-lip breathing, the FEV1 was 2.29 ± 0.58, Peak Expiratory Flow (PEF) 459 ± 198, and FVC 3.22 ± 0.53. In the Spo2 evaluation, a significant difference was observed within the groups, during and after the pursed-lip breathing and this improvement was observed due to PLB with a proportional increase of 2.05 per cent in the patient intervention group, healthy subjects (1.7%) and control group (0.75%). After the intervention and the 10-minute interval of rest, recovery was continued in the healthy intervention group (1.15%) and control group (0.55%), but in the patient intervention group, the patient experienced a decrease of (-0.9%), which was probably due to fatigue and weakness caused by the disease and inability to use of respiratory muscles or the immediate effects of the PLB intervention. Evaluation in the within groups with repeated measure test and follow-up LSD, there was a statistically significant difference only before and during the pursed-lip breathing, in Spo2 which indicates an improvement in the oxygenation state by intervention pursed-lip breathing.

According to the study conducted by Solomon [21], a statistically significant difference was found with a mean of 1.67 ± 1.35 in the pursed-lip intervention group in spo2 improvement. In the study conducted by Ramos et al., [20], showed a significant increase in SPO2 than before and after PLB, which is consistent with the findings of the study. The use of PLB training with oxygen therapy is an essential part of the treatment of patients with the obstructive pulmonary disease, and it is necessary whenever arterial oxygen saturation reaches less than 90 per cent [1]. There was a significant difference in the respiratory rate of the patient intervention group and healthy subjects in both stages during and after pursed-lip breathing. With comparison within-groups in the patient intervention group, a decrease of -0.65 and a decrease of -1.25 in healthy individuals during the pursed-lip breathing was observed in respiratory rate, which indicates a decrease in the number of respirations and increases in respiration depth by application of PLB technique. In the study conducted by Robert et al., [22], it was indicated that PLB decreases RR and increase in SpO2 and the use of PLB relief to dyspnea, increase in self-esteem, and reduced fear especially at night. The decrease respiratory rate in the PLB is probably due to an increase the Resistance air flow during exhalation and the use of muscles resulting from increased in tidal volume, improved gas exchanges and respiratory sufficiency [23]. In pulse rate index, there was a significant difference between groups during pursed-lip breathing. With comparing within groups, this difference before and during the PLB in the intervention group of patients was -1.60 bpm and -3.55 in the group of healthy subjects, which had a significant difference in the stage, before, during and after PLB. Therefore, PLB caused a decrease in heart rate, and this decrease was higher in healthy subjects. This impact is probably due to the stimulation of the autonomic nervous system and parasympathetic activity [24] [16]. The stimulation of vagus nerve causes relaxation and improvement of physiological parameters [25].

Similar to the findings of this study, in the study conducted by Solomon [21], heartbeat difference was reported in the intervention group of the PLB with the Mean and SD (-9.12 ± 6.20). In a study conducted by Silva et al. [26] on 18 patients with COPD there were no significant correlations between using PLB in four activities of walking on the treadmill, wearing shoes, lifting cauldron and taking a shower without using of PLB in Inspiratory Capacity (IC), SPO2, HR,

RR indexes. It seems that in PLB, the exhalation time is twice and longer than the inhalation, so it often results in a decrease in the heartbeat. In comparison between the groups, there was a significant difference in systolic, diastolic and mean arterial pressure before, during and after PLB, which was mainly due to high blood pressure in patients with COPD in the control group. In the healthy intervention group, the difference (3.35 mmHg) in comparing the systolic blood pressure was significant with pursed-lip breathing, and PLB resulted in an increase in systolic blood pressure, possibly due to the excitement and stress caused by spirometric results and stimulating the carotid receptors, which leads to an increase in systolic blood pressure, after the intervention. In the study conducted by Ramos et al., [20], no significant changes were observed in BP by doing pursed-lip breathing.

In the study conducted by Maind et al., [27] the systolic blood pressure before pursed-lip breathing was 144.32 ± 10.80 and after pursed-lip breathing 149.89 ± 8.08 ($P < 0.015$) and diastolic blood pressure changed from 77.35 ± 5.45 to 77.62 ± 5.47, respectively that is consistent with this study. Variation in BP can be due to changes in the chest compression due to respiratory movements, which compensate for the increase or decrease in systolic blood pressure fluctuations [5] [28]. The number and rhythm of respiration not only affects the respiratory system but also has direct effects on the cardiovascular system. It may be possible to adjust the blood pressure and pulse fluctuations and prevent cardiovascular complications through breathing PLB exercises [29]. The limitations of this study were reluctance some of the sampled subjects, especially healthy individuals to collaborate in our study, with sufficient descriptions of their satisfaction for participation. Also, the effects of acute and short-term PLB in 30 minutes were evaluated, which may be determined in the long-term phase with strengthening muscles and respiratory training. Therefore such research is recommended to be evaluated over a long time span.

In conclusion, the results of this study indicated that the pursed-lip breathing manoeuvre in comparison to normal breathing has an improving effect on the level of oxygenation. It can lead to significant positive changes in respiratory and cardiac parameters in COPD patients. Therefore, PLB as an easy, inexpensive, non- invasive and non-pharmacological method is considered as an important factor in improving the status of oxygenation and physiological indicators in patients with COPD and should be considered as an important part of rehabilitation programs for these patients.

Training pursed-lip breathing should be considered as nursing standards in nursing care so that patients at home can have beneficial effects from the PLB whether they are in a family-supported program and during follow-up of caregivers. In the education program of nursing student's, the importance of PLB in lung rehabilitation should be considered in teaching the theoretical classes and practice with implement nursing process.

Training pursed-lip breathing should be considered as nursing standards in nursing care so that patients at home can have beneficial effects from the PLB.

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Clinical Science

Comparison of the Effects of Omega 3 and Vitamin E on Palcitaxel-Induced Peripheral Neuropathy

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Abstract

BACKGROUND: Paclitaxel-induced peripheral neuropathy is the most important side effect limiting the use of this medication.

AIM: This study aimed to compare the effects of omega-3 and vitamin E on the incidence of peripheral neuropathy in patients receiving Taxol.

METHODS: In this clinical trial, 63 patients who were a candidate for receiving taxol, were enrolled based on inclusion and exclusion criteria. In group O, patients received 640 mg omega-3 three times a day, and group E, received 300 mg vitamin E two times a day. Patients took the supplements up to three months after the onset of Taxol. Group P received placebo for a similar period. All patients referred to a neurologist for electrophysiological evaluation before the onset of chemotherapy and at months 1 and 3. The presence of neuropathy and its progression was recorded by the neurologist.

RESULTS: Neurological examination in this study indicated that 6 patients (28.6%) in Group O, 7 patients (33.3%) in group E, and 15 patients (71.4%) in placebo group started peripheral neuropathy. There was a significant difference between intervention groups and the placebo group ($p = 0.0001$) and no significant difference between intervention groups ($p = 0.751$).

CONCLUSION: Our data suggested that vitamin E and omega-3 may significantly reduce the incidence of Paclitaxel-induced peripheral neuropathy. Routine administration of such supplements that have no special side effect for patients under chemotherapy may greatly enhance their quality of life.

Introduction

Taxol is one of the taxane-derived chemotherapeutic agents used to treat solid tumours such as breast, ovary, lung and Kaposi's sarcoma. The source of this drug is a plant called Taxus brevifolia, a native of North America. Taxol is anti microtubular agent that causes polymerisation of the tubulin, and it also leads to the formation of stable microtubules without dynamic instability [1] [2]. Peripheral neuropathy is likely to be the major side effect of Taxol. The exact pathology of this complication is still not well known. Due to the accumulation of microtubules in axons and Schwann cells, Taxol causes an axonal sensory peripheral neuropathy. This drug, even in more severe cases, may also cause fibre demyelination [1]. It usually takes three weeks to develop toxic, toxic signs of Taxol, which affects the sensory nervous system more than the autonomic nervous system (ANS). The most common symptoms of taxol due to neuropathy include anaesthesia, paresthesia and burning pain in the gloves. The onset of symptoms often occurs in the hands and feet simultaneously, and some patients also complain of discomfort [3] [4].

Omega-3 fatty acids, such as EPA and DHA, are unsaturated fatty acids present in phospholipid membranes of cells, including central and peripheral nervous system cells [5]. They have very beneficial effects in some psychiatric and neurodegenerative diseases. They play a decisive role in the biophysical properties of neuronal membranes and modulate the signal transduction by effects on ion channels and receptor function [6]. In addition, the production of

anti-inflammatory cytokines that cause neuropathy is reduced by these fatty acids, especially DHA [5] [6]. DHA-linked myelogenesis has been proven in recent studies [7]. Vitamin E is widely used to prevent diseases caused by oxidative stress. Its vital effects on neuronal function have been indicated in many diseases of vitamin E deficiency and central and peripheral nervous system manifestations. Studies have shown that neuropathic effects of cisplatin may have neuroprotective effects [8] [9]. Another study found that vitamin E was effectively and safely protected against neuropathy in cancer patients treated with taxol [10]. Most side effects of chemotherapy are eliminated after treatment discontinuation, but peripheral neuropathy may be somewhat reversible or irreversible in some patients, which can have adverse effects on the patient's quality of life. Therefore, this study aimed to compare the effect of omega-3 and vitamin E on the incidence of peripheral neuropathy in taxol recipients.

Material and Methods

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards, and was approved by Arak University of Medical Sciences Institutional Review Board (Protocol number IR.ARAKMU.REC.2015.219; November 23, 2015).

In this clinical trial study, 63 patients aged 30-70 years who received taxol from Ayatollah Khansari Hospital in Arak city were included in the study. Inclusion criteria included all patients with a solid tumour (lung, breast, ovaries, etc.), patients with a good liver and kidney status that received the first taxol treatment.

Exclusion criteria include patients with previous history of chemotherapy, the presence of neuropathy due to diabetes, AIDS, alcohol abuse, thyroid dysfunction and hereditary neuropathy, the use of any dietary supplement (fish oil, vitamins and minerals) at least three months before entering the study, smoking and drugs use, low back pain and the use of other chemotherapy drugs.

Blood samples were taken from patients for liver and kidney function, and BUN, creatinine, ALT and AST values were measured. Patient's history of treatment was also fully completed. Then, the patients were randomly divided into three equal groups according to the number table. In the first group (group O), patients received omega-3 capsules of 640 mg three times a day while receiving taxol (11), the second group (group E) received vitamin E supplements at a dose 300 mg twice daily (10). The duration of use of omega-3 and vitamin E was determined from the time of receipt of taxol to three months after its discontinuation. In the third group (group P), patients also used placebo capsules for the same period.

Before chemotherapy and one and three months later, all patients were examined by a neurologist who is responsible for the clinical and electrophysiological evaluation of the patients. Then the presence of neuropathy was recorded by the specialist. The person who registered the information was not aware of the patient's group, and the patients were not aware of their position in the relevant groups considering the medical ethics conditions.

After completing the checklist, data were entered into the SPSS software version 20. The analytical analysis was performed with appropriate methods such as independent T-test or nonparametric methods to test the difference in mean in different groups. Paired t-test was used to compare the quantitative parameters at the beginning of the study and after 4 months of treatment, while the qualitative data were analysed using the chi-square test.

Results

In this study, 69 patients with different types of cancers were studied. Six patients were excluded from the study due to discontinuation of the drug. Finally, 63 patients were evaluated in three groups. In the omega-3 group, 15 women (71.4%) and 6 men (28.6%) were present. In the vitamin E group, 16 women (76.2%) and 5 men (23.8%) were admitted, while 15 (71.4%) and 6 men (28.6%) were presented in the control group. Data analysis using chi-square test depicted that there was no significant difference between the three groups regarding gender ($p = 0.886$), (Figure 1).

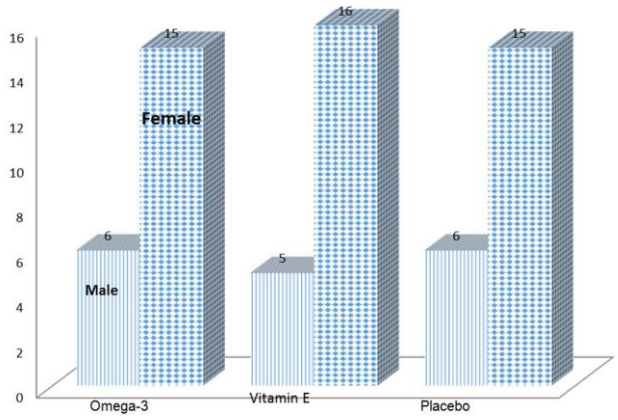

Figure 1: Frequency of sex in three groups

The mean age of patients in the group O was 51.5 ± 9.2 years, followed by 50.9 ± 10.4 years in the group E and 52.2 ± 10.1 years in the group P. The t-test revealed no significant difference between the age of three groups (p = 0.664), (Figure 2).

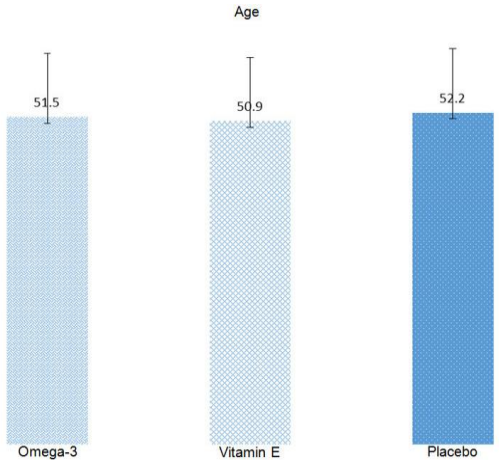

Figure 2: The mean age (± standard deviation) of patients in three groups of malignancy

The mean age (± standard deviation) of patients was shown in figure 2. Accordingly, 17 patients (27.0%) suffered from various types of lung cancer, followed by patients with breast cancer (57.1%, 36 individuals) and ovarian cancer (15.9%, 10 patients).

In the omega-3 group, 11 patients (52.4%) had breast cancer, followed by lung cancer (28.6%, 6 cases) and ovarian cancer (19%, 4 cases).

In the vitamin E group, 12 (57.1%) had breast cancer, followed by lung cancer (23.8%, 5 cases) and ovarian cancer (19%, 4 cases). In the placebo group, 13 (61.9%) had breast cancer, followed by lung cancer (28.6%, 6 cases) and ovarian cancer (9.5%, 2 cases), (Figure 3, and Table 1). Chi-square test exhibited no significant difference in the type of malignancy between the groups (p = 0.448).

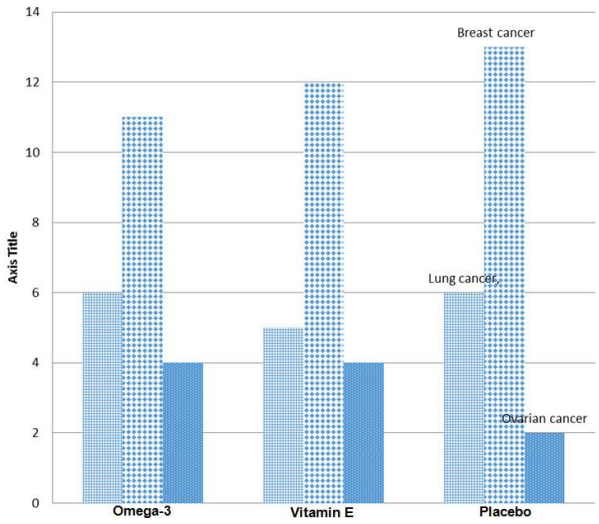

Figure 3: Malignancy in three groups

Table 1: Demographic and baseline variables in three groups

	Omega-3	Vitamin E	Placebo	P-value
Gender (female)	15 (%71.4)	16 (%76.2)	15 (%71.4)	0.861
Age (year)	5.51±2.9	9.50±4.10	2.52±1.10	0.664
Body mass index (kg / m2)	3.24±7.5	8.24±2.6	2.25±4.6	0.780
The type of malignancy				
Lung cancer	6 (%28.6)	5 (%23.8)	6 (%28.6)	
Breast cancer	11 (%52.4)	12 (%57.1)	13 (%61.9)	0.482
Ovarian cancer	4 (19.0)	4 (19.0)	2 (%9.5)	

There was no significant difference between the groups receiving the drug and the placebo group (p = 0.0001). However, the difference between the two groups of omega 3 and vitamin E was not significant (p = 0.751). Figure 4 shows the incidence and severity of peripheral neuropathy among the patients.

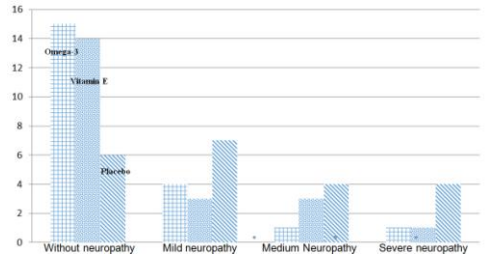

Figure 4: The incidence and severity of peripheral neuropathy in the three groups

In the present study, the peripheral nerves were examined electrophysiologically. To evaluate motor neurons, two ulnar and peroneal nerves were evaluated, and the sural and ulnar nerves were assessed for sensory function.

Table 2: Overall incidence and severity of peripheral neuropathy in patients in three groups

	Without neuropathy	Mild neuropathy	Medium Neuropathy	Severe neuropathy
Omega-3	15 (%71.4)	4 (%19)	1 (%4.8)	1 (%4.8)
Vitamin E	14 (%66.7)	3 (%14.3)	3 (%14.3)	1 (%4.8)
Placebo	6 (%28.6)	7 (33.3)	4 (%19)	4 (%19)

In these studies, motor delay (DL), amplitude (Amp), conduction velocity (CV) was measured. Electrophysiological variables of motor neurons and sensory nerves were indicated in Table 3 and 4.

Table 3: Electrophysiological variables of motor neurons during study period

	Group	Before chemotherapy	One month later	Three months later	P-value
Ulnar nerve					
DL (ms)	Omega-3	84.2	94.2	17.3	
	Vitamin E	81.2	98.2	16.3	0.720
	Placebo	77.2	14.3	26.3	
CV (m/s)	Omega-3	12.49	34.49	21.50	
	Vitamin E	14.49	37.49	28.50	0.431
	Placebo	09.49	42.49	11.50	
Amp (mV)	Omega-3	11.6	01.7	55.7	
	Vitamin E	05.6	97.6	49.7	0.446
	Placebo	14.6	01.7	87.7	
Peroneal nerve					
DL (ms)	Omega-3	31.6	43.6	57.6	
	Vitamin E	39.6	46.6	58.6	0.375
	Placebo	32.6	47.6	68.6	
CV (m/s)	Omega-3	35.44	84.44	51.45	
	Vitamin E	32.44	87.44	32.45	0.694
	Placebo	24.44	80.44	11.45	
Amp (mV)	Omega-3	16.5	31.5	71.5	
	Vitamin E	24.5	35.5	80.5	0.091
	Placebo	28.5	37.5	66.5	

Discussion

The results of this study clearly showed that the use of omega-3 or vitamin E-d supplements significantly reduced the incidence of peripheral neuropathy compared with placebo. However, there was no significant difference in the rate and severity of neuropathy in the two groups, and omega-3 and vitamin E did not have a significant superiority in this regard. Chemotherapy-induced peripheral neuropathy (CIPN) is a major clinical problem, which causes one of the dose-limiting side effects of some commonly used anti-neoplasms, such as taxol, cisplatin, and Vinca alkaloids.

Table 4: Electrophysiological variables of sensory nerves during study period

	Group	Before chemotherapy	One month later	Three months later	P-value
Sural nerve					
DL (ms)	Omega-3	31.4	94.3	87.3	
	Vitamin E	27.4	98.3	86.3	0.720
	Placebo	28.4	14.4	68.3	
CV (m/s)	Omega-3	24.40	31.40	29.41	
	Vitamin E	27.40	34.40	32.41	0.341
	Placebo	16.40	35.40	41.41	
Amp (mV)	Omega-3	17.6	38.6	84.6	
	Vitamin E	19.6	46.6	81.6	0.446
	Placebo	26.6	52.6	98.6	
Ulnar nerve					
DL (ms)	Omega-3	42.2	53.2	76.2	
	Vitamin E	46.2	58.2	83.2	0.375
	Placebo	53.2	63.2	95.2	
CV (m/s)	Omega-3	67.50	33.50	87.49	
	Vitamin E	84.50	41.50	84.49	0.694
	Placebo	73.50	20.50	31.49	
Amp (mV)	Omega-3	59.8	16.8	64.7	
	Vitamin E	67.8	14.8	54.7	0.091
	Placebo	64.8	02.8	13.7	

However, its onset may also affect the unpleasant quality of life in cancer patients, when CIPN is not a dose-limiting complication and causes chronic complications in these patients [12]. Studies show that 60-70% of patients receiving chemotherapy drugs are dose-dependent neurotoxicants [4]. Several studies have been conducted to reduce the incidence and severity of CIPN, but most of these studies have contradictory results. To best of our knowledge, no clinical study has compared the effects of omega-3 fatty acids and vitamin E on the incidence of chemotherapy-induced neuropathy.

On the other hand, until the completion of this study, only a few studies examined the effect of omega-3 on neuropathy. Meanwhile, Weymouth et al. only examined the role of omega-3 polyunsaturated fatty acids on diabetic neuropathy, which showed positive effects of omega-3 [13]. Another study specifically designed to evaluate the role of omega-3 fatty acids in the prevention of taxol-induced peripheral neuropathy [11]. The results of the aforementioned clinical trial suggested that 30% of patients receiving Omega-3 and 59.3% of patients in the control group exhibited peripheral neuropathy. These findings are consistent with our study because only 28.6% of Omega-3-receiving patients had peripheral neuropathy in the present study.

The findings of our study are consistent with previous studies that have examined the efficacy of fatty acids in diabetic neuropathy. These studies have shown that omega-3 fatty acids can reduce the severity of neuropathy in patients with type 2 diabetes [14] [15]. These micronutrients also reduced Na +/K + ATPase activity by reducing the neuronal conduction velocity in the sciatic nerve of the rats [16]. In a few pilot studies, vitamin E has been introduced as a neuroprotective agent [17] [18]. In a clinical study, patients undergoing paclitaxel-based chemotherapy received either chemotherapy with vitamin E [10]. The results of this Phase II clinical trial revealed that vitamin E, effectively and safely, could protect patients with cancer from paclitaxel-induced peripheral nerve damage.

Kottschade et al. demonstrated that vitamin E did not change the incidence of neuropathy in these patients [19]. In a meta-analysis study published by Eum and colleagues, it has been shown that daily intake of 600-300 mg of vitamin E has significant effects on the reduction of neuropathy induced by chemotherapy [20]. On the other hand, there are studies that violate the positive effects of this vitamin in improving neuropathy. Huang et al., in a meta-analysis study, believed that vitamin E could not reduce the incidence of CIPN [21]. They also said that a large sample size is needed to prove the effectiveness of this vitamin.

In conclusion, taken together, our findings suggest that the use of food supplements such as vitamin E and omega-3s may significantly reduce the paclitaxel-induced neuropathy. The routine use of such supplements, which do not add to certain side effects for patients undergoing chemotherapy, can greatly increase their quality of life.

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Clinical Science

Comparison of Dexmedetomidine and Fentanyl as an Adjuvant to Lidocaine 5% for Spinal Anesthesia in Women Candidate for Elective Caesarean

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Keywords: Dexmedetomidine; Fentanyl; Pain; Spinal Anesthesia; Lidocaine

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Abstract

AIM: This study aimed to compare the effect of Dexmedetomidine and fentanyl as an adjuvant to lidocaine 5% in spinal anaesthesia to increase post-operative analgesia among women candidates for elective caesarean.

METHODS: Eighty-four pregnant women candidates for caesarian were randomly divided into fentanyl and Dexmedetomidine groups. In the first group, 25 µg fentanyl was added to lidocaine 5% while in the second group, 0.5 µg per kilogram Dexmedetomidine was added to lidocaine 5%. After the operation, a pain score of the patients in recovery and within 4, 12 and 24 hours after the operation, the average length of analgesia and the average amount of the analgesics taken within 24 hours and after the operation were recorded.

RESULTS: The average length of postoperative anaesthesia and the average amount of the drug taken within the first 24 hours after the operation in fentanyl group was more than the Dexmedetomidine group (P = 0.01). Shivering in Dexmedetomidine group was more common than what was observed in the fentanyl group (P = 0.001). Higher rates of nausea-vomiting were observed in the fentanyl group (P = 0.001).

CONCLUSIONS: Fentanyl results in a longer period of postoperative analgesia and less consumption of drugs after the operation. Fentanyl is recommended in caesarian.

Introduction

The rate of caesarian operations has experienced a dramatic rise in developed and developing countries within the last 30 years [1]. Caesarian usually causes moderate to severe pain for 48 hours and requires favourable pain management after the operation that both enables the mother to move faster and plays a key role in post-caesarian rehabilitation [2]. Post-caesarian pain control enhances the mother's satisfaction and milking capacity, because, insufficient analgesia increases the level of plasma catecholamine [3]. Proper analgesia after caesarian helps the mother feel more comfortable and enhances her movement and ability to take better care of her neonatal [4]. Spinal anaesthesia is one of the most stable methods of anaesthesia in lower abdominal surgeries [5] and reduces the volume of blood lost during the operation and prevents Thromboembolic events after operation [6].

It is widely used for caesarian operations as it is safer for the mother, more useful for the fetus and results in greater satisfaction [7]. In spinal anaesthesia, various adjutants are used with local anaesthetics that prevent somatic and visceral pain and providing lasting postoperative analgesia [8].

It has been proved that spinal Opioids and local anaesthetics operate synergistically in the spinal level in the studies conducted on animals [9]. Adding

opioids may have unfavourable side effects such as respiratory depression, itching, nausea and vomiting [6]. Respiratory depression is the side effect of opioids which have the greatest fear for anaesthetists [10]. Fentanyl is structurally similar to local anaesthetics, and it has a local anaesthetic effect on primary C sensory afferents that facilitate analgesic effects. However, useful analgesia needs to be moderated based upon the maternal and fetal side effects like bradycardia, respiratory depression, hypotension, nausea, vomiting and itching [3] [8]. Dexmedetomidine is an elective alpha-two agonist that induces analgesia, tranquillity, and forgetfulness without depressing respiratory performance [11]. As it influences the locus ceruleus area which is associated with the frequency of sleeping and breathing, it has a minimal sedative respiratory depression effect [12]. Dexmedetomidine in the clinic was initially introduced as a short-term effect venous sedative. As the medicine represents the analgesic properties associated with connecting to a2-AR, various researches have studied it as a systematic analgesic adjutant particularly in the acute preoperative form. Considering the great lipophilic of Dexmedetomidine, the medicine will remain in the tissue of placenta and increase the frequency and range of vaginal contractions [13].

Considering the importance of trying to relieve the pain after the operation and wide utilization of opioids to reduce pain after operation and their major side effects and keeping in mind the fact that no comparison has ever been made between the analgesic effects of Dexmedetomidine and fentanyl in caesarian and due to the large prevalence of caesarian operation and the importance of reducing postoperative pain in the health and satisfaction of women candidates for caesarian, we decided to study the effects of adding Dexmedetomidine and fentanyl to lidocaine 5% in reducing the postoperative pain of caesarian among women candidate for it.

Material and Methods

A written letter of introduction was obtained from the authorities of the university for research centres, and the purpose of the research was described for all research centres, and their written consent was obtained. The information of the participants remained confidential with the research executive team. Throughout the research, all ethical statements stipulated in Helsinki Research and by the research committees of ethics of Arak Medical Sciences University were observed. This research was approved with the ethical code of IR.ARAKMU.REC.1395.138 on July 19th, 2016 (Table 1). This research is registered under the code IRCTz2017012320258N28.

Table1: The information of research variables

Name	Definition (scientific and applied)	Type of variable		Scale of variable		Variable measurement unit
		Based on research goals	Based on the type of variable	Qualitative	Quantitative	
Being placed in fentanyl and Dexmedetomidine group	Fentanyl and Dexmedetomidine are the two medicines used in this research	*	*	*		Questionnaire Fentanyl or Dexmedetomidine
The average length of analgesia after operation (average time to request the first painkiller)	The length of anaesthesia after an operation	*	*	*	*	Minutes Questionnaire
Average pain score in recovery, 4, 12, and 24 hours after the operation	Average pain based upon VAS	*	*	*		VAS ruler Number
The average amount of the analgesics taken within the first 24 hours after the operation	The average amount of the painkillers taken within 24 hours following the operation	*	*	*		Questionnaire Milligram
Age	Years passed	*	*	*		Questionnaire Years

This is a double-blinded, randomised clinical trial research conducted randomly on non-emergency patients resorting to Taleghani Hospital of Arak for caesarean. As many as 84 pregnant women candidates for elective non-emergency repetitive caesarean and had a single fetus signed the informed consent form and took part in the research and was randomly divided into fentanyl and Dexmedetomidine groups. All the qualified participants were randomly distributed into the above-said groups using blocked randomisation. (Method used to generate the random allocation sequence was blocked randomisation) Having signed the informed consent form, the patients entered the operation room and initially received 3 to 5 cc per kilogram crystalloid as compensatory volume expand (CVE). After conducting the necessary monitoring and checking the vital signals of the patient, the patient assumed a sitting position and cage 25 needles was sent through theL4-L5 or L5-S1 space, and they underwent spinal anaesthesia for caesarean. 25 µg fentanyl was added to lidocaine 5% in the first group, while 30 to 35 µg (0.5 µg per kilogram) Dexmedetomidine (equal 2 cc, each ml of a diluted vial of Dexmedetomidine was 15 mg) was added to lidocaine 5% in the second group.

The total volume of both solutions in each group reached 4 cc. After conducting spinal anaesthesia and entering the cerebrospinal fluid space, the above-said medicine was injected. After injecting the medicine, the patient was asked to assume a supine position, and after making sure about the spinal block, the operation started. Immediately after spinal anaesthesia, breathing, heart rate, blood pressure and percentage of oxygen saturation were recorded. To abide by the principles of a blind research, the solutions prepared for injection in spinal space were prepared by an anesthetist and given to the assistant resident. Considering the similar volume and appearance of both solutions and unawareness of the resident of the type of solutions used to inject the patients, the resident was blind to the medicines. The patients had no information of the injected material and were randomly divided between

the two groups. Finally, after the operation, the pain scores of the patients in recovery, 4, 12, and 24 hours following the operation were measured using Visual analogue scale (VAS). Considering the first demand for analgesics and the average amount of the analgesics used within 24 hours after operation, the average length of analgesia was calculated. The resulting information was analysed using SPSS 19, and the data was shown using statistical tables and charts.

Inclusion criteria: 1) The patients must apply for elective caesarean. 2) The patients must apply for second-time repetition non-emergency caesarean. 3) All mothers must be pregnant with a single fetus. 4) The patients must have filled the informed consent form to take part in the research. 5) They must agree to undergo spinal anaesthesia. 6) They should not be banned from undergoing spinal anaesthesia. 7) They should never be sensitive to local anaesthesia. 8) They should not be sensitive to drugs. 9) They should not be sensitive to Dexmedetomidine. 10) The operation should maximally last 60 minutes. 11) All operations need to be conducted by a single surgeon. 12) All patients must undergo spinal anaesthesia by a single anaesthetist.

Exclusion criteria: 1) All those patients whose spinal anaesthesia has failed and they have applied for general anaesthesia. 2) All patients are suffering from drug abuse. 3) Operations that last longer than 60 minutes. 4) Sensitivity to the drugs used in the project. 5) Having a certain indication of general anaesthesia. 6) Having a BMI equal to or larger than 35. 7) Twine or more patients. 8) Multiparous patients.

Based on the type of research, a clinical trial was chosen as the best method to conduct this research. In line with the principles of simple probability sampling, 84 patients were divided into two groups (42 each) using the randomised numbers table.

$$N = \frac{\left(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta}\right)^2 (\delta_1 + \delta_2)^2}{(\mu_1 - \mu_2)^2}$$

$Z_{1-\frac{\alpha}{2}}$ = 1.96, $Z_{1-\beta}$= 2.33, μ_1= 258.5, μ_2= 219.37, δ1 = 28.7, δ2 = 30.4, N = 42.

The total number of samples is 84 people. And the period of study was 8 months.

Statistical calculations were conducted using SPSS 16 (Chicago, IL, USA). The parametric variables were presented as Mean ± SD and analysed by the analysis of variance (one-way ANOVA) post-hoc test; non-parametric variables were analyzed by Kruskal-Wallis test, and qualitative data were analyzed by chi-square test. A P value less than 0.05 was considered statistically significant.

Results

According to Table 2, no significant difference was observed between the pain score in Dexmedetomidine and fentanyl in recovery and 4, 12, and 24 hours following the operation (0.05 ≤ P). As a result, the two groups were similar regarding pain score.

Table 2: Comparing the pain score of patients candidate for caesarean using spinal anaesthesia in recovery, 4, 12, and 24 hours after operation in both groups

Time	Dexmedetomidine	Fentanyl	P-value
Recovery	0	0	0.05 ≤
4 hours following the operation	5.1 ± 80.8	6.02 ± 1.2	0.05 ≤
12 hours following the operation	2.5 ± 1.03	2.1 ± 1.05	0.05 ≤
24 hours following the operation	1.0 ± 7.88	1.09 ± 0.75	0.05 ≤

Significant: p ≤ 0.05, non-significant: p ≥ 0.05.

As p-value = 0.01, a significant difference was observed between the two groups regarding the average length of analgesia. The average length of postoperative analgesia in fentanyl group was longer than what was observed in Dexmedetomidine group (Table 3).

Table 3: Comparing the average length of postoperative analgesia and the average amount of drug taken among patients candidate for caesarean with the spinal method in both groups

Time	Dexmedetomidine	Fentanyl	P-value
Average length of analgesia (hours)	1.2 ± 57.3	4.40 ± 1.4	0.01
Groups	Dexmedetomidine	Fentanyl	P-value
The average amount of drug taken (in milligrams)	148.26 ± 8.3	119.04 ± 23.3	0.01

Significant: p ≤ 0.05, non-significant: p ≥ 0.05

As p-value = 0.01, a significant difference was observed between the two groups regarding the average amount of drugs taken within 24 hours after the operation. The average amount of drug taken in fentanyl group was less than what was observed in Dexmedetomidine group (Table 3).

No significant difference was observed between the two groups regarding average blood pressure and heart rate before spinal anaesthesia (P ≥ 0.05) (Table 4).

Table 4: Comparing the average blood pressure and heart rate of patients candidate for caesarean using spinal anaesthesia before anaesthesia and immediately after anaesthesia and in recovery in both groups

Groups	Dexmedetomidine	Fentanyl	P-value
Average blood pressure	74.27 ± 12.2	73.92 ± 11.8	≥ 0.05
Average heart rate	85.32 ± 13.8	73.83 ± 10.9	≥ 0.05
Groups	Dexmedetomidine	Fentanyl	P-value
Average blood pressure	66.80 ± 8.8	66.69 ± 7.9	≥ 0.05
Average heart rate	28.74 ± 7.7	75.4 ± 9.8	≥ 0.05
Groups	Dexmedetomidine	Fentanyl	P-value
Average blood pressure	72.2 ± 8.6	73.3 ± 7.9	≥ 0.05
Average heart rate	81.4 ± 6.7	82.9 ± 8.7	≥ 0.05

Significant: p ≤ 0.05, non-significant: p ≥ 0.05.

No significant difference was observed between the two groups regarding average blood pressure and heart rate after spinal anaesthesia (P ≥ 0.05) (Table 4). According to Table 4, no significant difference was observed between the two groups regarding average blood pressure and heart rate in recovery (P ≥ 0.05).

A significant difference was observed between the two groups regarding the frequency of shivering in recovery with the shivering being more common and frequent in Dexmedetomidine group (P = 0.001). According to Table 5, a significant difference was observed between the two groups regarding nausea-vomiting with a higher frequency of nausea-vomiting observed in the fentanyl group (P = 0.001).

Table 5: Comparing the occurrence of shivering and nausea-vomiting among patients candidate for caesarean using spinal anaesthesia in recovery for both groups

Groups	Dexmedetomidine	Fentanyl	P-value
The frequency of shivering in recovery	4	2	0.001
The frequency of nausea-vomiting in recovery	1	4	0.001

Significant: p ≤ 0.05, non-significant: p ≥ 0.05.

No statistically significant difference was observed between the two groups regarding their average age (P ≥ 0.05) (Table 6). No statistically significant difference was observed between the two groups regarding the age of the pregnancy (P ≥ 0.05) (Table 6).

Table 6: Comparing the average age of the patient's candidate for caesarean in both groups

Average age	Dexmedetomidine	Fentanyl	P-value
Average age of patients	24.8 ± 8.7	25.1 ± 9.8	P ≥ 0.05
Average weeks of pregnancy	39.1 ± 7.8	39.5 ± 9.9	P ≥ 0.05

Significant: p ≤ 0.05, non-significant: p ≥ 0.05.

Figure 1 shows are comparing the average postoperative analgesic time and comparing the average amount of drug taken within 24 hours after an operation by patients candidate for caesarean using spinal anaesthesia in both groups.

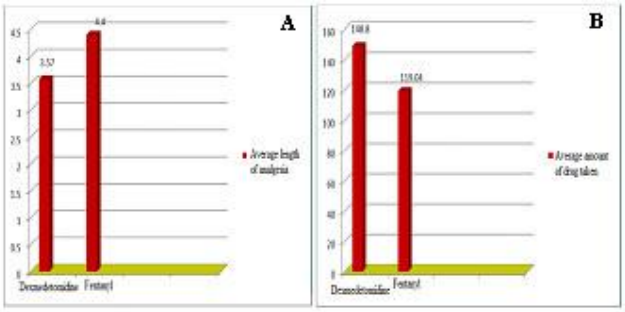

Figure 1: A) Comparing the average postoperative analgesic time; B) The average amount of drug taken within 24 hours after an operation by patients candidate for caesarean using spinal anaesthesia in both groups

Discussion

In this study, 84 pregnant women candidates for elective non-emergency repetitive caesarean were randomly divided into fentanyl and Dexmedetomidine groups. All the qualified participants were randomly distributed into the above-said groups using a randomised numbers table

Our data showed that there was no significant difference between the two groups regarding pain score in recovery, 4, 12 and 24 hours following the operation (P ≥ 0.05). In parallel, we have found between groups the terms of pain score similarly. Moreover, there is a significant difference between the two groups in terms of the average length of analgesia with a longer period of postoperative analgesia reported in fentanyl group (P = 0.01) as well as we have been indicated a significant difference among the two groups in terms of the number of drugs taken within the first 24 hours following the operation with less amount of drugs taken in fentanyl group (P = 0.01).

Furthermore, results demonstrate that there is no significant difference among the groups regarding the average blood pressure and heart rate of patient's immediately after spinal anaesthesia and recovery (P ≥ 0.05).On the other hand, we have also been exhibited a significant difference between the groups regarding shivering in recovery with higher rates of shivering reported in Dexmedetomidine group (P = 0.001). Regarding nausea and vomiting, the difference between the two groups was significant with a higher rate of nausea and vomiting observed in the fentanyl group (P = 0.001). A study was conducted by Mahendru et al., 2013 to compare adding Dexmedetomidine, clonidine and fentanyl to bupivacaine in an intrathecal manner with the goal of investigating the sedative factors and sensory and motor block and cardiovascular effects and reduction of anaesthetics in spinal anaesthesia. They concluded that Dexmedetomidine has a greater postoperative analgesic effect and a longer period of sensory and motor block with minimum side effects [14]. In the present research, fentanyl resulted in greater postoperative analgesia. This observation can be attributed to the fact that Dexmedetomidine and Fentanyl were both added to lidocaine in our research, whereas in Mahendru et al., 2013, Dexmedetomidine and Fentanyl were added to Bupivacaine. Our research was conducted on pregnant women candidates for caesarean, while Mahendru's study was conducted on lower limb operations.

Agrawal et al., 2016 researched to compare particulars of the block in spinal anaesthesia using intravenous Dexmedetomidine and clonidine. 5 cases of hypotension were observed in Dexmedetomidine group, while another 7 cases were observed in the clonidine group. The length of postoperative

anaesthesia was longer in Dexmedetomidine group. They arrived at the conclusion that better conditions were achieved as a result of using Dexmedetomidine [15]. In the present research, Fentanyl yielded a longer period of anaesthesia with less drug needed after the operation. This difference is probably due to the difference in basic medicine used in our research (lidocaine) and Agrawal's research (Bupivacaine).

Xiawei et al., 2017 studied the addition of Dexmedetomidine to a mixture of lidocaine and Ropivacaine in elongating the block. Sensory block was achieved earlier in Dexmedetomidine group, and a statistically significant difference was observed ($P < 0.05$). Motor block was also achieved faster in Dexmedetomidine with a statistically significant difference ($P < 0.05$). The length of sensory and motor blocks in Dexmedetomidine group was longer with a statistically significant difference ($P < 0.05$). They claimed that adding Dexmedetomidine to lidocaine and Ropivacaine helped elongate the length of sensory and motor block and sensory and motor blocks will be achieved faster [16]. In the present research, fentanyl elongated the postoperative analgesia.

In another research by Khezri et al., 2014 with the topic the effect of adding clonidine and fentanyl to bupivacaine has been indicated that there is a significant difference between the two groups regarding the length of anaesthesia and the length of the block. They have shown a statistically significant difference was observed between clonidine and fentanyl ($P = 0.006$). Anaesthesia and block were longer than placebo and clonidine in the fentanyl group, and a significant difference was observed between clonidine and fentanyl ($P = 0.001$) [17]. The amount of the drug taken up to 24 hours was less and postoperative analgesia was more in fentanyl group. Moreover, in a study else by Parmar et al., 2010 with the subject the effect of intravenous Dexmedetomidine in spinal anaesthesia using ropivacaine suggested that the blood pressure before and after this procedure was less in Dexmedetomidine group. The length of the block in Dexmedetomidine group was longer. A higher sedation score and more demand for Atropine was reported in Dexmedetomidine group. They pointed to the fact that Dexmedetomidine increased the length of the block with fewer side effects and gives the patient appropriate sedation. While using Dexmedetomidine, the anaesthetist must pay attention to bradycardia caused by Dexmedetomidine [18]. In the present research, Fentanyl was more effective than Dexmedetomidine. And also, Mahmoud et al., 2009 studied the effect of adding Dexmedetomidine to bupivacaine in spinal anaesthesia in Urology, they have found that sensory and motor block in 10 µg Dexmedetomidine group was longer, and it took much shorter to achieve block. They concluded that dose influences the effect on the block [19]. In the present research, Fentanyl had a greater influence than Dexmedetomidine.

In conclusion, considering the analysis conducted, the average length of postoperative analgesia in fentanyl group was longer than Dexmedetomidine group. The average amount of drugs taken after operation in fentanyl group was less than Dexmedetomidine group. The frequency of shivering in Dexmedetomidine group was less than the fentanyl group, and higher rates of nausea-vomiting were observed in the fentanyl group.

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Case Report

Giant Mushroom-Like Cutaneous Cylindroma of the Head

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Keywords: Dermal cylindroma; Head-and-neck-region; Surgery; Transposition flap

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Abstract

BACKGROUND: Dermal cylindroma is a rare benign skin tumour.

CASE REPORT: We report a giant stalked, mush-room-like cylindroma of the head-and-neck region in a 73-year-old female patient. A tumour was surgically removed, and the defect could be closed by a cheek transposition flap.

CONCLUSION: The mushroom-like growth pattern has yet not been described for cylindroma.

Introduction

Dermal cylindroma is a rare benign apocrine adnexal tumour with a female predominance. Most patients are Caucasians. The slow-growing tumours are usually small. The most common localisation is on the scalp although tumours off the head and neck region have occasionally been observed. Malignant transformation is rare and preferential in patients with multiple tumours [1] [2].

Histologically these tumours consist of rounded islands of basaloid cells which are arranged in a “jigsaw puzzle”-pattern. These islands have a Grenz zone to the epidermis. There is a cellular dualism with the palisading peripheral lining of smaller cells with more hyperchromatic nuclei and an inner population with larger, more differentiated pale cells and nuclei and small duct-like structures. Hyaline droplets can be present. A tumour is surrounded by a hyalinized sheath [3].

On immunohistology, expression of lysozyme and alpha l-antichymotrypsin, cytokeratin, epithelial membrane antigen (EMA) and EGF-receptor has been demonstrated. The presence of intermingled cells with coexpression of keratin and vimentin argues for a partial myoepithelial-like differentiation [4].

The genetics of sporadic and inherited cylindromas demonstrate a molecular heterogeneity. A central role has *MYB* which is an oncogene. *NFIB* is a transcription factor gene. When both of these genes fuse they form an oncoprotein. MYB-NFIB fusion oncoprotein and activated or overexpressed *MYB* are acted as a driver for the proliferation of cutaneous cylindromas [5].

Multiple cylindromas are characteristic in patients with Brooke-Spiegler syndrome, an autosomal dominant disease characterised by the development of multiple adnexal cutaneous neoplasms such as cylindroma, spiradenoma, spiradenocylindroma, and trichoepithelioma. Of these patients, 40% to 85% carry germline mutations in the tumour suppressor gene *CYLD*, but lack *MYB-NFIB* fusion transcripts. However, *MYB* activation has been

demonstrated in 69% of tumours [6].

Sporadic cylindromas may be associated with *MYB-NFIB* fusion transcripts or *MYB* activation in the absence of such fusions [7]. The treatment of choice is complete surgical excision [8] [9] [10].

Case report

A 73-year-old woman presented to our hospital with a slow-growing tumour on the face. A tumour had been there for more than 10 years. The patient had no remarkable medical history, no immunosuppression, and no previous cancer. She took no medications.

On examination, we observed a Caucasian woman with Fitzpatrick's skin type II. She had a 7 cm large, stalked tumour with a mushroom-like growth (Figure 1). A tumour was painless. No enlarged lymph nodes were detected. There was no family history of tumours.

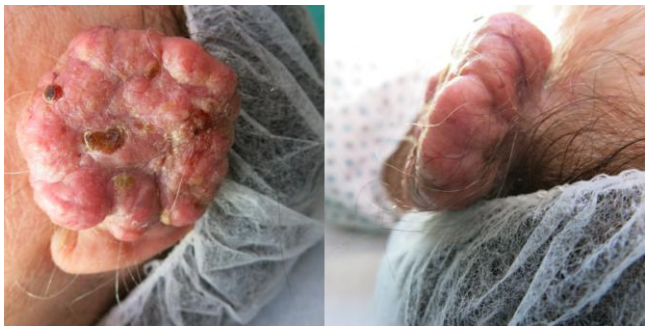

Figure 1: Clinical presentation of the giant cylindroma with a mush-room-like growth pattern

We suspected a non-melanoma skin cancer (keratoacanthoma-like squamous cell carcinoma) and suggested complete surgical removal by delayed Mohs surgery.

A tumour was excised in local anaesthesia. The stalk had pronounced vascularity that needed efficient hemostasis with bipolar tweezers. After resection, the defect was closed by a cheek transposition flap (Figure 2). Healing was unobtrusive.

Histopathology described a dermal tumour with a jigsaw appearance with nests of basaloid cells surrounded by a dense hyaline basement membrane. The basaloid cells with scant cytoplasm and dark nuclei palisaded around the edge of the nests. Larger cells with moderate eosinophilic cytoplasm and lighter staining nuclei were at the centre of the nests. Some of the larger cells were lumen-forming in a ring-like pattern.

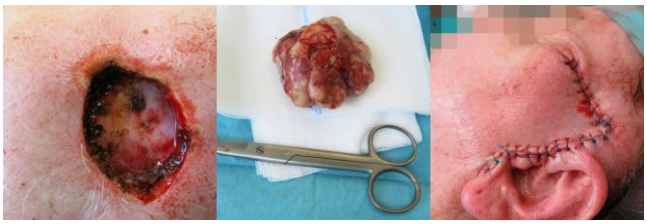

Figure 2: Surgical removal and defect closure: Resulting defect after complete excision (left). Tumor situs, view from the undersurface without the stalk (middle). Defect close by a cheek transposition flap (right)

There was no nuclear pleomorphism and no mitotic figures (Figure 3). The diagnosis of dermal cylindroma was confirmed, the resection status was R0.

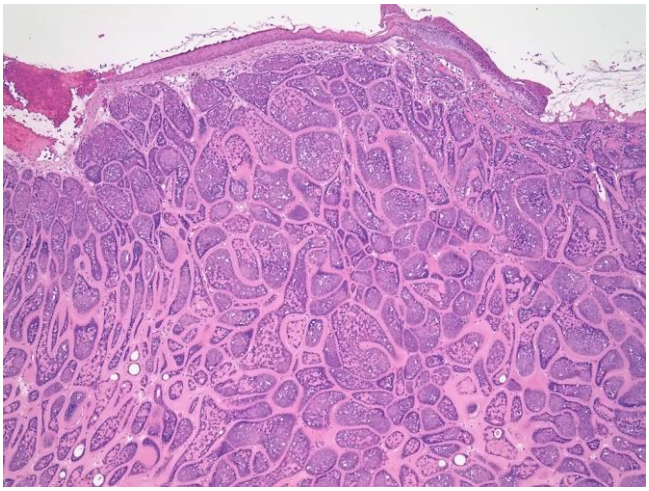

Figure 3: Dermal cylindroma – histopathology with basaloid cell nests in a jigsaw pattern (hematoxylin-eosin x 4)

Discussion

This case is remarkable. Giant tumours such as here are extremely rare. The mushroom-like growth pattern has yet not been described for cylindroma. It was originally described by Alibert for mycosis fungoides [11]. We observed this particular growth pattern with a stalk and exophytic tumour growth in basal cell carcinoma, melanoma, and Merkel cell carcinoma [12]. The present case demonstrates that dermal cylindroma can imitate skin cancer. Complete excision and careful, histopathological investigations are recommended. Fortunately, the true malignant transformation of cylindroma is rare [13] [14].

We had chosen the cheek transposition flap to close the defect after complete surgical removal since this flap offers a robust vascularisation and a perfect match in skin colour and texture. Operation time is short. In case the tumour had a wider base as

the top of the lesion suggested, a meshed graft would be an option for this elderly patient [15].

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Case Report

Behçet's Disease – Case Presentation and Review Literature

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Keywords: Aft syndrome; Behçet's syndrome; Mucosal ulceration; HLA-B51

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Abstract

BACKGROUND: Behçet's syndrome is associated with inflammation of various areas of the body. Sy. Behcet is a rare, chronic, recurrent disease characterised by changes in the: Arteries that supply blood to the body's tissues, veins that take the blood back to the lungs, the back of the eye's retina, brain, joints, skin and bowels. There is a close correlation between the geographical distribution of HLA-B51 and its prevalence. In the etiopathogenesis, there are indications of genetic susceptibility associated with environmental influence. Although aetiology is not yet known, it is thought of viral or autoimmune genes but is not yet confirmed by relevant analysis.

CASE REPORT: This was a case of a 29 years old young female presenting with recurrent oral and genital ulcers. Eye lesions usually start in one eye and then pass to the other eye. They are like iridocyclitis extending very quickly to another eye. Three months later, a few shifts were introduced in the form of small initial ulcers, which for 4-5 days have been enlarged and then epithelized by leaving the catapult in the genital mucus. In the skin of the lower extremities, papules appear to be as large as corn grain.

CONCLUSION: Diagnosis of Behçet's syndrome is determined based on eye changes, oral mucous and genital mucosa. Treatment of Behçet's syndrome depends on the severity and the location of its manifestations.

Introduction

Described in 1937 by the Turkish dermatologist Hulusi Behçet as a triad that associated uveitis with oral and genital aphthosis, it had been known since the fifth century BC, and its description can be found in the Hippocratic third book of endemic diseases. Since then, other manifestations have been described and added besides new aspects of epidemiology, immunopathogenesis, and treatment [1].

The highest of Behcet's disease (BD) prevalence ratios were found in Turkey, and its incidence is rare in the West (0.64/100,000 population in the United Kingdom and 0.12-0.33/100,000 in the United States).

In general, BD has a peak age of onset between 20-30 years and patients who develop the disease from a very young age present with more severe forms, with more organs affected. However, that study did not show the severity or extent of the disease [11].

Behcet's disease is a rare, chronic, recurrent disease characterised by an atopic artery in the mouth mucus, genital mucus ulcerations and eye changes (hypopyon-iritis, conjunctivitis) [1] [2] [3].

These are major symptoms, accompanied by other symptoms in the skin in the form of erythematosus, disseminated papillomas, pustules and purple and nodose shifts [4] [5]. In addition to the skin, other organs such as rheumatoid arthritis, thrombophlebitis, gastrointestinal disorders, kidney, respiratory system, CNS changes with symptoms such as psychiatric meningoencephalitis and psychogenic alterations [6] [7] [8] [9].

Aetiology: It is not known, though in the viral or autoimmune genesis, but is not yet confirmed by

relevant analysis [3].

Mucocutaneous manifestations are markers of BD, and their recognition may allow diagnosis and treatment [1] Oral ulcers are present in almost all cases. These lesions are the initial manifestation in up to 80% of the patients and precede subsequent ones in an average of 7-8 years. Changes in buccal mucus usually start one by one in the shape of the beds covered with a white layer of yellow that is surrounded by erythema. They are accompanied by pain; they are classified by size and arrangement into minor (< 1 cm), major, and herpetiform ulcers [1] [6] [7].

Genital lesions are similar to those in buccal mucous. However, they are less recurrent, have a greater tendency towards scar formation, and some exhibiting necrotic borders. Deeper lesions may complicate the onset of fistulas, especially in females with the possibility of being deeper and deeper [5] [6] [10] [12]. In women, ulcers in the vagina and colon can be oligosymptomatic. The most frequently involved region is the major labia [13]. Ocular disease, more common among men, affects the retina and uvea, occurring in 30-70% of patients, causing blindness in 25% of them. It usually appears 2-3 years after oral and genital ulcers, but maybe the first manifestation of the disease in 10-20% of cases. Eye lesions usually start in one eye and then pass to the other eye. They are like iridocycles associated with hypophysiology, extending very quickly to other eye structures and, consequently, blindness [8] [9].

Although cutaneous lesions are non-specific for BD, they are essential for diagnosis. Their frequency varies from 48-88% in diagnosed patients [14]. Cutaneous manifestations can be divided into papulopustular lesions, erythema nodosum lesions, thrombophlebitis, and varied cutaneous and vasculitic lesions. Erythema nodosum (EN) lesions occur in one-third of patients, typically affecting the lower limbs.

Neurological impairment occurs in 5-10% of the patients, affecting mostly men. It occurs about five years after the onset of the disease mainly affecting the central nervous system and the peripheral nervous system to a lesser extent.

Deep vein thromboses of the extremities are the most common form of vascular involvement together with recurrent superficial venous thrombosis. Men are more affected than women.

Joint involvement is reported in 45-60% of BD patients and includes arthralgia and non-erosive and non-deforming monoarthritis or polyarthritis. It affects knees, ankles, hips, elbows, wrists with neutrophilic and mononuclear inflammatory synovial infiltrates and thrombosis of small vessels.

The gastrointestinal tract is affected in 3-26% of patients, varying in different populations, being more frequent in Japan than in the Middle East and the Mediterranean.

Diagnosis is determined based on eye changes, oral mucus and genital mucus [2] [3].

The main objective of BD patient treatment is to induce and maintain remission and improve quality of life, preventing irreversible damage and exacerbation of mucocutaneous and articular disease [15] [16]. Its main premise is to eliminate inflammation and comprises the use of immunosuppressive agents in severe, life-threatening, and symptomatic manifestations in mucocutaneous and articular diseases [17]. Is not easy and sometimes does not give complete and quick results. Local: Fluorinated corticosteroids, nitric silver digestion, local anaesthetics are used. Systemic: Corticosteroids, sulfonate preparations (Dapsoni), gamaglobulin, large doses of vitamin C. [1] [2] [3].

The purpose of presenting this case with M. Behçet is to sensitise interdisciplinary cooperation for the benefit of the patient, to think more often in this disease, family doctors, dermatologists, dentists, ophthalmologists and gynaecologists.

Case Presentation

Patient X is 29 years old, housewife, married, and mother of three children. She is hospitalised at the Dermatovenerology Clinic.

Anamnesis morbi: Accepted in the clinic due to changes in mouth mucus and genitals and erythema nodosum in the lower extremities.

The disease started for the first time a year ago, first with mucus in the mouth on the inside of the lower lip, in the form of corrosion followed by the door, the temperature and the pain.

The changes have stayed for a week and have been withdrawn, but have also been introduced in other places of mouth and tongue mucus. While visiting the dentist has used some Hexoral solutions for disinfection, vitamin C and antibiotics, little changes are calm but not entirely.

Eye lesions usually start in one eye and then pass to the other eye. They are like iridocyclitis associated with hypophysiology, extending very quickly to another eye.

Three months later, a few shifts were introduced in the form of small initial ulcers, which for 4-5 days have been enlarged and then epithelized by leaving the catapult in the genital mucus. They have been associated with great pain in the affected area. Having been hospitalised in gynaecology because of these changes has been postponed for a month, received antibiotics in ampoules and has used vaginitis due to increased secretion. The situation has

slightly improved, but not entirely.

Figure 1: Conjunctivitis chronic recidivans

In the lower lip of the mucous membrane on the left side, there is erosion limited by a healthy mucous membrane, as large as a medium plaque. In the genital mucosa at the side of the left side there is a perforation with a diameter of 1 x 1 cm in round shape, and in the mucous membrane at the entrance of the vagina on the left side shows an erosion with a clear limit of irregular shape, diameter 1.5 x 0.5 cm with white suture on the yellow. On the right side of the large lips, a catheter is visible after the ulceration (Figure 2, 3).

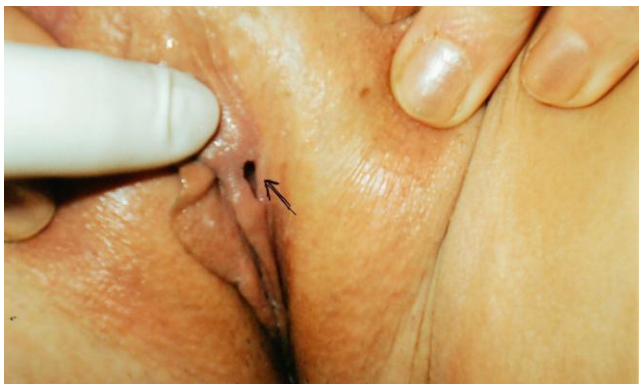

Figure 2: Ulcus vulvae (perforatio labia minor)

In the skin of the lower extremities, papules appear to be as large as corn grain, some above the skin level, some below the skin level, and hyperpigmentations at the stage of transition to the earliest poppies (Figure 4).

Laboratory analyzes: SE = 9/16; Leukocyte Formula: Er = 3.7 million cells/mcL, Ne = 60%, Ly = 32%, Mo = 4%, Eu = 4%; Hg = 74%, Urea = 4.6 mmol/L, Glycaemia = 5.3 mmol/L; Le = $14.3x10^9$/L Kreatinin = 60 mmol/L; Chol = 3.5 mmol/L; IgG = 1307 mg/dl (1800 mg/dl); IgA = 462↑ mg/dl (450 mg/dl); IgM = 225 mg/dl (230 mg/dl); IgE = 25.4 mg/dl (1-183 mg/dl); C3 = 161↑ mg/dl (135 mg/dl); C4 = 30 mg/dl; Anti DNA +.

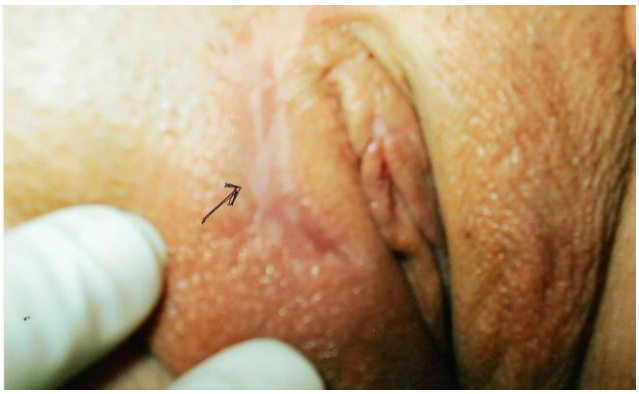

Figure 3: Cicatrix post ulcus vulvae

Urine: Turbid, yellow, sour, sediment; enormous amorphous urate salts, 8-10 leukocytes, frequent bacteria, 10-15 epithelial cells.

Vaginal stripe: *Enterococcus, Escherichia coli*. Stroke of the ulcer: *Staphylococcus aureus*.

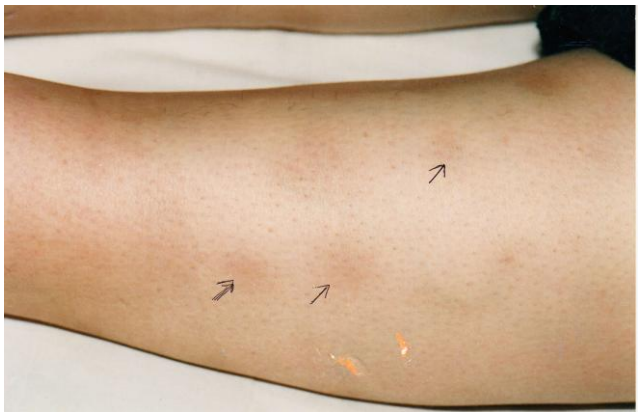

Figure 4: Erythema nodosum extremitas inferior billateralis

Ophthalmologist: Conjunctivitis chronica recidivans.

The patient was treated with: Amp. Nirypan 80 mg, 5 days/amp. 40 mg + Tab. Pronison a 5 mg/day reduces the dose every 5 days by 5 mg Nystatin salt for the oral, Tab. Erythromycin 500 4 x 1/two weeks, Vit C 3 x 2, vaginalete Geonistin 1 x 1 two weeks. The patient is released significantly improved.

Discussion

Is known that Behçet syndrome is a rare, chronic, recurrent disease characterized by three major symptoms: (1) stomatitis aphtous; (2) genital mutilation; (3) the conjunctiva, iridocyclitis in the eye, as well as accompanying symptoms in other organs

[1] [2] [3] [7] [8].

Based on the history and clinical picture it has been questioned whether it is pemphigus vulgaris, erythema exudative multiforme, or ulcus acutum vulvae [4] [5]. In our case - based analysis report, history and literature consultations, we have concluded that we are dealing with oculo-bucco-genital Behçet-Syndrome.

Behçet disease is a heterogeneous and yet intriguing disease. Despite the remarkable progress in research, many gaps need to be fulfilled. New knowledge regarding its immunopathogenesis, genetics, and epidemiology will greatly help in the development of laboratory tests, diagnostic criteria, activity indexes, and especially in the choice of the best treatment.

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Case Report

Lipoma of the Neck

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Keywords: Lipoma; Surgery; Conservative approach; MRI; Outcome

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Abstract

BACKGROUND: Lipomas are benign formations with the mesenchymal origin, which are found in the head and neck region in a very small percentage of cases. In these cases, they usually occur in the posterior neck and usually grow very slowly without causing complaints. However, when they cover the front of the neck, it is necessary to confirm the adipose origin of a tumour and to exclude the connection with the thyroid gland. Although in principle, lipomas are benign, there are three more unfavourable possibilities of 1) malignancy of lipomas, 2) the occurrence of de novo liposarcomas or 3) the association of lipomas with other tumours, such as retinoblastoma.

CASE REPORT: We present a 74-year-old woman with a painless subcutaneous formation in the neck. A Fine Needle Aspiration Biopsy (FNA) was performed, with the conclusion of the cytological result for lipoma. Surgical removal was planned under general anaesthesia.

CONCLUSION: Usually, lipomas do not require surgery, but when they engage muscles they become indicative of surgical treatment.

Introduction

Lipomas are benign tumours that are usually found in the subcutaneous tissue and can cover different parts of the body [1] [2]. They usually have a benign flowing course, but in rare cases, it is also possible to see a malignant transformation of the lipomas [3]. On the other hand, in some forms of lipomas, such as dysplastic lipomas, there is a possibility of an association between lipoma and another tumour, for example, retinoblastoma [4]. Although in most cases they are painless and slow-growing, sometimes lipomas can grow significantly and infiltrate musculature, leading to symptomatology and to be an indication of surgical treatment [5] [6].

Case report

A 74-year-old woman was hospitalised due to a subcutaneous painless tumour formation of the ventral neck (Figures 1a and 1b). Ten years ago, the patient developed a "nodule" in the area of the thyroid gland that gradually increased by size and was now the reason for diagnostics to clarifying its nature. The patient's medical history was remarkable for arterial hypertension treated with amlodipine 5 mg (0-0-1) and losartan potassium 50 mg (1-0-0), osteoporosis treated with denosumab 60 mg/ml x 1/day for 6 months, as well as chronic venous insufficiency. Additionally, we noted itchy, disseminated nummular partially pyodermic patches, located on the face, trunk

and extremities suggesting nummular or microbial eczema (Figures 1a and 1b).

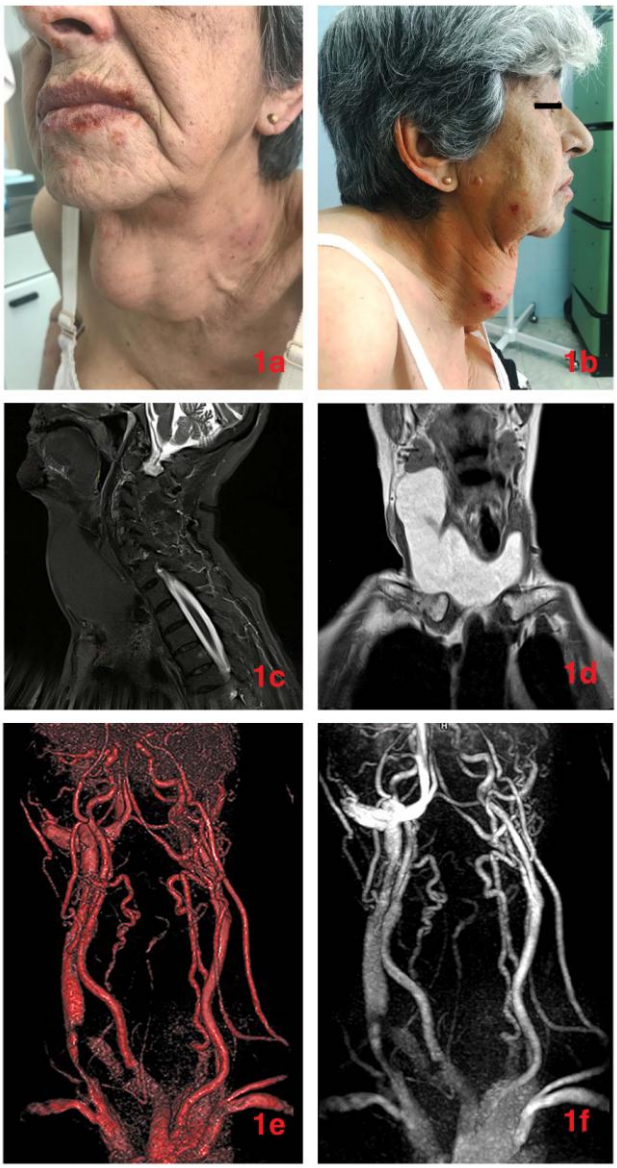

Figure 1: a, b) At the dermatological examination (front and sideways of the neck)-an oval lesion with the soft-elastic constellation. Clinical image of disseminated nummular partially pyodermic patches; c, d) Pre-surgical magnetic resonance imaging of the neck confirmed the presence of a lesion of the anterior cervical space. Tumour burdens from horizontal and vertical view; e, f) MRI contrasting blood system in the neck

During the examination, in the neck area, parathyroid was visualised a subcutaneous tumour formation with a relatively soft but also an elastic constellation, painless, consisting of 2 confluent subcutaneous nodules with a total size of 13 to 8 cm (Figures 1a and 1b). The results of the routine blood tests showed elevated cholesterol levels of 6.6 mmol/l, HDL-2.25 mmol/l, LDL-3.9 mmol/l. Ultrasound of the thyroid gland revealed a hypoechoic formation with streakiness and rounded adenomatous sections covering the right thyroid. Radiography of the neck area showed moderately arcuate displaced trachea to the left at the C4-C7 level. The cytological result of the ultrasound-controlled aspiration biopsy and subsequent specific lipid stains confirmed a benign lipoma. An MRI study was conducted which identified the presence of a large, subcutaneously located lesion of irregular shape and homogeneous structure, with a well-formed capsule (Figures 1c and 1d). The process penetrated insignificantly in the mediastinum of up to 11.5 mm caudally from the upper edges of the clavicles (Figure 1c).

Furthermore, there was an increase in the size of the right sublingual, submandibular and parotid glands. As a consequence, a dislocation of the carotid system and the right jugular vein was noted. There was a relative reduction of blood flow in the left sigmoidal sinus and left internal jugular vein but a compensatory collateral venous blood flow in the course of external jugular and submandibular veins to the left, as well as deep cervical veins (Figures 1e and 1f).

Discussion

Lipomas are benign mesenchymal tumours that usually engage the subcutaneous tissue but may occur in internal organs as well [1]. Each part of the body can be affected, with the anterior neck being one of the most uncommon locations [2]. This localisation requires further investigations to exclude a possible connection to the thyroid gland [2]. Intraglandular tumours of the parotid gland and other glands have been described in the medical literature [10] [11]. In our patient, the cytological data from the fine needle aspiration biopsy showed an origin from the adipose tissue with a benign nature. Imaging techniques (ultrasound diagnosis, X-ray and MRI of the cervical area) excluded a communication with the thyroid. Also, a giant parapharyngeal lipoma extending to the pterygoid region (anterior skull base) could also be excluded [7].

Lipomas are characterised by enlarged but truly benign adipocytes surrounded by a capsule. Some unconventional forms of lipomas, including chondroid lipoma, angiolipoma, pleomorphic / spindle cell lipoma and dysplastic lipoma may also be observed [4] [5] [8] [9]. The so-called dysplastic lipomas do not exert MDM2 gene amplification but are overexpressing p53 and may demonstrate abnormalities of the Rb1 gene as well as the probability of some cases being associated with other tumours such as retinoblastoma [4].

Even rarely, malignant transformation of lipomas is possible [3]. De novo developed liposarcomas should also be considered in a differential diagnostic aspect [3]. Sometimes the distinction between lipoma and sarcoma is extremely

difficult, but it is of paramount importance, as the therapeutic approach is radically different [5] [8].

Although they are slow-growing and usually painless, as with our patient, in rare cases lipomas can engage and infiltrate muscles, and especially in the cervical area, to induce symptoms that require the necessity of surgical treatment [5] [6]. Adequate preoperative diagnosis is important to assure adequate tumour control as well as optimal functional and cosmetic outcome since delicate structures a close in the neck region.

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Dental Science

The Contribution of Indian Endodontists in Rotary Endodontics to Pubmed Database, from 2000-2017

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Keywords: PubMed; Rotary Endodontics; Indian-authors; Endodontists publications

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Abstract

AIM: This study aimed at assessing the trends of publications of Indian Endodontists in the field of rotary Endodontics in the PubMed database from 2000-2017.

METHODS: The date of publication was set from 1st January 2000 to 31st December 2017, wherein keywords entered in the advanced search were “Indian” AND “Dental” AND “Rotary Endodontics”. From the collected articles the following criteria were noted: year of publication, the name of the journal, status of the journal, name of the first author, state of origin and the rotary Endodontic file system used.

RESULTS: All data was subjected for statistical analysis by SPSS software version 16. The data were subjected to chi-square test, and a statistically significant difference ($p < 0.001$) was obtained in the inter-6 yearly interval starting from 2000-2017; in the status of the journal; the state of origin and in the generation of rotary files which were published during the study period.

CONCLUSION: The plethora of publications by Indian Conservative Dentists and Endodontists is on the rise, and with the advent of better technology a greater interest in the mechanics and properties of these rotary file systems has invoked greater research work.

Introduction

PubMed-Medline is a widely used and an elaborate database devised by the National Centre for Biotechnology Information and National Institute of Health, USA. This database is updated almost daily and contains details of millions of manuscripts in the field of life sciences. It is the most widely used search tool for millions of health and life sciences researchers. PubMed-Medline is the NLM’s (National Library of Medicine, USA) premier online bibliographic database which is freely accessible and covers all the fields such as medicine, nursing, dentistry, veterinary medicine, health care system, and the preclinical sciences [1].

This kind of analysis may help inform the development of scientific and technological policies in dentistry; of special relevance in emerging economies that are currently undergoing rapid transformation [2]. This study aimed to assess the trends of research and publications of Indian Endodontists in the field of rotary endodontics in the PubMed database from 2000-2017.

The two main objectives of this study were: (i) to assess the total number of articles published by Indian Conservative Dentist and Endodontists, in the field of Rotary file systems in Endodontics from 1st January 2000 to 31st December 2017; (ii) and to understand the state-wise distribution; the status of journals in which articles are published and the frequency and most widely studied rotary file system used during the study period.

Methods

The search commenced on the first fortnight of January 2018 at 7:30 pm and ended at 7:35 pm.

The research was conducted in the PubMed database with a date limitation set from 1st January 2000 at to 31st December 2017. The keywords inserted in the advanced search were “INDIA” AND “DENTAL” AND “ENDODONTICS” AND “ROTARY”. After search completion, a total of two hundred and twenty articles were displayed (Figure 1). These articles were then subjected to a rigorous inclusion criterion wherein the First Author was of Indian nationality. Only the first author was considered for the study. The first author’s affiliation was to be a research institute belonging to an Indian state only. No other speciality except, Conservative Dentistry and Endodontics were included. No journal or types of article limitation were set. All articles, from all types of journals including basic sciences, clinical medical sciences, and dental journals were included. All the articles displayed were considered for the analysis. All articles pertaining to information about Rotary file systems were considered. Articles which included comparisons between Rotary and Reciprocating files systems were considered but only the Rotary systems used in the article were noted.

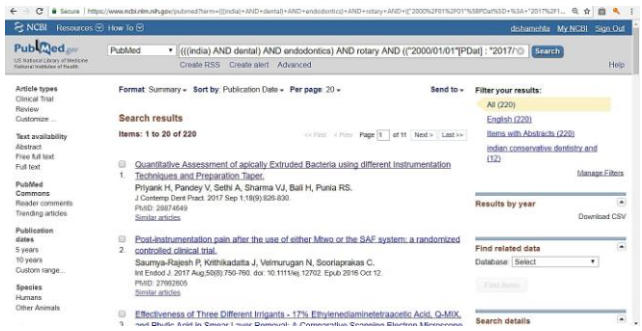

Figure 1: The result page after entering the keywords in advanced search option of PubMed Database, displaying a total of 220 results

On applying the necessary definitive inclusion criteria, a total of one hundred and twenty articles were shortlisted. From this collection of articles, the subsequent information was then charted out in a Microsoft Excel sheet for ease of assessment: the title of the published article, the year of publication, name of the journal, status of the journal (national or international), first author (Only conservative dentists and endodontists), state of origin and finally the type of rotary file system used (including rotary retreatment file systems).

Results

All data thus gathered were entered in SPSS software (version 16.0). From this database, the performance of Conservative Dentist and Endodontist was assessed. Descriptive analysis was performed and presented. Inter-annual distributions, State-wise distribution, the mean number of rotary files in each year, and the trend analysis using the present trend of articles published was charted in Microsoft Excel.

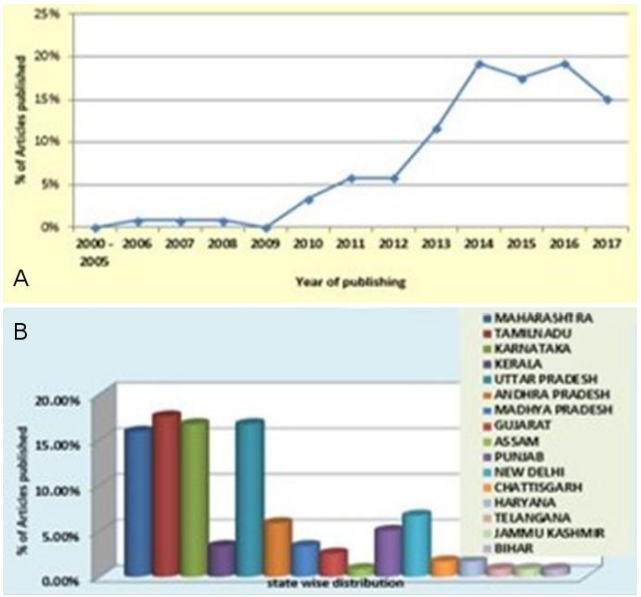

Figure 2: A) Represents the publication trends featuring in articles from 2000-2017 in the field of rotary Endodontics; B) State-wise distribution of the published articles

A trend analysis and an exponential growth in the publications featuring rotary endodontics was seen beginning from 2012 was observed; being highly statistically significant in the third interval (Figure 2a) (Table 1). National journals (72.5%) presented with a highly statistically significant difference over International Journals (27.5%) when subjected to Chi-square test.

Table 1: Comparison of the number of journals which had publications by Conservative Dentist and Endodontics during the study period (2000-2017)

6 Yearly Intervals	N (%)	Chi-square test	P value, Significance
2000 to 2006	1 (0.83%)		
2007 to 2012	20 (16.66%)	135.05	p < 0.001*, highly significant
2013 to 2017	99 (82.5%)		

**p < 0.001-highly statistically significant.*

The state-wise distribution of publication trends during the applied study period revealed the maximum number of publications were from Tamil Nadu (17.5%), Karnataka and Uttar Pradesh (16.66% each) and thirdly by Maharashtra (15.83%), (Figure 2b). Table 2 describes the distribution of a file system used in publications during the study period (2000-2017) based on Generations.

Chi-square test was the statistical analysis which revealed that the second generation of the Rotary files was highly statistically significant (p < 0.001).

Amongst all the second-generation files, 26.14% were ProTaper Universal file system (Dentsply) which contributed to being the most featured system.

Table 2: Distribution of file system used in publications by Conservative Dentists and Endodontists during the study period (2000-2017) based on generation; N-percentage of totally publications

Generation	N (%)	Chi-square test	P value, Significance
First generation	19 (7.19 %)		
Second generation	151 (57.19%)		P <0.001* , highly significant
Third generation	48 (18.18%)		
Fourth generation	10 (3.78%)	46.81	
Fifth generation	36 (13.63%)		

**p<0.001-highly significant.*

Discussion

Healthcare professionals usually establish their decisions on professional perspicacity, prevailing comprehensive clinical practice, peer consultation, dental schools, seminars and Continuing Education programs. Clinical know-how, technical prowess, and critical judgment are of utmost importance. Due to the complexity of information, scientific backing to clinical practice must be sought in the medical literature and derived from methodologically ratifying tools [3].

Therefore, it is necessary that surveys should be undertaken, for they provide a scientific basis to professionals in their search to deliver better quality treatment. The importance of bibliometrics has become increasingly useful in analyses of scientific applicability. It is that statistical support that allows gauging and generation of a variety of data and management indicators; particularly from the scientific, technological, productivity-related information gathering and communication systems.

These necessitate the planning, evaluation and management of given scientific context [4]. Publication analysis, as with any method chosen to assess scientific production, does not cover the entirety of the scientific production. However, a similar approach has been successfully used in other partial analysis of dental research production. The applicability of this scientific approach enabled us to understand the scientific productivity by Indian Endodontists in 17 years to understand and calibrate the publication trend specifically in the field of Rotary Endodontics [1] [5] [6] [7].

All the data gathered was from the PubMed database, starting from 1st January 2000 to 31st December 2017, spanning over 17 years. All the data gathered was entered into Microsoft Excel and based on the columns allotted, the articles published were distributed according to our analysis criteria. For ease of distribution the three-time intervals, of 6 years each was considered. The pattern of publications in the first yearly interval, only a single article was published by Indian Conservative Dentists and Endodontists in the period from the year 2000-2006. It was only in the second 6 yearly intervals beginning from 2007, one article per year was published barring 2009. The articles in this time frame were lesser, as Rotary Endodontics was fairly recent regarding a clinical application in the Indian scenario and the file systems used mainly belonged to the first generation.

Slowly and gradually with the increase in the popularity of rotary endodontics, there was a massive multiplication in the availability and application of the same, this is represented by the exponential growth in the third 6 yearly intervals, comprising of 82.5% of the total articles published in the period of study.

The drastic increase in publication in 2012 could also be due to the following reasons: (1) increase in quantity and quality of conservative dentists and endodontists, (2) increased technological advancements in rotary file systems (3) widespread awareness and ease of use of rotary file systems, (4) increase in number of journals published from India, are some of the common factor to support the same.

The state-wise pattern of publications revealed that the maximum number of articles were published by Conservative dentists and Endodontists from Tamil Nadu state (17.5%) followed by Karnataka and Uttar Pradesh (16.66%) taking the second place and Maharashtra (15.83%) in the third place. Based on the status of the Journal in which the articles were published majority articles featured in National Journals; that complied with 72.5% of the total articles published.

Root canal treatment is one of the most technically challenging procedures in dentistry, and the success depends on the diagnostic acumen, instruments used and the technologies adopted. The adoption of endodontic nickel-titanium rotary technology by Endodontists in India has increased two folds in the last two decades. Hence, this survey was conducted to understand the scenario of publication trends involving Rotary Endodontics by Endodontists in India.

The nickel-titanium rotary instruments are undoubtedly a massive leap in the field of endodontics. The clinical endodontic breakthrough was progressing from utilising a long series of stainless steel hand files and several rotary Gates Glidden drills to integrating nickel-titanium (NiTi) files for shaping canals. Regardless of the methods, the mechanical objectives for canal shaping were brilliantly outlined almost 40 years ago by Dr Herbert Schilder [9]. When properly performed, these mechanical objectives enhance the biological objectives for shaping canals, 3-D disinfection, and filling root canal systems.

The File-systems that featured in all the articles were assessed and then regrouped based on the Generations of evolution of Endodontic Rotary File systems beginning from the first generation to the fifth generation. The second-generation file systems collectively featured in 151 articles and therefore being the highest, this result complied with the trend in

publications seen in the second 6 yearly intervals.

Following the second generation were the third generation, fifth generation, first generation and finally the fourth generation. The least number of publications were with the fourth-generation files systems mainly because the only article containing the Self-Adjusting File system (ReDent Nova) was included as it uses a specialised type of handpiece which has two major functions of vibration and rotation [10].

The fourth generation of files mainly employed the use of single-file based cleaning and shaping protocol and the introduction of a new reciprocating type of motion. Hence, articles containing file systems with reciprocating function were excluded. Therefore, the top 5 file systems in decreasing order of being published in majority articles were ProTaper Universal, Mtwo, K3 & RaCe, ProTaper Next and 5th being profiles & twisted files.

In conclusion, a descriptive study of the contribution of Conservative Dentists and Endodontists' publications to Rotary Endodontics during 2000-2017 in PubMed database is presented. The locational variations and interannual variations are presented. The result of this study clearly shows the lacunae of Conservative dentists and Endodontists' contribution to Indian research in Rotary Endodontics and publications. Efforts should be made by Conservative Dentists and Endodontists in India to increase their global presence regarding scientific contributions.

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Dental Science - Case Report

Management of Maxillary Impacted Teeth and Complex Odontome: A Review of Literature and Case Report

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Keywords: Impacted maxillary canine; Impacted maxillary lateral incisor; Multiple impactions; Odontome; CBCT

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Abstract

BACKGROUND: Teeth impaction has become a common problem faced by orthodontic clinicians with the greatest incidence reported among third molars and maxillary canines. The great challenge lies in successfully treating these cases without deleteriously affecting the impacted as well as adjacent teeth while achieving acceptable functional and esthetic results. Several etiological factors have been associated with impactions including the presence of an odontome which is an asymptomatic odontogenic hamartomatous lesion.

CASE REPORT: This article presents a detailed orthodontic assessment and treatment of a 16 years old female having impacted right maxillary lateral incisor and canine caused by complex odontome.

CONCLUSION: Successful orthodontic treatment of multiple impactions can be achieved with minimal side effects even when odontomes are associated, through 3D radiographic examination, detailed evaluation as well as proper biomechanical control.

Introduction

Teeth impaction is a common clinical problem experienced by 25-50% of the general population [1]. The greatest incidence was reported for mandibular third molars, followed by maxillary third molars and maxillary canines [2], which show the prevalence of 0.8-2.8% in the general population [3], while mandibular canines were less frequently impacted (0.35%) [4]. Dachi and Howell [2] reported that the maxillary canine impaction is more than twice as common in girls as in boys with more common unilateral affection than bilateral.

Several etiological factors were associated with such a problem. Maxillary canines are commonly impacted due to their lengthy developmental period and long, tortuous eruption path [5]. Possible etiological factors can be categorised as either general primary causes or local secondary causes.

General primary causes include genetic factors [6] [7], endocrinal deficiencies, palatal clefts, developmental problems and irradiation [4]. Local secondary causes occur more frequently including dentoalveolar discrepancies, transverse maxillary deficiencies, prolonged retention or premature loss of deciduous canines. Additionally, congenitally missing or anomalous lateral incisors, ectopic tooth germ position, canines' root dilacerations, trauma as well as physical obstacles can cause maxillary canine impaction as well.

Physical obstacles comprise supernumerary teeth, ankyloses, cystic or neoplastic lesions and odontomes (odontomas). According to WHO, odontome is a congenital developmental defect, resulting from the growth of completely differentiated epithelial and mesenchymal cells, in which all kinds of dental tissues occur [8]. The term "odontome" refers to any tumour of odontogenic origin. However, it is nowadays accepted that odontome represents a

hamartomatous malformation rather than a true neoplasm [9].

Various classifications of odontomes have been proposed in the literature. According to the origin, Worth [10] classified odontomes as either of ectodermal origin (Enameloma), mesodermal origin (dentinoma, cementoma), or mixed ectodermal and mesodermal origin (complex composite odontome, compound composite odontome, geminated odontome and dilated odontome). According to the clinical presentation and location, odontomes present inside the bone are called central odontomes, those occurring in the soft tissue covering the tooth-bearing area are peripheral odontomes, and the last category are erupted odontomes [11].

Two main types of odontomes are acknowledged based on their appearance. Compound odontomes involve representation of all dental tissues orderly distributed forming tooth-like structures known as denticles. Complex odontomes comprise dental tissues but showing a disorganised distribution. Compound odontomes are twice as common as complex odontome and more commonly located in the anterior maxilla, whereas complex odontomes have a predilection for the posterior mandibular region [9].

Treatment of an impaction case can include one of two broad options. The first comprises the extraction of impacted tooth followed by either space closure and adjacent teeth substitution, or space opening and restoration or auto-transplantation of another tooth. The second treatment modality can be surgical exposure and orthodontic traction of the impacted tooth. The choice is based on the condition and location of an impacted tooth, the effect on adjacent teeth and structures, treatment duration and cost as well as the patient's preference.

Management of multiple impactions and odontome is highly challenging and requires careful assessment and treatment.

This article presents a case of impacted maxillary right canine, lateral incisor and complex odontome denoting the importance of detailed 3D (three dimensional) radiographic evaluation and orthodontic biomechanical control.

Case Presentation

A 16 years old female was presented to the outpatients' clinic with a chief complaint of a hard bulge at her upper right region and a delayed eruption of the corner tooth Fig. 1. The patient was medically free and had no history of previous dental trauma.

Extra-oral examination revealed the patient had a mesocephalic face with average vertical facial proportions, straight profile, competent lips, average nasolabial and mento-labial angles as well as a slightly retruded upper lip and prognathic chin.

Intra-orally, a hard vestibular swelling was found in the maxillary right canine region Figure 1. This was associated with retained maxillary right deciduous canine (UR C) with slight mobility, and absence of maxillary right lateral incisor (UR 2) and canine (UR 3) in the dental arch. Both maxillary and mandibular arches were symmetric and ovoid with mild crowding.

Assessment of the occlusal features revealed class I molars and incisors relationships with quarter unit class II left canine and undefined right canine relation. The upper midline was shifted 1 mm to the right relative to the facial midline with average overjet and overbite.

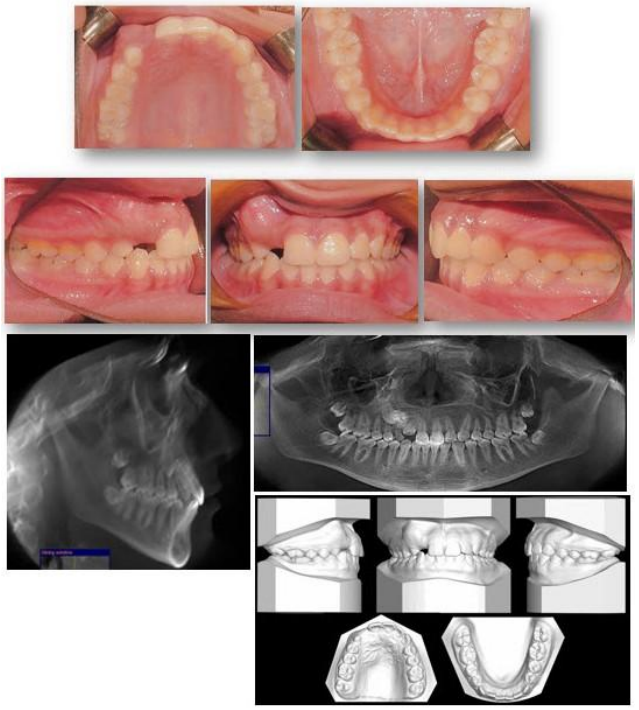

Figure 1: Pre-treatment records of a 16 years old female with impacted maxillary right canine, lateral incisor and odontome

Cone Beam Computed Tomography (CBCT) was ordered for this patient from which digital panoramic and lateral cephalometric radiograph were extracted Fig. 1. Radiographic examination showed that the hard bulge was caused by a complex Odontome present in the upper right canine region, with impacted UR 2 and 3 where both teeth appear to be favourably impacted with no signs of crown nor root resorption. No abnormal root morphology detected, except for retained UR C's root which was resorbed.

Three dimensional (3D) assessment of the CBCT (Figure 2) for odontome and impacted teeth revealed that: (i) complex odontome was present buccal to the impacted teeth, interfering with their eruption and causing palatal inclination of the

impacted UR 3. (ii) both impacted teeth appeared to be of favourable position, where: UR 2: Appeared to be: of favorable angulation, close to the alveolar ridge, having no signs of resorption, impeded from eruption only by the odontome; UR 3: Appeared to be: with its root apex in the line of the arch above the assumed canine position, of favorable angulation, forming around 15˚-30˚ relative to the sagittal midline, of average prognosis regarding its vertical height where it was located within the apical half area of the maxillary right first premolar (UR 4).

The cephalometric view findings confirmed the clinical findings where the patient had a mild class III skeletal base, in addition to retroclined lower incisors.

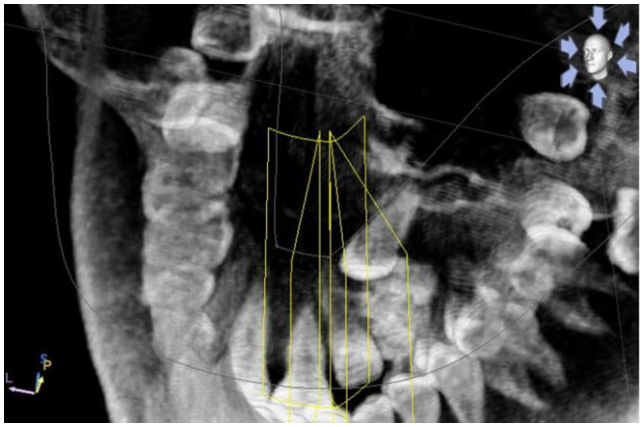

Figure 2: 3D rendered image of tilted occlusal view showing the relationship between impacted teeth & odontome, were the following detected: UR 2's root not touching the UR 3's crown. Proximity between the odontome & the crowns of the impacted teeth

Aiming to resolve this case's prioritised problem list, the main goals of treatment were to:

1. Extract the retained UR C and surgically remove the odontome followed by an observation period to allow for the normal eruption of impacted teeth.

2. Surgical removal and orthodontic alignment of both impacted teeth into the line of the arch.

3. Attain class I canine relation, coordinating the maxillary midline, achieving proper lower incisors' inclination, as well as achieving better upper lip esthetics.

The patient was sent to an oral surgeon for extracting the retained URC and surgical removal of the odontome. Afterwards, banding and bonding of fixed pre-adjusted orthodontic edgewise appliance (0.022" x 0.028" slot) Roth prescription was done for upper and lower teeth except UR 2 and 3. Upper and lower archwire sequence of 0.016" NiTi (Nickel-Titanium) followed by customised and coordinated 0.016" st.st (stainless steel), then 0.016" x 0.022" st.st was placed.

Upper right lateral incisor was seen to erupt spontaneously soon after removal of the odontome. It was followed up until it was close enough to the occlusal plane then it was bonded and engaged to 0.014" NiTi main archwire. The previous wire sequence was applied until inserting customised and coordinated 0.017" x 0.025" st.st upper and lower archwire.

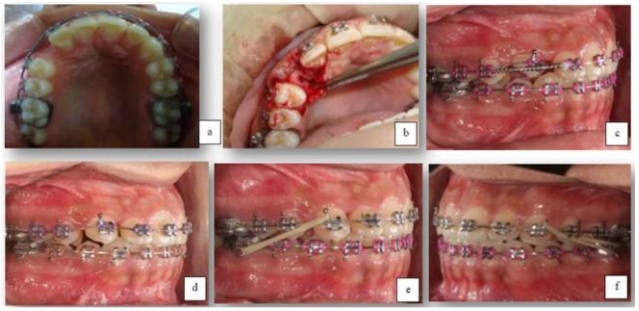

Figure 3: Canine alignment sequence; (a) Creating space for canine; (b) Surgical exposure; (c) Canine alignment with overlay wire; (d) Canine alignment with NiTi main archwire; (e) and (f) Residual space closure and settling of occlusion

Space for the impacted UR 3 was created using gradually activated open coil spring between UR 2 and UR 4 taking advantage of this technique to adjust the upper midline as well (Figure 3a). Finally, 0.019" x 0.025" st.st upper and lower archwire was inserted.

Surgical exposure of the impacted UR3 was done followed by bonding a button with a hole on the canine tip after engaging a heavy ligature wire. Then the flap was closed and the ligature wire released from the incision line (Closed technique) (Figure 3b). Power chain was then engaged to the attached ligature wire and then tied to the main heavy archwire to track the impacted UR3 to the occlusal plane. Gradual activation was done until UR3 appeared intra-orally.

When the span for further activation was short, a step-down bend was made in the main archwire thus facilitating UR3 traction. The canine was de-rotated by creating a couple through bonding a button palatally and ligating the palatal button to the UR4 after laceback from UR4 to UR6 was, and the labial button was pulled to UR2 after lacing from UR2 to UL2.

The canine was later bonded with a bracket with the same prescription, but the bracket was placed closer to the canine tip due to poor accessibility. Levelling of the UR 3 was achieved using 0.012" NiTi piggyback archwire (Figure 3c). Bracket re-positioning for UR3 was done after it became accessible followed by placement of 0.014" NiTi with full engagement (Figure 3d), then 0.018" NiTi and finally 0.018" st.st upper archwire with continuous power chain on the upper arch to close residual spaces and settling elastics (Figure 3e and f).

Radiographic assessment evaluating the teeth inclination and root uprighting upon approaching the end of treatment was performed in Figure 4. Finishing,

detailing and settling of occlusion was finally performed using upper 0.016" x 0.022" TMA with an aesthetic bend at UR2 to correct root angulation and settling elastics continued. Finally Upper and lower fixed retainers (0.0175 Dead Soft Respond® Wire (Ormco Co.)) from right to left canines were bonded.

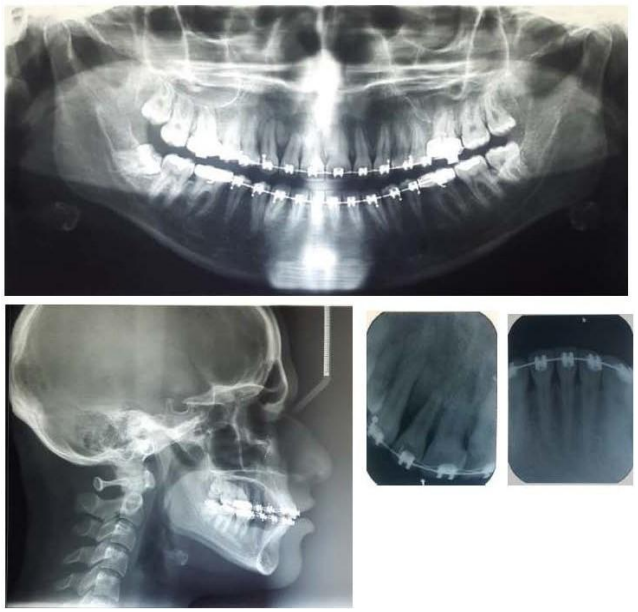

Figure 4: radiographic records taken upon approaching the end of treatment

The impacted right maxillary lateral incisor and canine were successfully positioned into proper alignment where the lateral incisor erupted after surgical removal of the complex odontome, while the canine was aligned through crown exposure and the elastic traction Figure 5. Ideal overbite and overjet, coordination of the midlines as well as class I canine relation were also achieved.

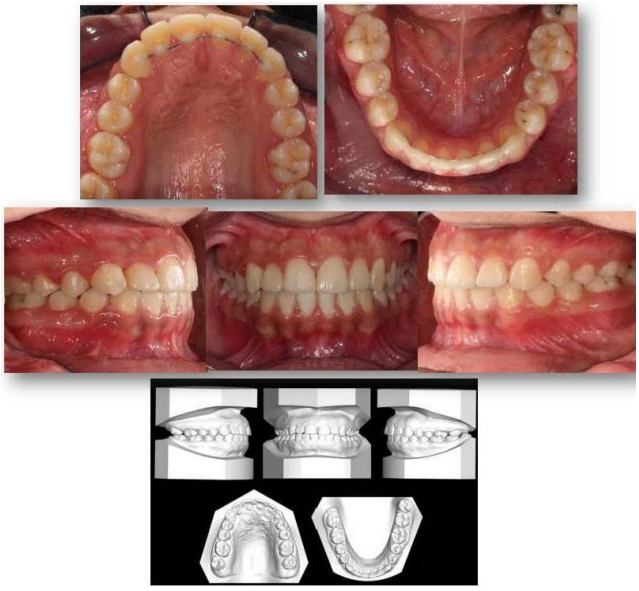

Figure 5: Post-treatment records

Radiographically, no change in root length was observed except for Grade I (slight blunting) root resorption in the upper right lateral incisor Fig. 4. Additionally, Upper and lower right 3rd molars were impacted, and the patient was referred to an oral surgeon for their extraction. The results of the cephalometric changes revealed camouflaged mild class III skeletal base relationship by correction of the inclination of the lower incisors and slight proclination of the upper ones.

Discussion

Giancotti et al., [12], Shastri et al., [13], Wen and Li [14], Ricchiuti et al., [15] as well as Pan et al., [16] have successfully treated impacted canines, yet only a few studies reported multiple impactions in the presence of odontomes [9] [17]. Although, 62% of compound odontomes are more frequently located in the anterior maxilla in association with impacted canines and 70% of complex odontomes are found in the mandibular first and second molar area [18], the current article reports a case with complex odontome in the maxillary anterior region causing multiple teeth impaction.

Treatment of such cases is usually by surgical excision of the odontome. The question lies in whether the associated impacted teeth are favourable for an eruption and later alignment with minimal side effects or not. In the current case report, 3D accurate evaluation of the site of impaction and pathology was done using CBCT rather than conventional radiographic methods as it was proved to have high diagnostic power influencing the outcomes of the treatment [19]. Detailed examination revealed the favourable position of the maxillary right lateral incisor regarding its angulation, location as well as root condition. Meanwhile, the moderate prognosis for the ipsilateral impacted canine was detected especially concerning its vertical height where it was located in the apical half of the adjacent first premolar [20].

Extraction of the retained UR C and surgical removal of the complex odontome was done initially. Bonding attachments over impacted UR 3 at the initial surgical exposure was not supported by the surgeon as it is relatively deep thus would necessitate excessive bone removal. So, it was scheduled later after the eruption of UR2 as it would probably achieve a better position and angulation. Regarding the UR2 there was a high probability of its spontaneous eruption without active pull, which would be more favourable for the periodontal health, bone support of the teeth as well as minimising undesirable risks of prolonged orthodontic forces.

Full orthodontic treatment was performed for upper and lower arches, and sufficient space was

created for the impacted canine. Surgical exposure of the impacted maxillary right canine was done using the “Closed technique” where according to recent Cochrane systematic review there was no sufficient evidence to support the use of open technique over the closed technique and vice versa; it was rather kept to the operator’s preference [21]. Bonding a button with a hole on the canine tip was done after engaging a heavy ligature wire which was then engaged to a power chain and pulled over the main heavy arch wire (0.019" x 0.025" st.st upper archwire) to avoid canting of the occlusal plane during upper right canine alignment. Eventually, an overlay wire was used to align canine while maintaining a light-traction force, thus reducing any side effects over the adjacent anchor teeth which were further reinforced by continuous lacing.

After impacted teeth were all aligned into the dental arch, residual spaces were eliminated by using continuous power chain from the maxillary right to the maxillary left first molars on 0.018” st.st main archwire. Labial root torque was needed at the upper right lateral incisor and canine, thus round st.st wire was used to facilitate this movement. After taking the panoramic radiograph, root approximation between the upper right central and lateral incisors was detected with mesial root tip of the lateral incisor. This was then corrected with an esthetic bend to achieve the desired root parallelism.

Assessment of the post-treatment photographs and study models (Figure 5) revealed that the maxillary right lateral incisor had a slightly increased labial crown torque, which had to be corrected via labial root torque, however this was avoided because this tooth showed apical root resorption and torquing the tooth against the labial cortical plate might cause additional root resorption [22] [23].

Meanwhile, no change in root length was detected except for Grade I (slight blunting) root resorption in the UL2 which was considered to be normal [22]. Furthermore, the incidence of upper lateral incisor root resorption associated with impacted upper canines is around 12% [24]**.** It was discovered later that this percentage is underestimated with the plain radiographs, where more recent C.T (computed tomography) studies showed that 48 % of upper lateral incisors demonstrate a degree of root resorption [25]**.**

According to Ericson and Kurol [26], the risk factors for resorption of lateral roots are; female patient with age less than 14 years, cases with advanced canine root development, horizontal palatally impacted canines and impactions with the canine’s crown medial to the midline of the lateral incisor. Accordingly, the current patient showed the moderate risk of upper lateral incisor root resorption as most risk factors were not fulfilled in her case except for the advanced canine root development and the gender.

In conclusion, successful orthodontic treatment of multiple impactions can be achieved with minimal side effects even when odontomes are associated, through 3D radiographic examination, detailed evaluation as well as proper biomechanical control.

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Dental Science - Review

Sugar Substitutes: Mechanism, Availability, Current Use and Safety Concerns-An Update

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Abstract

BACKGROUND: Dental caries has continued to be the major oral disease in the past, as well as the present scenario. Cariogenic sugars in the presence of specific bacteria *Streptococcus mutans* over a period have been attributed as the major etiologic agent for dental caries. The association between sugar consumption and dental caries has been well documented.

AIM: Hence, the dental profession shares an interest in the search for safe, palatable sugar substitutes.

METHODS: Therefore, the use of a suitable sugar substitute can help in combating dental caries.

RESULTS: Out of the various sugar substitutes available, xylitol is the most widely used. It is available in various forms. It decreases the plaque formation, bacterial adherence and inhibits the growth of Mutans Streptococci.

CONCLUSION: This article provides a comprehensive review of the sugar substitutes, present-day availability, role in the prevention of dental caries and their safety concerns.

Introduction

Sweetness is the taste that is strongly identified with affection and reward. Indulgence in sweets has been described as a “universal human weakness.” Carious lesions were sparse in ancient times but increased dramatically in the industrialised world. Epidemiological studies in many parts of the world support the hypotheses that increase in dental caries was associated with dietary changes. The classical evidence from Vipeholm, Hopewood house and Turku sugar studies has shown clearly the importance of diet in the carious process [1].

Many oral bacteria utilise sucrose, glucose, fructose and other simple sugars to produce organic acids (lactic, acetic and propionic) in sufficient concentration to lower the pH of plaque to levels that may result in some demineralisation of enamel [2].

Sucrose – An arch-criminal

Sucrose refined from sugar canes or sugar beets is the most common dietary sugar. A large variety of other common food like most breakfast cereals, many milk products, some meat and fish products, etc. also contain sucrose. It is also naturally present in fruit [3].

Sucrose has been called the arch-criminal in dental caries (Newbrun, 1967) [1]. This is because it is only from sucrose, that most oral bacteria can synthesise both soluble and insoluble extra-cellular polymers which increase the bulk of plaque and facilitate the attachment of bacteria, especially *Streptococcus mutans*. Unlike other sugars, sucrose can serve directly as a glycosyl donor in the synthesis of extracellular polymers.

The dietary sugars all diffuse into the plaque rapidly and are fermented to lactic and other acids or can be stored as intracellular polysaccharide by the

bacteria. The high free energy of hydrolysis of sucrose permits this reaction to proceed without other sources of energy. Thus, sucrose favours colonisation by oral microorganisms and increases the stickiness of the plaque, allowing it to adhere in larger quantities to the teeth. This property along with the high specificity of the enzymes involved in the synthesis of the extracellular polymers has led some workers to regard sucrose as having a unique role in caries. Therefore, sucrose may be expected to be somewhat more cariogenic than other sugars [3].

The prevalence of dental caries in children is declining, but children at high risk of developing dental caries are still an important public health concern. Dental caries has an age-specific characteristic in that ageing populations are also at risk of root caries. The use of non-cariogenic sweets can be recommended by professionals in these clinical settings as an important adjunct in reducing dental caries risk in these individuals. Many medicines have been found to have the side effect of producing a dry mouth (xerostomia), and prolonged use of such drugs contributes to an increased risk of dental caries, using non-cariogenic chewing gum to promote salivation would be beneficial in these cases.

Sugar substitutes

A sweetener is a food additive, which mimics the effect of sugar on taste. Therefore, they are called sugar substitutes [4].

To be an acceptable sweetener of commercial utility, a substance must:

1. Have sufficient sweetening power [1] [5].
2. Have no unpleasant aftertaste [1].
3. Be non-carcinogenic and non-mutagenic [1] [5].
4. Be reasonably inexpensive [1] [5].
5. Be thermostable (i.e. resist cooking temperatures) [1] [5].
6. Have little or no calories [5].

Classification of sweeteners

Sweeteners, which give food a sweet taste, are classified as carbohydrate sweeteners (caloric) and non-carbohydrate sweeteners (non-caloric). Caloric sweeteners are also called nutritive/bulk sweeteners and include sugar and sugar-alcohols. Sugar alcohols are erythritol, sorbitol, mannitol, xylitol, maltitol, lactitol, and reducing starch syrup [6].

The noncaloric sweetening agents are also called nonnutritive sweetening agents that have no caloric value and are not fermented by microorganisms of the oral cavity. The noncaloric sweeteners are generally much sweeter than sucrose and can, therefore, be used in smaller amounts. The high-intensity sweeteners are non-caloric, non-acidogenic. E.g., Aspartame, Saccharin, Acesulfame. They are further divided into chemically synthesised sweeteners, including saccharin, aspartame and sucralose, and those obtained from plants, including stevioside, thaumatins, and monellin [7].

There is another way to classify the sweeteners, based on the time of origin. Saccharin, cyclamate and aspartame which were the earliest known sweeteners are called as ‘first generation sweeteners”. The newer sweeteners such as acesulfame-K, sucralose, alitame and neotame are categorised as second generation sweeteners [8].

The sweeteners approved by the Food and Drug Administration (FDA) of the United States are aspartame, acesulfame potassium, saccharin, sucralose and neotame only [4] [6] [9]. Also, stevia, a natural sweetener made from extracts of a plant, has been approved for limited use [10].

Prevention of dental caries by sugar substitutes

When the general health is concerned, sugar substitutes are a useful aid to maintain reduced energy intake and body weight and decrease the risk of type-2 diabetes and cardiovascular diseases compared with sugars. Further, they also facilitate the maintenance of a nutritionally balanced diet by satisfying a diabetic person’s desire for sweets and assisting in the control of caloric intake [4].

The dentist often has the opportunity to provide advice regarding the importance of diet and the role of sugars in caries formation. Reducing the amount of sugar in the diet of humans, especially children, is an important consideration in preventing caries. Non-cariogenic sweeteners offer an alternative to sugar if used in moderation. The identification of new, safe, palatable, heat stable, non or low-caloric sweetener substitutes for the more cariogenic sugars such as sucrose, glucose, fructose and maltose would be extremely helpful in combating dental caries.

The use of sucrose substitutes in sweets is believed to have contributed in part to the decline in the prevalence of dental caries in industrialised

countries.

The anticariogenic effects of sugar substitutes include:

A. Inhibition of insoluble glucan synthesis from sucrose by Mutans Streptococci (MS).

B. The decrease in MS numbers in whole saliva and plaque.

C. Increase in the buffering capacity and pH of dental plaque

D. Interference with enamel demineralisation and an increase in enamel remineralisation [11].

Sugar substitutes of clinical importance are being elaborated here.

Sugar alcohol (polyol)

Important benefits of sugar alcohols include their none or low fermentability in human dental plaque and their ability to promote remineralisation of demineralised enamel. However, except for erythritol, the general demerits of sugar alcohols are side effects such as abdominal discomfort, flatulence, softened stools, and diarrhoea when taken in excess. Hence, they are not recommended for children less than three years of age [12].

Sorbitol (D – glucitol)

It is moderately sweet (about half that of sucrose) and relatively inexpensive. Practically all strains isolated (96%) of caries-inducing mutans group of streptococci will ferment sorbitol (and mannitol) in vitro to give a final pH of below 5. The failure of sorbitol to appreciably lower pH of plaque can be explained by the fact that, although Streptococcus mutans ferment sorbitol, the rate of acid production is much slower compared to other fermentable hexoses and disaccharides. This permits salivary buffers to neutralise acid and end products as they are formed [5] [6]. Candies and chewing gum sweetened with sorbitol are available commercially. Sorbitol-sweetened gums reported having low cariogenicity when they were chewed three times a day [12].

Xylitol

The sugar corresponding to xylitol is xylose. It is a non–fermentable, pleasant tasting, non–cariogenic polyol. It has sweetness similar to that of sucrose and has a cooling effect on the mouth. It is primarily used in chewing gum. Regular use of xylitol-containing chewing gum reduces the amount of dental plaque as well as increases the salivary flow [11].

Dental benefits of xylitol were first recognised in Finland. The first chewing gum developed with the aim of reducing caries and improving oral health was released in Finland in 1975 and the United States shortly after. The first xylitol studies in humans known as the Turku Sugar studies demonstrated the relationship between dental plaque and xylitol as well as the safety of xylitol for human consumption [1] [3].

Xylitol reduces plaque formation and bacterial adherence (i.e., it is antimicrobial), inhibits enamel demineralisation (i.e. reduces acid production) and has a direct inhibitory effect on Mutans Streptococci. The continuous-culture biofilm model showed that within a young biofilm, sucrose significantly promotes whereas xylitol reduces bacterial colonisation and proliferation. The results indicate that xylitol affects the ability of certain S. mutans strains to adhere to the hydroxyapatite [13].

Prolonged use of xylitol appears to select for a “xylitol resistant” mutant of the MS cells [14]. These mutants appear to shed more easily into saliva than the parent strains, resulting in a reduction of MS in plaque. Xylitol has been credited in reducing the transmission of cariogenic bacteria from mother to infant and has been shown to have bactericidal qualities [12] [15]. A recent Cochrane review concluded that Xylitol also increases the production of saliva and reduces the growth of acidogenic bacteria in the oral cavity [16].

Xylitol currently is available in many forms (e.g. gums, mints, chewable tablets, lozenges, toothpaste, mouthwashes, cough mixtures). Xylitol is approved for food, cosmetics, and pharmaceuticals in about 40 countries. It is used as a sweetener mainly in noncariogenic confectionery (chewing gum, candies, gumdrops, in pharmaceutical products (tablets, throat lozenges, vitamin tablets, cough syrup), and occasionally in dentifrices. A significant deterrent to the widespread use of xylitol as a sweetener is its cost, currently ten times that of sucrose [2].

Long lasting effects have been demonstrated up to years after years of using xylitol chewing gums [17].

Alanzi et al., [18] conducted a study to measure the xylitol content in the sugar-free chewing gums available in the market of the Gulf Cooperation Council (GCC) countries in the Middle East. The mean measured xylitol content/piece was 0.33 ± 0.21 g. Xylitol content was < 0.3 g/piece in 9 products, 0.3 g in 7 and > 0.5 g in 5 products. They stated that majority of xylitol chewing gums sold on the GCC market do not provide the consumers with the recommended daily dose of xylitol for caries prevention. They also recommended that clear, accurate labelling for xylitol chewing gums.

AAPD Recommendations (2014-15) for the use of xylitol in caries prevention [17]:

1. It supports the use of xylitol in caries

prevention. Clinicians may recommend its use in moderate to high-risk caries patients.

2. Dosing frequency should be a minimum of two times a day, not to exceed 8 grams/day.

3. Chewing gums, mints and hard candies have been the predominant modality for xylitol delivery. In children, less than four years, xylitol syrup 3 to 8 gms/day in divided doses should be given. In children above four years of age, the same dosage in an age-appropriate product such as chewing gums, mint or lozenges can be given.

A recent meta-analysis proved xylitol to be an effective self-applied caries preventive agent [19].

Lactitol

Lactitol is disaccharide alcohol of galactose and sorbitol obtained by the dehydrogenation of lactose. It has a sweetness that is 30-40% of sucrose, and its quality and taste resemble that of sucrose. It is not easily metabolised by acidogenic and polysaccharide forming oral microorganisms [1] [5].

Maltitol

Maltitol also termed reducing maltose, is disaccharide alcohol of glucose and sorbitol obtained by the hydrogenation of maltose. The sweetness of maltitol is 75-80% that of sucrose, and its quality of taste resembles that of sucrose [11]. In-vivo studies have shown that maltitol does not lower plaque pH [20]. A recent study showed that maltitol in chewing gums significantly reduced the concentration of cariogenic bacterial species (S. mutans, S. sobrinus, A. viscosus and Lactobacillus) in dental plaque compared to gum base [21].

Aspartame

Aspartame sold under the brand names of Nutrasweet and Equal, is a dipeptide methyl ester discovered in 1965 by James Schaltter. It is an artificial, non-saccharide sweetener [4]. Aspartame was accidentally discovered to have a pronounced sweet taste, is about 180 times sweeter than sucrose in aqueous solution [1].

Aspartame was the first sweetener to be approved by the FDA in 1981. It is the most commonly used non-cariogenic artificial sweetener. Its primary use is in diet soft drinks, yoghurt, puddings, gelatin and snack foods [5].

The manufacturers are required to label aspartame and to indicate that it contains phenylalanine, and its intake is restricted for individuals with phenylketonuria.

Based on government research reviews and recommendations from advisory bodies such as the European Commissions Scientific Committee, aspartame has been found to be safe for human consumption by more than ninety countries worldwide [22]. Despite, of some unscientific assumptions, there is no evidence that aspartame is carcinogenic [23].

Saccharin

Saccharin was discovered accidentally by Remsen and Fahlberg in 1879. Saccharin was the first artificial sweetener discovered and was well accepted during the World Wars I and II because of its low production cost and shortcoming of regular sugar [24]. It is 200 to 500 times sweeter than sucrose [1] [2]. It is an aromatic organic compound used mainly in the form of its sodium salt. Most commonly it has been used as tablets containing 15, 30, or 60 mg of sodium saccharin. Saccharin is pharmacologically inert and untoward effects are very rare [1].

There have been bladder cancer-inducing effects of saccharin from animal studies in the rat; however epidemiological studies in human did not find such effects [23].

Acesulfame – K

Acesulfame–K is 130 times as sweet as sucrose. It is stable in the temperature, pH and storage range that is likely to be encountered in foods and beverages. Safety studies have found no evidence of carcinogenicity, mutagenicity, cytotoxicity or teratogenicity [1].

In 1988, the FDA approved acesulfame-K for use in dry food products, including chewing gum, dry mixes for beverages, instant coffee, instant tea, gelatins, puddings, and non-dairy creamers. Acesulfame-K has been approved in twenty other countries, where it is also used in soft drinks, candies, toothpaste, mouthwashes and pharmaceutical preparations [1] [2].

Stevioside

Stevioside is an intensely sweet, naturally occurring compound found in the leaves of a small shrub, *Stevia rebaudiana* Bertoni, also called *yerba dulce.* It is 150-300 times sweeter than sucrose. It is a steroid glycoside.

Stevia is calorie-free, non–cariogenic sweetener. Stevioside is heat stable, resistant to acid hydrolysis and non-fermentable that makes them advantageous over the non-caloric sweeteners [25]. In 1995, the FDA approved the import and use of stevia as a dietary supplement, but not as a sweetener. Steviol glycoside has been extensively

tested to demonstrate safety for use for humans [26]. A recent study showed that the inhibitory effect of Stevia rebaudiana extract against Streptococcus mutans was superior when compared with chlorhexidine [27]. Brambilla et al., [28] evaluated the effects of S rebaudiana extracts on in vitro S mutans biofilm formation and in vivo pH of plaque. Higher in vitro S mutans biofilm formation was observed with sucrose solution. Also, in-vivo sucrose rinse produced a statistically significant lower pH value compared to S rebaudiana extracts.

Neotame

Neotame is a derivative of a dipeptide compound of the amino acids aspartic acid and phenylalanine. It is 7000 to 13,000 times and about 30 to 60 times sweeter than sugar and aspartame, respectively. It was approved by the US FDA as a general purpose sweetener in July 2002 [29].

Alitame

Alitame is an intense sweetener with sweetness potency 200 times greater than that of sucrose. It is a dipeptide of L-aspartic acid and D-alanine with a terminal N-substituted methylthietanyl-amine moiety [4].

Palatinose

Palatinose is a disaccharide of glucose and fructose. The sweetness of palatinose is forty-two per cent that of sucrose and quality of taste resembles sucrose, but the sweet taste disappears faster. It is considered an excellent sweetener for sweets and drinks for infants, children and diabetic patients [11].

Little or no acid production activity by some serotypes of mutans streptococci and other oral streptococci has been demonstrated following fermentation of palatinose, and acid production by dental plaque suspensions was noticeably lower in the presence of palatinose compared with sucrose. It has also been found that the plaque suspensions produce little or no lactate following fermentation of palatinose [7]. Candy and dairy product drinks containing palatinose are being marketed today.

Sucralose

Sucralose is a non-nutritive, non-caloric trichlorinated derivative of sucrose. It is chemically synthesised from sucrose. Sucralose is 600 times sweeter than sucrose and has been approved for use in some products. Results from various studies have shown it to be non-cariogenic [5] [11].

Sucralose is widely used throughout the world in many food products such as tea and coffee sweetener, carbonated and non-carbonated beverages, baked goods, chewing gum and frozen desserts. No health concerns have been reported with sucralose [5].

3, 6-Anhydro-l-galactose (AHG)

3, 6-anhydro-l-galactose (AHG) is a rare sugar obtained from red macroalgae. The inhibitory effects of AHG and xylitol were evaluated on S. mutans. In the presence of 5g/l of AHG, the growth of S. mutans was retarded. At a concentration of 10g/l of AHG, the growth and acid production by S. mutans were completely inhibited; whereas, 40gm/l of xylitol still showed the growth of S. mutans. These results suggest that AHG can be used as a new anticariogenic sugar substitute for preventing dental caries [30].

Safety aspects of the use of sugar substitutes

Extensive scientific research has demonstrated the safety of the six low-calorie sweeteners, ie. Stevia, acesulfame –K, aspartame, neotame, saccharin and sucralose currently approved for use in the US and Europe; if taken in acceptable quantities daily [31]. According to the current literature, the possible risk of artificial sweeteners to induce cancer seems to be negligible [23]. In studies done on the pediatric population, using aspartame and placebo, no differences in blood pressure, glucose, or lipid profiles between the two groups were observed [32]. In another study, on teenage girls using sugar-sweetened or artificially sweetened soda no differences between groups in blood pressure, waist circumference or lipid profile was seen [33]. Hence, it can be concluded that sugar substitutes have no untoward effect on the general health and metabolism of an individual.

For each sweetener, the FDA establishes an Acceptable Daily Intake, (ADI) [34] in mg per kg body weight, which is the amount of sweetener thought to be safe to consume every day for a lifetime. The ADI is typically 100 times lower than the dose of the sweetener that caused toxicity in animal studies. The acceptable daily intake ADI for sucralose in the US is 5mg/kg body weight/day. The ADI for neotame in the US is 18mg/person/day [35].

Aspartame, saccharin, sucralose and neotame are classified as food additives by the FDA, while stevia is classified as Generally Recognized as Safe (GRAS), meaning that similar data consistent with its safety exist as for food additives [36].

Recent scientific evidence indicates that routine and long-term consumption of beverages with non- nutritive sweeteners are associated with an increase in risks for type 2 diabetes, cardiovascular disease, hypertension and stroke [37]. However, for its anticariogenic properties, sugar substitutes are used for a comparatively shorter duration of time; hence these side effects would not be seen.

In conclusion, dental caries is a matter of concern worldwide, and so effective measures must be taken at grass root level to prevent it. Considering diet as a factor, sugar substitutes can be used as an effective measure to control caries, especially with the sugar-free chewing gums as they have a dual role. Sugar substitutes can play an important role in shifting the caries process in favour of maintaining dental health, and they should be recommended as part of overall preventive treatment for patients at high risk of developing caries. Although sugar substitutes have anticarcinogenic properties, there is not sufficient evidence to recommend them as a first-line anticaries strategy in light of the large body of evidence on the effectiveness of topical fluorides and dental sealants. However, they should be recommended as an adjunct to other preventive intervention strategies.

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A Qualitative Study on the Importance and Value of Doctor-Patient Relationship in Iran: Physicians' Views

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Abstract

BACKGROUND: Doctor-patient relationship [DPR] refers to verbal and non-verbal communication between doctor and patient, which is of great importance in consultation sessions.

AIM: Therefore, the present study attempts to explore the importance and value of DPR in Iran.

MATERIAL AND METHODS: The method used in the study was conventional content analysis. The data were collected from 21 faculty members (FMs) of Shiraz University of Medical Sciences (SUMS), Shiraz, Iran, who participated in three focus group discussions (FGDs). Transcribed data were analysed using Conventional Content Analysis (CCA) which identified condensed meaning units, subthemes, and themes.

RESULTS: Four themes were extracted from 198 meaning units, 87 condensed meaning units, and 17 subthemes. These included gateway [the role of DPR]; nonlinearity [the nature of DPR]; distortion (quality of DPR in the context); and dysfunctional system (weakness in health system). Generally, results showed DPR to be the gateway to consultations based on non-verbal communication and doctor empathy. The study showed distorted DPR which was due to the dysfunctionality of the health care system.

CONCLUSION: As indicated DPR plays an important role in medical contexts, but if distorted it leads to an unsuccessful outcome. Therefore, to promote DPR, it is necessary to reinforce its structure. Thus, the infrastructure has to be modified and developed at all levels.

Introduction

DPR is an important topic for discussion [1] [2] [3]. It includes verbal and non- verbal communication between doctor and patient [4] [5], which lead to a bilateral exchange of views. Each clinical communication has three aims including a good personal relationship, exchanging information and making decisions [4]. Thus DPR plays an important role in healthcare systems [6].

Scholars have presented different DPR, having three diverse models with three features. These include activity-passivity, guidance-cooperation and mutual participation [4]. According to scholars, DPR is situational, and each of its three models is only suitable for a particular condition. Also, Emanuel and Emanuel have presented additional models which are paternalistic, informative, interpretive, and deliberative. They believed that in the deliberative model physician helps patients explore health-related values and choose a treatment based on these values, which is best suited for DPR [7]. At the same time, there are two models of medical encounter including socioemotional and task-oriented versions. Satisfaction is considered a socioemotional result and recall and compliance are regarded as task-related outcomes [8].

Generally, these models could be classified into two groups including physician-centred and patient-centred models. The physician-centred model is recognized by the domination of physicians with the exchange of biomedical information, whereby the patient input in medical dialogue is the least [9] [10],

asymmetrical and dominated by the power of the physicians [11] based on absolute knowledge and medical standards [10]. This view legitimates asymmetrical power relationship. However, in a patient-centred model doctor communicates with the patient according to his or her views and values [12] [13] [14] [15] [16]. This view is based on an equal power relationship.

In the sociology of medicine, there are two rival approaches. On the one hand, there is a Parsonian view which legitimises the asymmetrical power relationship [17] [18]. Parsons theory of social system is based on a phrase of sick role [19] [20]. A sick role is a functionalist approach of medical institutions [21] [22], where illness and disability are defined as social deviance [19] [23] and abolished on patients' gaining ability to return to society [24].

On the other hand, there is critical sociology which theoretically criticises modern medicine by focusing on the critical point of DPR [10] [25] [26] [27] [28]. Michel Foucault is a pioneer sociologist who has criticised the knowledge-power discourse of modern medicine [29]. In his writing on The Birth of Clinic he indicated that the clinic is constantly praised for its empiricism, the modesty of its attention, and the care with which it silently allows things surface to the observing eye without being disturbed by discourse which owes its real importance to being a reorganization in depth, not only in medical context but because of its most likely impact on discussion about disease [30]. Jürgen Habermas is another critical sociologist whose theory criticises the medicine as a symbol of the modern institution and expert power [31].

Generally, these two rival theories view medicine from different aspects. The important point is that DPR is of pivotal importance in, where it plays a crucial role in medicine by its specific impact on doctor-patient satisfaction [32] [33] [34] and promoting the efficiency of consultation, a milestone in the present study. Despite its significance, this issue has been neglected in Iran and has only been the subject of a doctoral dissertation prepared in teaching hospitals affiliated with SUMS [1] [3] [9]. Additionally, the past few years have witnessed some critical discussions about the relationship and communication problems regarding doctor-patient interaction in Iran's public sphere, a reality that reflects the importance of present investigation.

Methods

The data of this qualitative research were collected from April to September 2014. The Department of Ethics and Philosophy of Medicine at Shiraz University of Medical Sciences [SUMS] invited 40 academics to discuss DPR. The data for this study were collected from target groups between April to September 2014. The method used for sample collection was heterogeneous sampling or maximum variation sampling because the study intended to detect high variations in perspectives. The participant received letters of invitation to the study and briefed on the research objectives. The inclusion criteria were more than 3 years of teaching and clinical experience in different medical fields, being members of the university during this study, and not being retired or from outside.

The focus group discussion [FGD] method was used for the collection of data. The reason for using this technique was access to heterogeneous perspectives and collective opinions of the participants. FGD included a moderator and a note taker who recorded the points discussed. The moderator brought up the questions which were then discussed and answered by the participants. Two fundamental questions raised in the discussion group were as follows: *[1] How important is the communication between physicians and patient? [2] What is the situation of doctor-patient communication in Iran?* The moderator led the participants to the issue concerned whenever they deviated from the main topics. Before starting discussions and for ethical considerations, the verbal consent of the participants was obtained regarding the digital recording of the topics under discussion.

Based on saturation criteria, three focus group discussions attended by 21 participants were concluded with the aim of obtaining maximum information. Of three focus groups, two lasting 1.15 and 1.25 hour were held in the department of medical ethics, and one, lasting 1.05 hour, in the conference room of Nemazi hospital.

The information obtained was transcribed and analysed using Conventional Content Analysis [CCA]. As a whole, there are three methods for content analysis. These are conventional, directed, and summative approaches. In the conventional method, the researcher analyses the data, regardless of the previously determined theoretical framework about the subject [34]. The coding of data was performed by the constant back and forth movement across the data and by interpreting the statements made by the participants. This was a continuous process and performed by back and forth movement of analysts between data, concepts and extracted codes. The concepts and codlings were directly extracted from the data. The greater the level of movement toward pivotal codes, the lager was the level of data segregation. Thus data were interpreted to explore condensed meaning units [brief meaning of the interpretation], sub-themes [the initial abstracted concept that explored the related condensed meaning units], and themes [an abstracted concept about some subthemes]. Accordingly, all concepts obtained from research data, through back and forth movement, had

the highest level of categorisation.

The present research was validated by member check method [35] whereby participants were informed about the extracted concepts, and their approval constituted the authenticity of the research. The credit-rating or trustworthiness was another issue which attracted the attention of participants. This was observed during the study by maintaining subjectivity and reflexivity, adequacy of data, and efficiency of interpretation strategies [36]. The selection of conventional content analysis and considering the research objective formed the basis for stepwise collection and analysis of data. The credibility of coworkers was also considered about research design, methodology and analysis of data. The credibility was ensured by members check and peer debriefing, transferability by thick description, and conformability through reflexivity. Also, reflexivity was observed with drawing attention to the production of knowledge and minimising the prejudgments of researchers.

The present study was based on ethical research code of the Helsinki declaration and conducted according to the ethical committee of SUMS. Also, alongside obtaining the consent of participants to take part in the study, attempts were made to observe the anonymity of participants, including all stages from data collection to the final research report.

Results

Twenty-one physicians with 11 specialities participated in this study (Table 1).

Table 1: Participants' characteristics

Field of Specialty	Number of participants [s[	Number of focus groups
Surgery	1	1
Psychiatry	5	1-2-3
Internal Medicine	1	2
Gynaecology	2	2
Dentistry	3	2
Endocrinology	1	2
Pathology	2	1,2,3
Psychology	2	1,3
Radiology	1	1
Anesthesiology	1	1
Nephrology	2	3

The results of 198 meaning units, 87 condensed meaning units, 17 subthemes and 4 themes are demonstrated in Table 2.

Generally, results showed that DPR is an important part of medical care. All participants agreed that a proper DPR should lead to mutual satisfaction in both parties. Thus, the doctor feels that his/her treatment is on the right path. At the same time, patients feel that they have achieved their goals in the consultation. Furthermore, because of mutual understanding, the patient feels that the consultation has been effective. All participants emphasised the importance of DPR. As shown in Table 2 four themes extracted from the coding processes include *gateway, nonlinearity, distortion,* and *dysfunctional system.*

Table 2: Conventional content analysis of the doctor-patient interaction

Meaning unit	Condensed meaning unit	Subtheme	Theme
I think interaction is the key to treatment domain. We communicate to enter this domain by asking questions.	Interaction is the key	Speciality of DPR	Gateway
Physical examination needs to be more interactive than communicative.	Importance of interaction		
In Psychiatry, interaction is the key. A good relationship means good interaction.	Good DPR is equal to good interaction	DPR is key to good interaction	
I have two Iraqi patients are unable to communicate in the Persian language, and I do not speak Arabic either. But we have complete trust in each other.	Trust is created with unknown events	The unplanned trait of relationship	Nonlinearity
We have a good relationship with each other which leads to empathy .But most of the times we do not know how this form of communication has happened and why it has evolved	Both parties do not know how good relationship and empathy occur	Humanistic DPR	
By nodding your head or saying um… and with eye contact, the patient feels that the doctor understands him/her and has no feeling of being ignored.	Nonverbal communication has important role		
Even today, there are still some physicians, even with good reputations, who do not even talk to their patients	Silent doctors	DPR without meaning	Distorted DPR
A mechanical relationship means that no attention is paid to the patient's psyche; the person in front of you is a human being whose soul and psyche should be taken into account	Non-humanistic relationship		
The doctor performs an examination and then decides without any communication	Treatment without communication	One-dimensional relationship	
Some doctors are inclined to ask questions, and when a person sits in front of them, they start asking questions in an interrogative tone.	Interrogator relationship		
At present, there is no course or workshop for medical ethics, and even now, such courses are not offered during our residency period!	Education in DPR and ethical issues are ignored.	Malfunction and dysfunction of medical education.	Dysfunctional system
In the clinics, students learn from their academics how to give consultation without having to communicate	Passive DPR Hidden curriculum.		
There is no control over doctors. Some specialists work late until 2 AM. I know a surgeon specialist in Tehran who has employed 10 surgeons to work for him anonymously.	Doctors are not controlled and supervised.	A system without monitoring and controlling	
We do not have any screening plan for selecting medical students.	Lack of monitoring and controlling.		
The biggest obstacle is the system. Our flawed health system forces the attending physicians to visit 50-60 patients per day.	There is no balance between the number of patients and doctors.	Irresponsive system	
When I have to visit 100 patients in a Hospital, I cannot even understand what the thirtieth or the fortieth patient is saying, or I simply refer him/her to my residents.	The high volume of patients and weak communication.		

Gateway refers to the importance of DPR in good treatment. Without the gateway, an inefficient treatment is natural because the doctor cannot enter the private domain of a patient. Thus, for better treatment, a physician needs to communicate with the patient. He needs to ask about the history of illness and its signs. Also, a successful treatment hinges on a complete knowledge about the patient, which is achieved by an active DPR. Thus, DPR is a gateway of medicine

"I think interaction is the key to treatment domain. We communicate to enter this domain by asking questions. Physical examination needs to be more interactive than communicative. The dialogue should be done in the second stage, after gaining the patient trust in a way he/she accepts the physician" (Surgeon of FGD 1).

This interaction is a stepping stone, which if not present the treatment will not be successful.

"In our field, interaction is the key. A good relationship means good interaction. The first step in DPR should be based on a good Rapport. It means

that the doctor has to have a warm, empathic relationship with patients. He/she has to sense the patient's problem and needs" (Psychologist of FGD 2).

As these statements show, DPR is the key or the gateway of interaction. It means that without good relationship doctor cannot have an effective treatment, and will not gain the patient's trust.

DPR is not a linear path which can be planned. It is an ongoing and nonlinear process. It is a flexible and complex phenomenon due to its humanistic structure. Generally, an important trait of DPR is non-verbal communication that causes nonlinearity in a relationship. The initial eye contact is very meaningful that tells both parties how this interaction will continue. Meanwhile, greeting, posture and doctor's tone of voice are the non-verbal communications that produce a communicative package. Undoubtedly, the first eye contact plays an important role and establishes the quality of the relationship.

"During the initial eye contact empathy is shaped, then the next interaction occurs that opens the path of diagnosis and progressive treatment" (Psychiatric of FGD 2).

Thus, a good DPR is heavily dependent on the first interaction. In this stage oral communication does not have any significant role.

"By nodding your head or saying um… and with eye contact, the patient feels that doctor understands him/her and does not have the feeling that he/she is talking to a wall" (Psychologist of FGD 1).

Non-verbal communication and *empathy* lead to the nonlinearity of communication. This means that both parties had not decided on how to communicate with each other beforehand.

"I have two Iraqi patients who were unable to communicate in the Persian language, and I do not speak Arabic either. But we have complete trust in each other. We liked each other which led to empathy. However, most of the times we didn't know how this form of communication had happened and why it developed" (Surgeon of FGD1).

Another theme is called distortion which is related to the quality of DPR in the context of this study. All participants were concerned about the quality of DPR in Iran. They believed that DPR is flawed and derogatory to patients. A distorted relationship is one-sided and regulated by the doctor's personal experiences, without verbal and non-verbal communication that does lead to a mutual understanding. Such a relationship would ignore the scientific principles of diagnosis and treatment. The doctor, instead of engaging in a dialogue or any verbal or active non-verbal interaction, finalises the consultation within several minutes. The statements have shown that a distorted DRP, within the context of this study, had turned into a *norm*, where the doctor may even conduct his consultation without any verbal communication at all:

"Nowadays, there are still some physicians, even those with good reputations who do not even talk to their patients (Infectious infant specialist of FGD 3) As witnessed, in the governmental system,, verbal communication has sharply declined and it has leaned towards a situation where the doctor performs an examination and then makes a decision without any type of communication" (Psychiatric of FGD 3).

"Don't even talk" indicates a total absence of verbal communication between the doctor and patient. In such situation, diagnosis and treatment are mainly based on the doctor's experiences or laboratory data. In extreme cases, the patient may feel that the doctor does not consider him/her as a human being in need of treatment. A distorted relationship is mechanical and passive in which no interaction takes place. Also, the doctor would not gain any understanding of the patient's perception of the illness, and thus, the patient feels that part of his/her existence has been ignored. As such, the interaction is governed by an instrumental relationship.

"A mechanical relationship means that no attention is paid to patient's psyche; the person in front of you is called a human being whose soul and psyche should also be taken into account. By considering psychological issues, the doctor can treat many of his physiological problems, and the psychological aspects should not be ignored or suppressed, or treat the patient as an object. Treating a patient is not like repairing a car. The doctors do not look at their patients, but they only look at the lab tests papers" (Psychiatric of FGD 2).

So, a distorted relationship deviates from its natural pathway for transferring the meanings, and thus it cannot contribute to a common understanding of the disease, its diagnosis and possible treatment. Under such circumstances, diagnostic and therapeutic purposes are ignored. Another characteristic of a distorted DPR is inequality, where the doctor makes a one-sided diagnosis and prescribes a treatment, while the patient leaves the office without any interaction. There is even a higher level of inequality in doctors' conduct and their patterns of questioning, shaping an interrogator style of dialogue:

"He is not supposed to act like an interrogator! Some doctors are just like that, and when a person sits in front of them, they start asking questions in such atone" (Psychiatric of FGD 1).

In an interrogative interaction, the patient faces a multitude of closed questions which should be answered with Yes/No. If the patient wishes to change the direction of the consultation, for example by asking a question about diagnosis or therapy, the question is simply suppressed and ignored. As such, either the doctor prevaricates, or if the patient insists,

he/she may receive an unpleasant answer. Beside, dissatisfaction and ineffectiveness, the main outcome of distorted DPR is patient' deception.

"Deception frequently happens, specifically in the field of Gynecology which includes differential diagnosis. The patients who come to us are young and healthy and do not have any medical problem. Despite this, deception is frequently taking place" (Specialist in OB-GYN *of FGD2).*

The dysfunctional system refers to the weakness of the healthcare network in the systematic management of DPR. All participants agreed that there is a structural failure in managing the medical institutions which have led to distorted DPR. Healthcare system is lacking strategy regarding macro and middle levels of medical systems in providing a theoretical framework for a good DPR. Due to a large number of referrals, doctors are forced to give consultation to many patients considering standard protocols.

"When I have to visit 100 patients in the Hospital, I cannot even understand what the thirtieth or fortieth patient is saying, or I simply refer him to my resident. How much strength should I have to visit 100 patients!?" (Nephrologist of FGD 3).

This problem is not only related to admitting patients, but there is a similar situation in the clinic. For instance, a psychotherapist has to spend at least thirty minutes with each patient, but those in charge have different expectations.

"The biggest obstacle is the system. Our flawed health system forces the attending physicians to visit 50-60 patients, while everything is dependent on interaction, and if non-existent, even in case of emergency, no matter how much I try to help, it won't work" (Psychiatric of FGD 1).

Also, the health system focuses on the number of services, merely by increasing the number of consultations. In such a situation, the criterion for professors is simply promotion and the number of papers they have published, while their performance is not evaluated.

"In Professors' Promotion Form, there's no item for quality, and the whole story is about the number of published papers. The best academic that I've seen with numerous papers receive half a dozen of patients in his room, and never allows them to talk, and for the sake of his promotion, quality was not considered, and the only thing which was important was the number of published papers! I wonder how much these papers are going to help patients in reality" (Internist of FGD 2).

Another characteristic of the dysfunctional system is poor control/supervision, or even lack of surveillance overtreatment, which adversely affects DPR processes. Lack of control and supervision on physicians' work seems to be intentional with its benefits.

"…I just wanted to say that we are not satisfied ourselves, because there is not even a system to put us within a framework, and this is a serious obstacle" (Surgeon of FGD 1).

Dysfunctional system neglects to teach ethics and philosophy of medicine. In this structure, DPR skills are not taught, and ethical problems are not explored.

"During our time, there was no course or workshop for medical ethics, and even now it is missing during our residency period! So, teaching such issues is not important, and professors do not expect students to know about them. The only important thing is the lab test results and medical procedures, and not the way you treat the patient". (Psychiatric of FGD 2).

Finally, there is no effective screening system to select medical students.

"Here, no attention is paid to medical students when they are accepted; most of them should not even be allowed to choose medicine as their major in the first place and are fit for other majors" [1].

Discussion

This study aimed to explore the condition of DPR according to views of FMs. Results showed that DPR is the key to successful diagnosis and treatment. It plays an important role in medical interactions where if not present physicians cannot provide appropriate treatment and enter into the patient's private domain. Also, DPR is a nonlinear phenomenon which presents interaction between two human beings. Nonlinearity means that both parties do not know how and where the interaction begins and how it continues. This also means that interaction is heavily dependent on non-verbal communication specifically the eye contact and empathy. Despite the importance of DPR and its characteristics, the unequal and distorted DPR has become the norm. Distorted DPR is more related to the dysfunctionality of health care system which is due to lack of strategy about a successful DPR.

Our study showed that a good DPR has two main components which are non-verbal communication and empathy. As Friedman mentioned, non-verbal communication through touch, facial expression, voice tone, etc. is essential for a successful patient-physician interaction [38]. Also, non-verbal mode characterised by nodding, forward lean, direct body orientation, uncrossed legs and arms, arm symmetry, and less mutual gaze is shown to be positively associated with outcomes of DPR [39].

Regarding the distorted DPR, our findings were confirmed by other studies [1] [40]. However, there are different perspectives about distorted DPR. Sadati et al. showed that the asymmetrical power relationship is related to modern medicine discourse [1], the domination of paraclinical standards [10], and dysfunctional healthcare system [3]. Additionally, Mishler's classic study showed that this form of interaction is related to the separation between the voice of medicine and the voice of lifeworld [36]. Barry et al. showed that type of illness could affect the quality of DPR [38]. This study showed that asymmetrical and distorted DPR is due to the dysfunctionality of health care system which is in line with Sadati et al., a study [41], a situation leading to several interactional problems in DPR. [42].

Our study revealed that dysfunctionality of health care system plays a pivotal role in the formation of distorted DPR. Thus, distorted DPR is related to the nature of modern medicine and its voice, but it is also associated with the health care system approach to DPR. When the health care system does not have any strategy to address this issue, medical students have no clue as to how to initiate a good relationship. Also, due to lack of active surveillance in the system, doctors do whatever they wish.

According to our results and concerning functionalist theory or Parsonian theory a poor form of DPR is due to the dysfunctionality of the system. In this context, we are witnessing a hidden conflict between the structure and the agency. Also, the agencies are tremendously powerful because there are no plans to manage, control and survey the inherently fragile structure. According to the critical theory, we can say that the powerful discourse of modern medicine is naturally suppressive and leads to distorted DPR. According to the results of other studies [1] [41], an asymmetrical power relationship is expected that includes different shapes and forms of suppression.

In conclusion, the results of this study showed that the gateway to a successful diagnosis and treatment is active DPR, which is a nonlinear phenomenon and related to non-verbal communication and doctor's empathy. In this context, the dysfunctionality of the healthcare system leads to distorted DPR. Knowing that the system suffers from this problem, there is as yet no strategy to deal with this issue. Therefore, if a powerful and strengthened DPR is desired, appropriate measures should be taken to reinforce its structure. This can be achieved by modifying and redeveloping the underlying infrastructure. Finally, system surveillance has to be promoted in addition to the fundamental revision of medical ethics.

Limitation and recommendation: The main limitation of this study was that it only presented the subject from a physician's perspective. Thus, future studies are warranted with a variety of views including patients, doctors of private hospitals, nurses and other caregivers of the health system. Also quantitative studies on this subject are proposed.

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A Dermatological Questionnaire for General Practitioners with a Focus on Hidradenitis Suppurativa

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BACKGROUND: Hidradenitis suppurativa (HS) is a skin chronic inflammatory disease typically located in several areas such as perianal, inguinal and axillary regions. In 40% to 70% of cases, general practitioners (GPs) are the first health care professionals consulted by patients suffering from HS. The role of GPs in HS management could be more substantial than it has been in the past.

AIM: We developed a questionnaire to assess the knowledge of HS by GPs and to evaluate if in their perception the dermatologist is the reference medical doctor for pathology above.

METHODS: The data were processed by a univariate descriptive statistical analysis.

RESULTS: Our study showed GPs could recognise patients affected by HS. They have proven to know the main features of HS. Nevertheless, the second part of the questionnaire has highlighted the considerable confusion of GPs about who the reference figure is.

CONCLUSION: The data registered regarding therapy and follow up too, only show a mild preponderance of dermatologist compared to other professional figures, such as a surgeon, GPs and plastic surgeon.

Abstract

Introduction

Hidradenitis suppurativa (HS) is a chronic inflammatory disease, commonly characterised by painful, deep dermal abscesses and chronic, draining sinus tracts. Lesions are typically located in several skin areas such as perianal, inguinal and axillary regions [1] [2]. The prevalence rate is not finally defined yet: it varies between 0.0003% and 4% depending on the study population [3] [4]. Estimates from insurance databases suggest a prevalence of < 0.1% [5] [6].

This variation strongly suggests a significant selection bias or misclassification, and it may be speculated that not all patients ask any healthcare consultation. Also, diagnosis of HS may usually be delayed for years, and even when diagnosed, is challenging to treat [7].

The healthcare system identifies the general practitioners (GPs) as the first reference figures for the citizen in the health care. The situation does not change for HS.

In 40% to 70% of cases, GPs are the first health care professionals consulted by patients suffering from HS [8]. The role of GPs in HS management could be more substantial than it has been in the past. Moreover, GPs are still the primary

caregivers for 15% of patients after an HS diagnosis is received [7].

The main goal of the study is to assess the knowledge of HS by GPs and to evaluate if in their perception the dermatologist is the reference medical doctor for pathology above.

Methods

At the Department of Clinical Medicine and Surgery, Section of Dermatology of University Hospital Federico II of Naples, we developed a questionnaire (Table 1) on HS, structured as follows:

- 6 knowledge questions about the pathology;
- 5 related questions to HS diagnosis, therapies and follow up.

The paper mentioned above questionnaire was filled by 150 GPs from Campania, Italy.

The results were expressed as a percentage.

Table 1: Six knowledge questions about HS (first part of the questionnaire) and five questions about HS diagnosis, therapies and follow up (second part of the questionnaire)

QUESTIONNAIRE (first part)
HS manifest with painful skin lesions
HS manifest with inflammatory nodules
HS manifests with abscesses
HS manifests with draining fistulas
HS manifests with scars
HS occurs with lesions typically localised in the following regions: axillary, inter-inframammary, inguinal, perineal, gluteus
Options: I do not agree – I do not know – I partially agree - I fully agree
QUESTIONNAIRE (second part)
The diagnostic suspicion is supported by
In the diagnosis of HS, the reference figure is
In the HS therapy setting, the reference figure is
In the management of drug therapy (topical/systemic), the reference figure is
In the follow up of HS patients, the reference figure is
Options: Dermatologist – Surgeon – General Practitioner – Plastic surgeon

Results

The first part of the questionnaire consists of 6 general questions about HS. The collected responses showed a good knowledge of the disease by GPs. More than 80% (partially agree + fully agree) showed to know that HS manifests with painful skin lesions, with inflammatory nodules, abscesses and draining fistulas.

A smaller percentage of respondents (75% = partially + fully agree) proved to be aware of the anatomic sites involved by the disease and that HS is also characterised by scarring (Figure 1).

In the second part of the survey, according to GPs involved in the study, diagnostic suspicion is supported by a dermatologist (24%), surgeon (26%), GP (38%), plastic surgeon (12%).

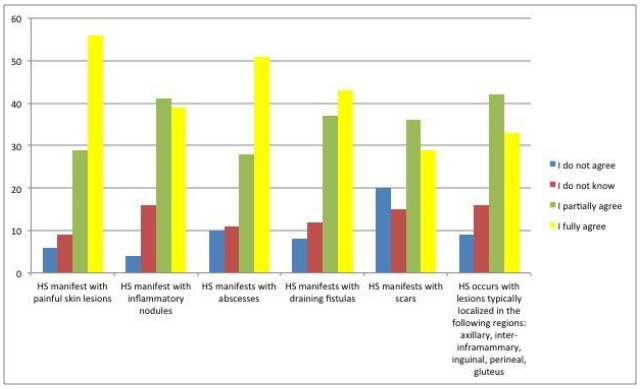

Figure 1: Results of 6 knowledge questions about HS (first part of the questionnaire). The collected responses showed a good knowledge of the disease by GPs. More than 80% showed to know that HS manifests with painful skin lesions, with inflammatory nodules, abscesses and draining fistulas even though GPs were not aware that HS is also characterised by scarring

In the diagnosis of HS, dermatologists are slightly most involved (33%) compare to the surgeon (24%), GP (26%) and plastic surgeon (17%).

In the HS therapy setting the reference figure is dermatologist (39%), surgeon (20%), GP (26%), plastic surgeon (15%).

In the management of drug therapy (topical / systemic) the reference figures are as follow: dermatologist (44%), surgeon (16%), GP (24%), plastic surgeon (16%).

In the follow up of the HS the reference figures are as follow dermatologist (30%), surgeon (24%), GP (31%), plastic surgeon (15%) (Figure 2).

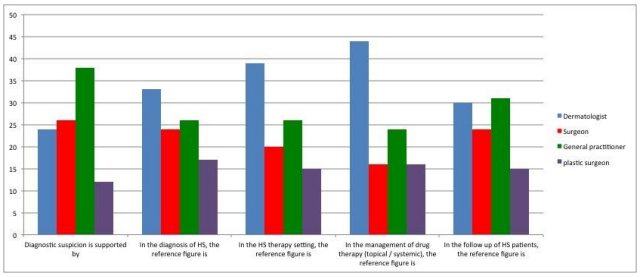

Figure 2: Results of 5 questions about HS diagnosis, therapies and follow up (second part of the questionnaire). This part of the questionnaire has highlighted the considerable confusion of GPs about who the reference figure is for HS

Discussion

The diagnosis of HS is based on the presence of recurrent painful or suppurating lesions more than twice in 6 months in the considered 'typical' areas of

the body, including axilla, genital area, perineum, gluteal area and, in women, infra-mammary area. Long delays in diagnosis are common since HS is frequently misdiagnosed as a simple infection [9].

Our study showed that GPs could recognise patients affected by HS. They have proven to know the main features of HS. However, initial or mildly severe clinical frameworks can easily be confused with other pathologies whose overall management is different. This data affects the problem of delayed diagnosis and contributes to determining worsening conditions [10].

The second part of the questionnaire has highlighted the considerable confusion of GPs about who the reference figure is.

The data registered regarding therapy and follow up too, only show a mild preponderance of dermatologist compared to other professional figures, such as a surgeon, GP and plastic surgeon. Inadequate management is more frequently associated with a wrong diagnosis. The consequence is the worsening of clinical manifestations with an increase of severity degree.

It is well known that it is easier to treat mild forms of HS, compared to severe forms that are less responsive to therapies. Also, the disease progression has a significant impact on the quality of life of patients. Furthermore, the literature shows that inadequate management of HS patients causes worsening not only of skin but also of systemic clinical conditions [11] [12].

Indeed, HS has been considered a systemic disease because of the possible association with several comorbidities like endocrine disorders, such as diabetes and hyperinsulinemia, acromegaly and Cushing disease, cardio-metabolic comorbidities, metabolic syndrome, obesity and other conditions like inflammatory bowel diseases (especially Crohn disease), spondyloarthropathy, genetic keratin disorders associated with follicular occlusion and squamous cell carcinoma [13] [14] [15] [16] [17] [18] [19] [20]. Early recognition of the HS associated diseases and a timely therapy improve disease outcome and can prevent long-term complications.

The worsening natural history of the disease, together with co-morbidities, makes necessary a multidisciplinary approach to HS. The multidisciplinary assessment of patients allows a complete evaluation of the disease and a more comprehensive treatment approach compared with traditional consultation.

In a shared and multidisciplinary approach, the GP plays a key role, as the first physician interfaces with patients suffering from HS.

The GP should be able to recognise the dermatologist as the reference figure in the treatment of HS patients, notwithstanding the contribution of the other medical figures indispensable for the proper management of a multidisciplinary pathology, such as HS.

In conclusion, this study emphasises the need for education of GPs to make an accurate and early diagnosis, to initiate treatment and obtain the best management of HS patients.

Future objectives include submitting the same web-based questionnaire to the largest number of general practitioners on a national scale.

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Sexual Desire and Related Factors in Middle-Aged and Elderly Married Women: A Cross-Sectional Study in Iran

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Abstract

BACKGROUND: The sexual desire in the middle-aged and senior women is one of the factors affecting their quality of life and psychological well-being.

AIM: The present study was aimed to assess the sexual desire and related factors among married women aged 50-70 years.

MATERIALS AND METHODS: In this descriptive-analytical study, 210 married menopausal women aged 50-70 years were selected by cluster sampling method. Data were collected using a demographic profile questionnaire and Sexual Desire Inventory (SDI), and analysed by SPSS version 22 software using descriptive statistics and independent t-test, ANOVA and Pearson correlation coefficient tests.

RESULTS: The mean age of women was 59.40 ± 5.93 years, and the mean sexual desire score was 22.66 ± 17.78 (out of 112). There was a significant relationship between sexual desire score and age, educational level, occupation of women and spouses, age of menopause, marital history, number of pregnancies and children, individual health and pain level ($P < 0.001$). The sexual desire score had a significant association with diabetes, hypertension, heart disease, high cholesterol, chronic pain, gastrointestinal problems, chronic ulcers, bladder and intestinal problems, joint and bone disorders, taking cardiac medications, antihypertensive, anticoagulant, insulin, cholesterol-lowering drugs ($P < 0.001$).

CONCLUSION: The low sexual desire score and its reduction with age and the presence of various diseases and factors affecting sexual desire highlight the importance of diagnostic screening, family related educational planning and the role of health care providers in the health status of the older adults.

Introduction

Elderly is described as one of the most important periods of life that has been neglected. The changes in the physical appearance and the roles of older adults often led the community to forget that the older adults are not an isolated generation [1]. Health has a variety of dimensions, one of which is sexual health [2]. The World Health Organization (WHO) defines sexual health as "a state of physical, emotional, mental, and social well-being in relation to sexuality; it is not merely the absence of disease, dysfunction or infirmity", but it is a relatively good sexual state requiring a positive approach to sexuality and sexual relationships and a pleasant experience without fear, shame and coercion [3]. Neglecting elderly people and their needs, such as sexuality, affects their quality of life and the feeling of being "good" [2] [4]. Sexual desire has been considered as one of the fundamental human needs, although this need goes beyond the biological realm and also has physiological, social and spiritual dimensions [2] [3]. The sexual desire can promote the growth of individual personality and relationship, and contribute to overall life stability, as well as provide opportunities

to access new advancements and gain experience. The sexual desire of the elderly people is in a challenge with a kind of indifference around the world because the culture of society and the general public does not acknowledge this need in elderly people and perceive sexual interests in elderly people as a deviant behavior [3] [5]. Sexual desire is not just for young people but it is one of the important dimensions of the lives of elderly people, especially those who had been sexually active throughout life. Despite decreasing sexual relationships with age, feeling of interest, need for intimacy, communicating and sexual desire remain strong among elderly populations and is a motivator for continuing and improving the quality of their life. The elderly people through meeting these needs can take more pleasure in their lives [6] [7] [8] [9]. Gott et al., found that despite the benefits of the sexual desire physically and psychologically, there are many barriers to the sexual desire for elderly people, as the most common cases are the absence of a sexual partner through death, divorce or illness and that the attitude of elderly towards the sexual desire is formed on the basis of the cultural and social context existing in the society and not based on their belief [10]. It has shown that women, compared to men, in the aging course are more concerned with cases such as the quality of the relationship or the rational health, among which the physiological factors of the relationship are more important to them and that the absence of the partner makes the elderly to seek alternative relationships [11] [12].

The results of a study showed that the sexual desire of older adults is extensively influenced by the emotional and physical satisfaction of the individual, as well as the length of the relationship between the elderly and spouse, has been reported to be an important issue. Moreover, the elderly following many years of life alongside the spouse can be sad because of death and is no longer involved with sex [7] [13]. The results of a study showed that the sexual desire enhances the need of the elderly to interact with each other and respect for the partner [14]. Moreover, it has been shown that attitude toward the sexual desire is heavily influenced by the beliefs and attitudes of the ancients. The ancients spoke less explicitly about the sexual desire as if sexual pleasure was only special for men at the time and the role of women was the only sexual satisfaction of husband or reproduction [3] [5].

According to the abovementioned introduction, the sexual desire has merged with personality and identity and plays an important role in adapting and improving the quality of life of the elderly.

Further, because the sexual desire is ignored in older adults and is affected by certain factors, the present study was conducted to investigate the sexual desire and its related factors among married women aged 50-70 years old who referred to Health centres in Sabzevar (Iran) in 2016.

Materials and Methods

The present descriptive and analytical research was conducted on married menopausal women aged 50-70 years who met the inclusion criteria. The sample size was estimated to be 210 people using a pilot study and a sample size formula of $n = \frac{Z^2_{1-\frac{\alpha}{2}}\delta^2}{d^2}$ at 95% confidence interval and 90% test power. After obtaining approval from the Ethics Committee of Bojnurd University of Medical Sciences, the samples were collected by systematic cluster sampling method. Thus, first, the city of Sabzevar was divided into four main clusters based on the statistics of households with married women aged 50-70 years, the sample size was then calculated systematically in each health centre, and some medical cases were randomly selected at each centre. The female researcher referred to their address given in the case, and the sample was selected based on the criteria listed in the sample selection checklist after completing the informed consent form and measuring blood pressure and blood glucose (to gain the trust of cooperation).

Exclusion criteria included the absence of depression and psychological illness known in the individual and the spouse, the absence of any physical, mental and motor disabilities, the lack of physical constraints and dependence of physical movement of women and spouses in the daily activities, no history of oophorectomy, hysterectomy and mastectomy in women, and no history of prostatectomy in a spouse. Before interviewing and completing the questionnaires, people were given explanations about the confidentiality of the information, the objectives and methods of study as well as how to answer the questions, in the condition that people had a mental readiness to answer the questions.

Data were collected using a demographic profile and SDI questionnaires. Demographic information questionnaire included a general profile of women and spouses (age, occupation and educational level), characteristics of marital life (menopause age, years of marriage, number of pregnancies and number of children), and questions on underlying diseases, drug use, and health status of the individuals. The validity and reliability of SDI were used by Tracee and Moore in a study on older adults, whose validity was confirmed by content validity and whose reliability by Cronbach's alpha of 0.86 [15]. The questionnaire consisted of 14 questions, including nine items for the sexual desire for the interpersonal relationship, four items for assessing the individual sexual desire and one question for any sexual desire in the individual. For questions 1, 2, 10 and 14, the respondent needed to select one out of eight options available. A number must be selected for other questions on an 8-point Likert scale as 0 (= 'not at all')

means no sexual desire and 7 (= 'more than once a day') means the maximal sexual desire. The final score of the questionnaire was calculated by adding scores, giving a maximum score of 112. For each question, the respondents were asked to choose which of the options is most similar to their thoughts and feelings of interest and desire for sexual activity in the last month.

The content validity method was used to verify the validity of the questionnaires. Thus, this form was set by studying the latest references in the field of research under the supervision of the supervisor and then introduced to 10 experts including the supervisor, consultants and faculty members of the Faculty of Nursing Midwifery at the Bojnurd University of Medical Sciences. The questionnaire was utilised after including the suggestions and amendments necessary to ensure its comprehensiveness.

The reliability of the questionnaire was confirmed by Cronbach's alpha (r = 0.91). At last, the descriptive statistics were used to describe the demographic variables using mean, standard deviation, frequency and percentage. The independent t-test was used to investigate the relationship between sexual desire score and disease and to compare the mean sexual desire scores in two age groups under 60 years and over 60 years; the analysis of variance (ANOVA) test to compare the sexual desire score for multivariate variables; Pearson correlation test to examine the relationship between quantitative variables such as the relationship between sexual desire and age of menopause, marital history, number of pregnancies and number of children according to the normal distribution of data. All data were analysed by SPSS version 22 software.

Results

The present study was conducted on 210 married women aged 50-70 years. The mean age of women was 59.40 ± 5.93 years. These women were mostly housewives (61%), and most of them (41%) had the education level of high school. The age of menopause, marital history, number of pregnancies and number of children are listed in Table 1.

Table 1: Demographic variables of research units

Age (Mean ± SD)		59.40 ± 5.93
Age of menopause (Mean ± SD)		50.3 ± 3.25
Years of marriage (Mean ± SD)		38.51 ± 7.74
Number of pregnancies (Mean ± SD)		4.29 ± 2.20
Number of children (Mean ± SD)		3.74 ± 1.63
Occupation/employment status Number (percent)	Full-time	1 (0.5)
	Part-time	31 (14.8)
	Retiree	50 (23.8)
	Housekeeper	128 (61)
	Illiterate	18 (8.6)
Educational level Number (percent)	<High school	56 (26.7)
	High school	86 (41.0)
	Associate's degree	34 (16.2)
	Bachelor's degree and higher	16 (7.6)

The mean sexual desire score of women was 22.66 ± 17.78; the comparison of sexual desire score at middle-aged and elderly subjects showed a significant relationship between these two age groups (Table 2).

Table 2: Comparison of the mean sexual desire score in the two age groups of less and more than 60 years

Groups	Frequency	Mean score	Standard deviation	F	P
< 60 years	104	30.37	18.54	6.85	0.000
> 60 years	106	15.10	13.20		
Total	210	22.66	17.78		

The underlying diseases were diabetes (53.3%), hypertension (56.7%), heart disease (13.9%), high cholesterol (21.9%), chronic pain (40%), gastrointestinal problems (33.8%), chronic ulcer (8.1%), bladder and intestinal problems (25.2%), joint and bone problems (42.9%) and urinary tract infection (9%). The used medications were anti-hypertensive (42.4%), anticoagulant (21.4%), insulin (20.5%), cholesterol-lowering (38.6%) and cardiac (21.9%). The household income level in the majority of participants (48.1%) was between 10 and 20 million Rials per month. The description of most participants (42.4%) of their health status was in the "good" cluster. Most participants (30%) reported mild pain in the last four weeks. The results of Pearson correlation coefficient showed a negative and significant correlation of the sexual desire score with age of menopause, years of marriage, number of pregnancies and number of children (Table 3).

Table 3: Relationship of the sexual desire score with age of menopause, years of marriage, number of pregnancies and number of children

Variables	Pearson correlation coefficient	P value
Age of menopause	-0.16	0.01
Years of marriage	-0.43	0.00
Number of pregnancies	-0.37	0.00
Number of children	-0.38	0.00

It was also found that there was a significant difference between the sexual desire score of women with age (p = 0.00), educational level (p = 0.03), spouse's education (p = 0.02), spouse's age (p = 0.000), spouse's occupation (p = 0.000), diabetes mellitus (p = 0.000, t = 5.51), hypertension (p = 0.0005, t = 5.64), heart disease (p = 0.00 and t = 3.78), high cholesterol (p = 0.00, t = 6.69), chronic pain (p = 0.005 and t = 5.80), gastrointestinal problems (p = 0.00, t = 4.56), chronic ulcer (p = 0.00, t = 4.66), bladder/intestinal problems (p = 0.00, t = 4.90), joint and bone problems (p = 0.00 and t = 3.69), taking anti-hypertensive (p = 0.000 and t = 5.61), anticoagulants (p = 0.000, t = 3.91), insulin (p = 0.000 and t = -4.2), cholesterol-lowering (p = 0.000, t = 6.16), and cardiac (p = 0.000 and t = 4.03), the individual description of health status (p = 0.00) and the pain level in the last four weeks (p = 0.00). The results of this study showed that there was no

significant relationship between the sexual desire score and occupation of women (p = 0.3), household income level (p = 0.07) and urinary tract infection (p = 0.06 and t = 1.87).

Discussion

According to the present findings, the sexual desire among middle-aged women was almost twice as high as senior women. In a study on the elderly in the United States [15] using the same tool, the mean sexual desire score was reported to be higher than that of our study. Delamater et al. also reported a decrease in the sexual desire level in both women and men. Meanwhile, the mean sexual desire in men was more than in women [16]. In a study Beigi et al., on sexual dysfunction in menopause, the sexual desire dysfunction was 62.6% in postmenopausal women [17].

Similarly, da Silva et al., in a review study found that 60-year-old women had sexual dysfunction in different dimensions in comparison with 30-year-old women and 72.2% of postmenopausal women were involved in sexual activities merely because of their thousand's satisfaction, and they were unwilling to participate in these activities [18]. Sheikhan et al. concluded that 66.3% of postmenopausal women had undesirable sexual desire level [19]. A review study of Palacios et al. revealed that the sexual function was strongly influenced by age so that the incidence rate of sexual dysfunction increased from 10% in women aged 49 years to 22% in women aged 50-65 years [20]. The sexual desire was decreased in postmenopausal women to 47% in the UK, 54% in Italy, 42% in France and 24% in Germany [5] [15], while Leiblum et al., in the US reported that 62% of women believed in enhanced sexual desire after menopause [21]. With the ageing of postmenopausal women in traditional societies, they often tend to be involved in caring for children, descendants and religious during this period, and the sexual activity is in their next priorities [22]; these results were different from those obtained from some other developed societies [23] [24]. In the review study, Palacios et al. found that the sexual function was strongly influenced by age so that the incidence rate of sexual dysfunction increased from 10% in women aged 49 years to 22% in women aged 50-65 years [20].

The sexual response, the sexual desire and the frequency of sexual intercourse in menopause are decreased with age, leading to the sexual dysfunction in women. This can be an important factor in reducing sexual function in postmenopausal women [25]. Ponholzer et al., examined the risk factors and the prevalence of sexual dysfunction in 703 Australian women and found that 22% suffered from sexual desire disorder, 35% had a sexual arousal disorder, and 39% expressed orgasmic problems; all of these problems had been significantly increased with age [26].

The results of this study showed that despite the high mean sexual desire score in employed women (full time), there was no significant relationship between the sexual desire score and the occupation of women. Further, the results of a study on 846 women aged 40-60 years in Maryland (USA) [27] confirmed the results of our study. In a study, employed women had the sexual desire higher than homemakers, which could also affect the sexual satisfaction [28]. The results of this study showed a significant relationship between the sexual desire score and the educational level of women. The women who had a Bachelor's degree and higher revealed higher mean sexual desire score.

Other studies showed that female sexual function is reduced by increasing educational level [29] [30]. In the current study, there was no significant relationship between female sexual desire score and household income level, although it was close to the significance level. However, the lowest mean sexual desire score was related to the household income level "below 10 million Rials". It has been shown that sexual function score was significantly higher in women who had an adequate household income level [23] [17], but Tomic et al., in Maryland reported that the household income level was not a predictor of sexual function in middle-aged women [27]. In the present study, there was a significant relationship between the sexual desire score of women and spouse's occupation, and the highest sexual desire score of women belonged to the group whose spouses worked "full time" and the lowest sexual desire score was seen in the group of women whose husbands were "unemployed". Sheikhan et al., [19] and Valadares et al., [29] both reported that the postmenopausal women whose husbands were self-employed had more sexual arousal.

This can be attributed to the fact that the adequate income provides peace of mind and subsequently sexual satisfaction [19] [31]. There was a significant relationship between the sexual desire score of women and the educational level of the spouses. The women whose spouses had the associate's degree had the highest mean sexual desire score. In the study of Sheikhan, there was also a significant relationship between the sexual arousal and the educational level of the spouses, as Gonzalez et al., confirmed this finding [19] [30]. The results of this study indicated a negative relationship between the sexual desire score of women and age of menopause, marital history, number of pregnancies and number of children. In a study of Gutbrie et al., which lasted nine years, it was found that the sexual dysfunction increased from 42% to 88% from the beginning to the latter phase of the menopausal transition possibly due to a sharp drop in the sexual hormones [32]. It has been reported that the overall

sexual function decreased from 88% in the first year after menopause to 34% after eight years of menopause [18]. On the other hand, the sexual response in the postmenopausal women is more likely to result from the need for intimacy than the sexual arousal [25]. However, some studies have claimed that the marriage does not affect women's quality of life [33].

The results of this study revealed that the sexual desire score in women had a significant association with diabetes, hypertension, heart disease, high cholesterol, chronic pain, gastrointestinal problems, chronic ulcers, bladder and intestinal problems, and joint and bone disorders. No significant relationship was found between the sexual desire score and the urinary tract infection, although close to the significance level, due to the small sample size. In a study female sexual function was affected by some conditions including cancer, high cholesterol, chronic ulcer care, bladder and intestinal problems, diabetes, poor vision, gastrointestinal problems, hypertension, major surgery, joint and bone problems, and general health status [31]. DeLamater et al. underlined that the older adults with hypertension have, the lower sexual desire; as well as prostatic hypertrophy among men had a significant relationship with decreased sexual desire. Diagnosis of diabetes, arthritis and depression was associated with a decrease in the sexual desire of women [16].

Our results demonstrated a significant relationship between female sexual desire score and taking five groups of drugs including antihypertensives, anticoagulants, insulin, cholesterol-lowering and cardiac drugs. In similar studies, such as the study of DeLamater et al., a significant correlation was observed between the sexual desire score and regular taking four groups of drugs, including anticoagulants, cardiovascular drugs, cholesterol-lowering and antihypertensive [16]. We found a significant relationship between the sexual desire score and self-reported individual health status and those who rated their health in the "very good" group had a higher mean sexual desire score and those with "poor" option had the lowest mean sexual desire score compared to the rest of the groups. Besides, there was a significant relationship between the sexual desire score and pain level in the last four weeks, meaning that people who selected "never" option for their pain levels in the last four weeks had the highest mean sexual desire score compared to other groups. Lindau et al. expressed that men and women with a better level of health are more sexually active as compared to those who are at lower health levels, and their sexual desire is higher as well [34].

In conclusion, as the age increases, the sexual desire of married women aged 50-70 years decreases, and this downward trend in our country is significant compared to similar studies in other countries. Also, the sexual desire score in middle-aged women is about twice as high as senior women, suggesting the effect of cultural beliefs of the individual and the society on their sexual desire. The findings of this study can be hopefully an effective step forward to raise the awareness of individuals and consequently the community towards this important need in elderly people, as well as a warning to the society that ignoring the need for sexuality in elderly people can suppress this need and thus reduce the hope and happiness of life in this group of population.

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Do Personality Characteristics Constitute the Profile of Burnout-Prone Correctional Officers?

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Keywords: Burnout; Correctional officer; Eysenck personality questionnaire; Maslach burnout inventory; Prison staff

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Abstract

AIM: This study examined the relationship between personality characteristics and burnout syndrome among Bulgarian correctional officers.

METHODS: The cross-sectional study took place through individual, voluntary and anonymous interviewing of 307 employees from three district prisons. Maslach burnout inventory, Eysenck personality questionnaire and demographic characteristics were administered.

RESULTS: The personality predictors of emotional exhaustion were low emotional stability and low level of dimension extraversion. The predictors of depersonalization were high levels of neuroticism and psychoticism and low level of extraversion.

CONCLUSION: This research helps to identify employees who are at risk for developing burnout as a result of their personalities. In Bulgaria, there is still no official information about studies in this area.

Introduction

Employees of prisons, for their job nature and work environment, are exposed to high levels of occupational stress [1] [2]. Armstrong and Griffin [3] contend that "very few other institutions are charged with the central task of supervising and securing an unwilling and potentially violent population". Researchers have found that the perceived dangerousness of the job, as a result of threats and inmate violence is a significant cause of stress for much correctional staff.

Over time, these prolonged or chronic stressors in the workplace can lead to strain and ultimately to burnout among many prison staff [4] [5] [6].

Over the last years, researchers focus on the issue of job burnout among prison staff in various ways. Some authors study correctional officers in general [6] [7] [8], others focus on the relationship between personality variables and burnout [9], gender difference in stress and burnout [10], impact of correctional officer job stress and burnout [2] [11], relationship between supervisor and management trust and job burnout among correctional staff [12].

The limited study of burnout in the field of corrections has found that this is a serious problem for many officers and other prison staff, and one that needs more scientific attention [7] [13]. Cieslak et al.,

[14] contend that correctional staff burnout is less frequently studied than burnout of teachers or medical personnel, particularly regarding identifying and understanding its potential causes. Neveu [13] indicates that he has found only 16 published studies on correctional staff burnout and not all of these explore possible causes of burnout.

There is currently a paucity of research that examines personality characteristics and their association with employee burnout within correctional officer samples. During the last two decades, quite a few studies have indicated the possibility that personality plays an important role in the development of burnout. In Bulgaria, there is still no official information about studies in this area. Correctional officers are a little studied professional group that works in totally closed institutions that are difficult to access for investigations. To date, the psychological support offered in Bulgarian prisons is commonly directed to inmates. Thus, the present research will help us to identify employees of penalty system who are at risk for developing burnout as a result of their personalities. This dataset offers an opportunity to gain new insight into Bulgarian corrections and Bulgarian correctional officers.

This study aimed to examine the relationship between personality characteristics and burnout syndrome among correctional officers. The focus was to investigate if personality characteristics constitute the profile of burnout-prone prison staff.

Methods

There are twelve prisons in Bulgaria of which eleven are for male inmates, and only one is for women. The prisons in Plovdiv and Pazardzhik for male inmates encompass the building of the prisons itself and four open type correctional dormitories. The Plovdiv District Prison is the second largest prison in Bulgaria. The Pazardzhik District Prison is mainly for recidivists convicted of major crimes. Working there involves high-risk work with hostile individuals, high crime rates and the size of the community, a high incidence of physical ailments and psychological problems that affect their work performance. In Bulgaria, there is only one prison for women and one correctional dormitory for minor girls, both located in Sliven. The staff of the prisons and the affiliated dormitories is distributed in the following divisions: custodial work and guarding activities, social activities and educational work, financial division, human resources and medical centre.

A cross-sectional study was carried out with 307 correctional officers working at three prisons in Bulgaria. The all available staff at prisons in the Plovdiv District Prison (N = 106), the Pazardzhik District Prison (N = 100) and the Sliven District Prison (N = 101) were surveyed. Given that the data come from three prisons comprising 25% of all prisons in Bulgaria, the sample selected was considered representative of the Bulgarian prison system context.

To conduct the survey, we received a statement of approval and permit for admission to the respective prisons from the General Directorate "Execution of Penalties" (GDEP) at the Bulgarian Ministry of Justice (with Reg. No. 11518/22.12.2011).

The participation of the prison employees was voluntary without any financial compensation. The questionnaires were accompanied by a letter in which the goal of the study was briefly introduced, and the confidentiality and anonymity of the answers were emphasised. Data were collected between June 2012 and April 2014. The only qualification in the sample selection was that the employee has direct contact with inmates. The exclusion criteria included an unwillingness to cooperate and incorrect completion of the questionnaire. Some staff members were off duty (e.g., sick leave, vacation or administrative leave) during the period of the survey. The response rate was 85.28%.

Six demographic characteristics were selected. Gender was coded as female = 0 and male = 1. Age was measured in continuous years. Marital status was divided into married, unmarried, divorced, and widowed. Education was measured as a dichotomous variable representing whether the respondent had a university degree or not. Tenure was measured as the number of years the respondent had worked at the prison. Job position was measured as a dichotomous variable representing whether the respondent was a correctional officer or inspector (supervisory officer).

The most widely used and validated instrument for the measure of burnout is the Maslach Burnout Inventory (MBI) developed by Maslach and Jackson [15]. The Bulgarian version of the MBI adapted for the Bulgarian population by B. Tzenova [16] was used to measure the three core dimensions of burnout – emotional exhaustion, depersonalization and reduced personal accomplishment. Emotional exhaustion (representing a lack of energy and feelings of being over-extended and depleted of emotional and physical resources; the basic stress component of burnout) was measured using nine items. Depersonalization (representing feelings of detachment and unresponsiveness about the job; the depersonalization aspect of burnout) was measured using five items. Finally, personal accomplishment (feelings of incompetence, a reduced ability to do the job, and lack of accomplishment; the self-evaluation dimension of burnout) was measured using eight items. The items of personal accomplishment were reverse scored (lack of professional efficiency). The response choices, rated on a 7-point frequency scale, were never (0), few times in year (1), once a month or

less (2), few times in a month (3), once a week (4), few times a week (5), every day (6). High scores on emotional exhaustion and depersonalization and low scores on personal accomplishment were indicative of burnout.

The Bulgarian version of the Eysenck Personality Questionnaire (EPQ) was used to assess the personality traits of a person [17]. The questionnaire contains 86 items and answers are given on a Yes-No scale with regards to whether respondents agree or disagree with the given statement. Eysenck initially conceptualises personality as two, biologically-based independent dimensions of temperament measured on a continuum:

1. *Extraversion-Introversion:* Extraverts, according to Eysenck's theory, are chronically under-aroused and bored and are therefore in need of external stimulation to bring them up to an optimal level of performance. Introverts, on the other hand, are chronically over-aroused and jittery and are therefore in need of peace to bring them up to an optimal level of performance. Most people fall in the midrange of the extraversion-introversion continuum; an area referred to as ambiversion.

2. *Neuroticism-Stability:* Neuroticism or emotionality is characterised by high levels of negative affect such as depression and anxiety. According to Eysenck's theory neurotic people who experience negative affect (fight-or-flight) in the face of minor stressors, are easily nervous or upset. Emotionally stable people who experience negative affect only in the face of very major stressors, are calm and collected under pressure.

Further research demonstrates the need for a third category of temperament:

3. *Psychoticism-Socialization:* Psychoticism is associated not only with the liability to have a psychotic episode (or break with reality) but also with aggression. Psychotic behaviour is rooted in the characteristics of tough-mindedness, non-conformity, inconsideration, recklessness, hostility, anger and impulsiveness.

EPQ assesses three basic personality dimensions-extraversion-introversion, neuroticism and psychoticism, and, additionally, the tendency to provide socially desirable answers-the lie scale.

The Statistical Package for the Social Sciences version 17.0 for Windows (SPSS Inc, Chicago, IL) was used for the statistical analysis. A descriptive analysis was conducted on the sample (the results were presented as mean ± SEM). A Pearson correlation coefficient was used to explore the relationship between burnout subscales and personality traits. A multiple linear regression analysis with burnout subscales was used to examine the impact of personality characteristics. The emotional exhaustion, depersonalization and personal accomplishment were the three dependent variables. The main independent variables in this study were personality characteristics. These were taken as predictors to determine whether introversion, neuroticism and psychoticism made significant independent contributions to the three dimensions of burnout. A p-value < 0.05 was considered as statistically significant level. All p-values were two-tailed.

Results

Concerning the demographic description of the sample, the mean age of participants was 40.59 ± 0.48 years, of which 68.02% (n = 209) were male. Of the total number, 237 officers (77.20%) were married, 124 (40.39%) had a university education, 79 (25.73%) were inspectors, and the mean number of years of service at the prison was 11.37 ± 0.44.

The three personality dimensions were significantly correlated with burnout subscales (Table 1). The strongest correlation was between neuroticism and emotional exhaustion and depersonalization, and negatively correlated with personal accomplishment. Low values of extraversion correlated strongly with low scores of personal accomplishment, and with high scores of emotional exhaustion and depersonalization. Dimension psychoticism was positively correlated with high values of depersonalization.

Table 1: Pearson Correlations between Personality Variables and Burnout Subscales

MBI Subscales Personality	Emotional Exhaustion	Depersonalization	Personal Accomplishment
Extraversion	-0.45**	-0.41**	0.55**
Neuroticism	0.76**	0.59**	-0.49**
Psychoticism	0.35*	0.56**	-0.34*

Note. *p-value ≤ .05. **p-value ≤ .01 (two-tailed).

A series of analyses were performed using multiple linear regression (using the Enter method) to identify the predictors of the MBI scales. For every single dependent variable, we developed an independent regression model. First, we conducted a linear regression analysis with the personality variables as the predictors and emotional exhaustion as the dependent variable (Table 2). The coefficient of determination (R^2) is 0.391, which means about 40% of the variance in emotional exhaustion was explained by the variance of high levels of neuroticism and low levels of extraversion. The high level of neuroticism was the factor that had the most significant influence on emotional exhaustion.

In a second regression analysis, we regressed depersonalization on the independent variables. The personality traits neuroticism, psychoticism and extroversion, explained 38% of the variance in depersonalization (see values in the

second column in Table 2).

Table 2: Effects of Personality Traits on Burnout among Bulgarian Correctional Officers

Independent Variable	Emotional Exhaustion		Depersonalization	
	B	β	B	β
Extroversion	-0.205	-0.096*	-0.209	-0.134**
Neuroticism	1.106	0.575**	0.717	0.514**
Psychoticism	0.448	0.089	0.780	0.212**
R^2		0.391**		0.388**

Note. N=307. *p-value ≤ .05. **p-value ≤ .01.

We performed the third regression analysis using personal accomplishment as the dependent variable. No significant relations were found with personality traits. All variables that had a statistical significance level of $p > 0.05$ were excluded from the model.

Summary of the multiple linear regression model determined that the important statistically significant predictors of emotional exhaustion were a low level of dimension extraversion and low emotional stability. Depersonalization was best predicted by low level of extraversion, high levels of neuroticism and psychoticism.

Discussion

All the previous researchers reported that prison environment affects emotional well-being of the prison staff, which in turn brings about increased stress among them and culminates into job burnout among many prison staff [1] [6] [18]. In our previous study, we have revealed that the Bulgarian prison employees do suffer from burnout [19]. Based on Maslach's categorisation of burnout 10.42%, 25.73% and 50.49% of our respondents experience high levels of job burnout in the emotional exhaustion, depersonalization and reduced personal accomplishment subscales, respectively. This result is in line with other previous studies on similar populations [6] [20].

Undoubtedly correctional work is a high-risk occupation for developing burnout. Lambert et al., [21], in a review of 55 published studies on burnout, conclude that various workplace stressors play a role in leading to correctional staff burnout. But what factors contribute to burnout? Why do some employees report high levels of burnout whereas others in the same environment do not? Personality characteristics are important in explaining burnout among correctional officers. The theories of personality suggest that individuals' dispositions affect their interpretations of and reactions to their environments. The risk of burnout may differ not only across situations but also across individuals. The three personality factors that constitute the basic structure of personality in Eysenck's system are most important in determining how individuals experience and adjust to the stressful events in their lives. In our study, the application of the Eysenck personality inventory shows that introversion has the strong effect on emotional exhaustion and depersonalization. Our results are consistent with the findings of similar studies in the field [22]. The workers higher in positive affectivity (a component of extraversion) experience less burnout [23] [24].

Neuroticism, introversion and psychoticism appear to be the most consistent predictors of burnout. A tendency to underestimate self-performance and a tendency to react with strong emotions and self-criticism in stressful situations seem to be associated with a higher vulnerability to all symptoms of burnout [25] [26]. We find that correctional officers who are higher in neuroticism experience higher levels of emotional exhaustion and depersonalization. These findings resonate with another recent study among a sample of UK prison officers, in which higher neuroticism emerges as a risk factor for burnout [27].

Schaufeli and Enzmann [28], in a comprehensive review of more than 250 studies on burnout, reported that neuroticism is one of the strongest personality correlates of burnout. For instance, when exposed to high job demands employees with high levels of neuroticism are more prone to burnout than those with lower levels [29]. According to Eysenck and Eysenck [30], individuals who score high on neuroticism are prone to emotional, anxious and fearful responses, and these disproportionally pronounced feelings of distress may lead to emotional exhaustion. Thus more neurotic individuals combined with low extraversion perceive a given work environment as more stressful compared to less neurotic individuals [31].

Psychoticism is positively associated with depersonalization. Some authors see depersonalization as a coping strategy in itself, rather than as a manifestation of burnout. Hobfoll and Freedy [32] expect psychoticism to affect depersonalization directly, rather than through coping strategies.

Although conditions in the work environment contribute to burnout, our findings suggest that burnout is also associated with employee personality. Personality traits are considered to be rather stable and unchangeable throughout life, and difficult to modify directly. Personality characteristics may predict which employees experience increases, decreases, or constant levels of burnout over time. Personality types may also be used as indicators for individuals in need of support in the workplace.

In closing, emotional exhaustion is predicted by low levels of extraversion and high levels of neuroticism. Depersonalization is predicted by low levels of extraversion and high levels of neuroticism and psychoticism. Thus, correctional officers who are

high in neuroticism and low in extraversion deserve special attention and need to benefit from training programs in preventing and reducing burnout.

Limitations: This article is a part of a large study on burnout among prison employees in Bulgaria. It should be noted that the results presented in this article were from only one study and longitudinal studies should be undertaken in future to confirm the conclusions obtained in this study.

Future studies need to research the impact of different job and organisational characteristics on correctional staff burnout. Our next step is to offer some detailed coping strategies to decrease job burnout among correctional officers. We hope that preventive care to the mental health of correctional officers benefits not only the prison staff but also their families and the inmates also.

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The Quality of EBM Sources Perceived By Belgian Family Physicians

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Abstract

BACKGROUND: Belgian family physicians use several local and international sources for evidence-based medicine (EBM).

AIM: This study aims to investigate the quality of these EBM sources according to the Belgian family physicians.

METHODS: A sample of Belgian family physicians completed a digital survey on the quality of EBM sources.

RESULTS: Respondents evaluated the quality of the information for the major part of the local and international EBM sources good to excellent. More than 50% of the respondents found in the major part of the sources an answer to the question. More than half of the respondents found the necessary information in less than 5 minutes in most of the sources. Younger participants self-evaluated their search skills better than older participants.

CONCLUSION: The quality of most frequently used EBM sources in Belgium is evaluated as good and client-friendly. More than half of the respondents found an answer to their questions in most of the sources and this within 5 minutes.

Introduction

Evidence-based medicine (EBM) has grown to one of the most important aspects of clinical decision making during the past decades. EBM is defined in 1996 by Sackett as: “Evidence-based medicine is the conscientious, explicit, and judicious use of current best evidence in making decisions about the care of individual patients” [1].

The quality of international sources seems to be guaranteed by international procedures in the development of guidelines and other EBM sources. Thereupon we can assume that the widespread usage of EBM forms an external control for these sources. Besides the international sources, many countries develop local guidelines and other EBM sources for which the quality control is less obvious.

Belgian scientific organisations also developed specific local EBM sources which are adapted to the Belgium healthcare system. Minerva publishes reviews of recent international papers [2]. The Belgian Centre for Pharmacotherapeutic Information (BCFI) provides EBM information on drugs and their indications. They publish the “Commented Drugs Repertory”, the “Folia Pharmacotherapeutica” and the “Transparency sheets” [3]. Recommendations for good practice are published by the French-speaking Scientific Society for General Practice guidelines (SSMG) [4] as well as by their Dutch-speaking counterpart Domus Medica [5].

The Belgian Centre for Evidence-Based Medicine (CEBAM) [6] makes among others two EBM sources available to Belgian healthcare workers: the Digital Library for Health (CDLH) and the Evidence Linker [7]. The Evidence Linker is integrated into the Electronic Medical Records and provides EBM sources based on the clinical complaint or diagnosis.

EBMPracticeNet is another portal for easy access to EBM sources [8]. Several other EBM sources exist such as the "Belgian Guide for anti-infectious treatment in the ambulatory practice" (BAPCOC) [9], Guidelines for the use of medical imaging from the Belgian Medical Imaging Platform (BELMIP) [10], the consensus papers from the National Institute for Health and Disability Insurance (RIZIV) [11], a database on medication before and during pregnancy and breastfeeding (Cybele) [12], the project Farmaka.Be [13] providing EBM information to physicians on medication, the "Formularium for the Homes for the Elderly" [14], a website reviewing medical information from the lay press "Gezondheid en Wetenschap" [15], thematic advice from the "Superior Health Council" [16] and the Belgian Healthcare Knowledge Centre (KCE) [17].

Two Dutch EBM sources are widely used in Belgium: the "Dutch College of General Practice guidelines (NHG) [18] and the guidelines from the "Dutch Institute for Healthcare Improvement (CBO)" [19].

The abundance of local and international EBM sources guarantees their quality neither their user-friendliness nor their user-satisfaction. Although EBM is considered by most physicians as positive, there are some aspects that might hamper its use and implementation. Difficulties in finding the right source may cause long search times and consequently loss of time which cannot be remunerated for the physician [20]. This could undermine the future use of EBM or at least of specific sources. The lack of performant search skills is one of the most important barriers to use EBM [21]. Several studies have investigated the access and barriers to EBM in Belgium and several other countries [21] [22] [23] [24] [25] [26]. However, the perceived quality, the user-friendliness and the user-satisfaction have hardly been studied in Belgium.

This study aims to find out (1) how much time was spent to find the source, (2) how the sources were evaluated regarding the quality of the information and (3) how satisfied the physicians were with these sources.

Methods

In Belgium, the government and the Board of Physicians have the (email) addresses of all physicians but for privacy reasons, these addresses can not be used for research and consequently it is difficult or almost impossible to invite all physicians to participate in a survey. We tried to short-circuit this by sending an email to all Dutch-speaking local organisations of family physicians (n = 54) and by asking them to forward our email with an invitation to participate in our study.

Our department of family medicine and chronic care has another 377 email addresses of family physicians who participated previously in training our research and who were willing to receive new invitations. They received an email to participate in the study. Invitations were sent in December 2015, and a reminder was sent in February 2016. Data entry was stopped on February 22, 2016.

It is not known how many local associations forwarded the email neither do we know how many physicians received the email. For these reasons, we can not estimate the response rate.

The study was limited to Dutch-speaking family physicians living in Belgium. They needed a computer to answer the questions. Physicians without a computer, physicians who did not comprehend Dutch or who were not family physicians, were excluded. Surveys who were completed partly were excluded from the analyses.

An online survey containing 27 questions was build after analysing the literature. It enquired about demographics, general questions about EBM and questions about the personal appraisal on EBM sources. A list with commonly used (EBM) sources was developed including the international and Belgian sources. For each source the questionnaire asked about the perceived quality, the time used to find the information and the user-satisfaction.

During the survey, only information related to physicians is gathered, and no medical information from patients neither physicians was registered. For that reason, permission from an ethics committee was not necessary. The anonymity of the participants was guaranteed because no personal identifying characteristics were recorded.

The collected data were stored in a protected database in LimeSurvey that also generated the descriptive statistics. An SPSS file was extracted from LimeSurvey permitting the analyses in IBM-SPSS-v23. T-tests and ANOVA-tests were used for univariate analyses of continuous variables with a normal distribution and Mann-Whitney-Wilcoxon-tests and Kruskal-Wallis for continuous variables without a normal distribution. Chi-square tests were used for categorical variables.

Results

In total 77 men (54%) and 66 women (46%) fully completed the survey (Table 1). Forty other participants did not answer all questions and were excluded. The mean age of the participants was 45 years (SD 14) ranging between 25 and 71 years. The mean age of men was 52.9 years and of women 35.8 years with men significantly older ($P < 0.001$) (Table

2). The mean time of activity as a family physician was 18.4 years (SD 14). A correlation of 0.953 was observed between age and the years of activity.

Table 1: Demographics of the participants (n = 143)

	N	%
Gender		
Men	77	53.8
Women	66	46.2
Type of practice		
Solo practice	43	30.1
Duo practice	25	17.5
Group practice	75	52.4
Practice as part of a network		
Yes	36	25.2
No	94	65.7
Unknown	13	9.1
Place of practice		
Urban	57	39.9
Semi-rural	56	39.2
Rural	30	20.9
Type of family physician		
Trainee	15	10.5
Involved in Master after Master programme	27	18.9
Trainee supervisor	53	37.1
None of the above mentioned	66	46.2
The receiver of telematics premium by the government		
Yes		
No	136	95.1
Unknown	4	2.8
	3	2.1
Usage of technology on a visit		
Laptop	34	23.8
Tablet	15	10.5
Smartphone	35	24.5
None of the above mentioned	67	46.9
Not applicable (no visits)	5	3.5
Access to a wireless Internet connection, when using technology		
Yes	57	80.3
No	13	18.3
Unknown	1	1.4

More than 50 % of the participants worked in a group practice, 17.5% in a duo practice and 30.1% worked solo. One-quarter of the participants worked in a network with family physicians in other practices. Forty per cent of the physicians worked in urban practice, 39% in a semi-rural setting and 21% in a rural area. Ten per cent of the participants were trainees, and 19% was involved in the master-after-master training.

Table 2: Age and time active in family practice for the participants

	Men (N = 77)	Women (N = 66)	P	All (N = 143)
Age				
Mean (SD)	52.9 (10.9)	35.8 (11.5)	< 0.001	45.0 (14.0)
Median	56	30		48
Min	26	25		25
Max	71	64		71
Years active				
Mean (SD)	26.2 (11.4)	9.7 (11.2)	< 0.001	18.4 (14.0)
Median	30	4		20
Min	1	1		1
Max	47	38		47

All physicians used a computer in their practice, 24% used a laptop on housecalls, 11% a tablet and 25% a smartphone. Ninety-five per cent received a telematics premium from the government for the use of medical informatics, and 80% had access to the internet during house calls.

The physicians who worked in solo practice were significantly older (55 years) than those who worked in group practices (40 years) ($p < 0.001$). There was no significant difference between the age of those who worked in a rural area (48 years) and those who worked in an urban area (43 years) ($p = 0.499$) (Table 3).

Table 3: Age differences per type of practice and type of area

	GP in solo practice (N = 43)	GP in duo practice (N = 25)	GP in group practice (N = 75)	P
Age				
Mean (SD)	54.6 (9.4)	42.4 (14.1)	40.3 (13.6)	< 0.001
Median	57	46	33	
Min	27	25	25	
Max	71	64	69	
	Urban area (N = 69)	Semi rural area (N = 63)	Rural area (N = 31)	P
Age				
Mean (SD)	42.6 (14.0)	45.7 (14.6)	48.1 (12.8)	0.499
Median	39	51.5	53	
Min	25	25	25	
Max	71	68	64	

Most of the respondents (91%) believed that patients might benefit from EBM. Not less than 59% of the respondents attended an information session on EBM, and 55% estimated their search skills good to excellent.

Table 4: Perception and willingness to learn about EBM sources

	N	%
Will patient benefit from the use of EBM practice		
Yes	130	90.9
No	2	1.4
Unknown	11	7.7
Self-evaluation of search skills		
Very bad to bad	23	16.1
Neutral	41	28.7
Good to excellent	79	55.2
Previously attended an info session about searching with (an) EBM source(s)		
Yes	84	58.7
No	59	41.3
Willingness to improve search skills		
Yes	110	76.9
No	33	23.1

Self-evaluation of search skills	Age		Number of years active	
	Mean	SD	Mean	SD
Very bad to bad	55.5	6.1	29.8	6.3
Neutral	46.0	13.4	19.7	13.7
Good to excellent	41.5	14.5	14.5	13.9
P value	< 0.001		< 0.001	

Younger respondents self-evaluated their search skills in EBM sources better than older participants. Similarly, respondents with a newer activity as family physician self-evaluated their search skills in EBM sources better than those with a longer activity (Table 4) (Figure 1).

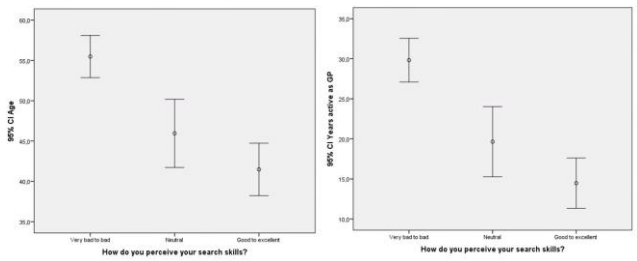

Figure 1: Error bar plots (95% C.I.) for the perceived search skills, respectively for age and number of years active as GP

More than 70% of the responders used BAPCOC, BCFI, CEBAM, Domus Medica and NHG.

Less than 30% used BELMIP, CBO, CIPIQ-S, “Gezondheid en wetenschap”, Google Scholar, Superior Health Council, SSMG and UpToDate (Table 5).

Table 5: Time spent on average to find information

Source	Users N	<1 minute	1 - 5 minutes	5 - 10 minutes	10 - 20 minutes	>20 minutes
BAPCOC	104	50%	44%	5%	0%	1%
BCFI	129	43%	48%	7%	1%	1%
BELMIP	10	10%	30%	40%	20%	0%
CBO	5	0%	20%	40%	40%	0%
CEBAM	106	4%	48%	38%	8%	2%
CIPIQ-S	1	0%	0%	100%	0%	0%
Clinical Evidence	45	0%	40%	42%	14%	4%
Cochrane Library	72	1%	26%	34%	29%	10%
RIZIV	57	2%	23%	54%	14%	7%
Cybele	51	39%	49%	10%	2%	0%
Domus Medica	122	3%	57%	37%	2%	1%
EBMPracticeNet	92	7%	67%	21%	4%	1%
Evidence Linker	50	12%	60%	20%	4%	4%
Farmaka.be	72	7%	60%	28%	5%	0%
Formularium for Elderly	49	8%	69%	17%	4%	2%
Gezondheid en Wetenschap	19	5%	53%	32%	5%	5%
Google	92	20%	52%	24%	3%	1%
Google Scholar	18	6%	50%	32%	6%	6%
Superior Health Council	35	9%	22%	40%	20%	9%
KCE	68	6%	19%	40%	26%	9%
Medline	70	1%	24%	26%	30%	17%
Minerva	75	5%	31%	39%	15%	10%
NHG	108	6%	50%	36%	6%	2%
SSMG	10	0%	50%	10%	30%	10%
UpToDate	42	7%	40%	29%	17%	7%

Fifty per cent or more of the participants could find their information within five minutes in sources such as BAPCOC, BCFI, CEBAM, Cybele, Domus Medica, EBMPracticeNet, Evidence Linker, Farmaka. Be, Formularium for the Elderly, Gezondheid en Wetenschap, Google, Google Scholar and NHG. For BAPCOC and BCFI the information was even found within the first minute for 50% and 43% of the users, respectively.

Searching in Medline takes relatively longer time. Seventeen per cent needs 20 minutes or more in Medline, and 47% searched for 10 minutes or more. Similar search times were found for SSMG and Cochrane Library.

Table 6: User-satisfaction for different EBM sources

Source	Users	Always	Mostly	50/50	Seldom	Never
BAPCOC	104	41%	51%	6%	2%	0%
BCFI	129	37%	59%	4%	0%	0%
BELMIP	10	10%	30%	50%	10%	0%
CBO	5	0%	20%	60%	20%	0%
CEBAM	106	3%	52%	36%	7%	2%
CIPIQ-S	1	0%	0%	100%	0%	0%
Clinical Evidence	45	0%	33%	54%	13%	0%
Cochrane Library	72	1%	26%	49%	21%	3%
RIZIV	57	0%	28%	54%	14%	4%
Cybele	51	39%	53%	6%	2%	0%
Domus Medica	122	7%	60%	23%	10%	0%
EBMPracticeNet	92	9%	58%	25%	9%	0%
Evidence Linker	50	8%	56%	26%	10%	0%
Farmaka.be	72	3%	44%	43%	10%	0%
Formularium Elderly	49	12%	51%	22%	14%	0%
Gezondheid en Wetenschap	19	0%	58%	32%	10%	0%
Google	92	8%	44%	41%	7%	0%
Google Scholar	18	0%	39%	61%	0%	0%
Superior Health Council	35	0%	43%	34%	23%	0%
KCE	68	0%	33%	42%	23%	2%
Medline	70	1%	30%	46%	20%	3%
Minerva	75	1%	36%	43%	17%	3%
NHG	108	13%	61%	23%	2%	1%
SSMG	10	0%	10%	50%	40%	0%
UpToDate	42	7%	43%	38%	10%	2%

The user-satisfaction was measured by the proportion of participants that reported to have found an answer to their question. In 80% of the cases BAPCOC, BCFI and Cybele could answer. CBO, Cochrane Library, Superior Health Council, KCE, Medline, Minerva and SSMG delivered an answer in half or less than half of the cases when being used (Table 6).

More than 70% of the questioned physicians found the quality of most sources good to very good. Only for BELMIP and Google, the quality was evaluated less good: 20% and 17%, respectively, received a poor score which in contrast to most other sources (Table 7).

Table 7: Quality of the EBM information

Source	Users	Very good	Good	Neutral	Poor	Very poor
BAPCOC	104	57%	39%	4%	0%	0%
BCFI	129	55%	41%	3%	1%	0%
BELMIP	10	20%	40%	20%	20%	0%
CBO	5	20%	40%	40%	0%	0%
CEBAM	106	35%	50%	14%	1%	0%
CIPIQ-S	1	0%	100%	0%	0%	0%
Clinical Evidence	45	24%	53%	22%	0%	0%
Cochrane Library	72	24%	50%	26%	0%	0%
RIZIV	57	21%	56%	21%	2%	0%
Cybele	51	49%	39%	10%	2%	0%
Domus Medica	122	34%	52%	14%	1%	0%
EBMPracticeNet	92	27%	51%	22%	0%	0%
Evidence Linker	50	30%	46%	24%	0%	0%
Farmaka.be	72	40%	47%	13%	0%	0%
Formularium Elderly	49	41%	41%	18%	0%	0%
Gezondheid en Wetenschap	19	26%	53%	16%	5%	0%
Google	92	5%	30%	47%	17%	0%
Google Scholar	18	0%	39%	56%	6%	0%
Superior Health Council	35	17%	49%	31%	3%	0%
KCE	68	16%	60%	19%	4%	0%
Medline	70	13%	50%	34%	3%	0%
Minerva	75	36%	40%	23%	1%	0%
NHG	108	46%	48%	5%	1%	0%
SSMG	10	30%	40%	30%	0%	0%
UpToDate	42	21%	55%	21%	2%	0%

Discussion

The problems with the sample population were already mentioned in the methods section. We do not claim to report on a representative sample of the Belgian family physicians because we recruited Dutch-speaking physicians only, the respondents in our study were younger, and they needed a computer to participate [27]. However, our research did not focus on the participants but on the quality of the EBM sources itself. Consequently, the results provide valuable information on the quality and the user-friendliness of the included EBM sources.

We are aware that the methodology of a user survey has its limitations. We could not measure the real time that was spent to find a certain source but the time was estimated by the participant. It was recorded whether an answer was found in the source. We did not evaluate the correctness of the answer. We could neither evaluate the quality of the sources with a validated method, but we asked for the subjective impression of the physicians.

BAPCOC, BCFI, CEBAM, Domus Medica and NHG were the most frequently used sources. They were freely accessible, in Dutch and focused on the

Belgian situation, which may explain the high number of users.

Google was also mentioned among the EBM sources because from our pilot questioning we understood that many physicians used Google in their search for medical information although it is not an EBM source. It is possible that Google was used to access real EBM sources by adding the name of the source in the search strategy. However, this was not investigated in our study.

Most of the EBM users thought that there was a need for improvement or clarification of the available sources. This confirms previous studies indicating that there exist significant barriers to work with EBM because of a large number of sources, the English language, the lack of user-friendliness and contradictions between sources [20] [21].

For most of the sources, the information is found within 5 minutes. These EBM sources had some points in common: they provided practical information, the information was of direct clinical interest and specific, the websites were user-friendly permitting to click through to the topic of choice in an easy way, and the information was concise. These sources did not require to read long articles to find the information. These sources also scored high in the frequency of use, which also can explain that the information was quickly found by intuition.

A small proportion of the participants found the information on BAPCOC, BCFI, BELMIP, Cybele, Evidence Linker and Google even within 60 seconds. These sources focussed on specific informations such as antibiotics, medication and radiographic examinations, respectively.

The sources which needed more search time were used less frequently. Some focussed more on policy support, others contained less specific information, were provided in a different language (Clinical Evidence, Cochrane Library) or contained more extensive and detailed information (UpToDate, Medline). These sources with comprehensive information certainly had their value but were less suitable to use online on-the-spot.

More than 50% of the respondents found an answer to their question in most of the EBM sources. However, only 20% of the participants seldom to never found an answer in CBO, Cochrane Library, Superior Health Council, KCE, Medline, Minerva and SSMG. This may indicate a lack of knowledge about these sources or difficulty in finding the information available. Sources, where it was difficult to find the answer, were not necessarily bad source.

Physicians should make a considered choice for which clinical question a particular source may be used. A bad search strategy may have led to a non-response, even though the source was, on itself, of good quality. This is in line with the study by Davies et al., [26] where the importance has been emphasised that physicians know where to find good guidelines.

The respondents found the quality of the information in the majority of the sources good to excellent. Only for BELMIP and Google, the quality was considered poor by at least 17% of the responders. It must be said that Google is not an EBM source but a search engine that gives access to a huge number of sources whose quality is not assured. It is not surprising that physicians evaluate its quality as poor. According to Tang and Ng, Google shows the correct diagnosis in 58% of the searches [28]. This corresponds with a wrong diagnosis in 42% of the cases.

Younger physicians self-evaluated their search skills better than older physicians. Several reasons may underlie these findings.

The recent introduction of some new sources such as EBMPracticeNet and Evidence Linker [22] and the recent incorporation of courses on EBM in the medical training [29] have probably contributed to the fact that younger physicians have better search skills for these sources. Younger physicians have less clinical experience and need to search for more information, even in the presence of the patient. Older physicians may more rely on their experience and do less appeal to EBM [21]. Moreover, a shorter search time makes searching on-the-spot easier. And finally, the more experience a physician has with a resource, the quicker the searching.

The fact that some sources were used more frequently suggests that there was a better understanding of the usefulness of these sources and that experience with a source contributes to better search strategies. This may explain the higher user-satisfaction and higher perceived quality by younger physicians. Physicians should know the specific goals of a source and the kind of information available in the sources to choose the most suitable source. This will increase the perceived user-friendliness and quality of the source.

In conclusion, physicians evaluate the quality of the information on most of the EBM sources as good to very good. Only for BELMIP and Google, the quality was considered as poor by at least 17% of the physicians. More than half of the respondents found an answer to their questions in most of the sources. For some sources of known high quality [for example Cochrane and Medline), the answer to the question was rarely or never found in 20% of the cases.

More than half of the respondents found the necessary information in less than 5 minutes in most Belgian sources but also in Google and NHG. The fact that all these sources were available in Dutch, the language of the participants, may play a role. Thereupon is the information in these sources very concise, of direct clinical relevance and adapted to the local situation. We advise the developers of EBM sources to take these points into account by the

development or modification of new EBM sources.

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Public Health

Falsification of Type at Work: Assessment of Prevalence and Investigation of Predictors

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Abstract

BACKGROUND: Occupational-stress, job-satisfaction and poor health outcomes are closely related and strongly pertain to individuals' mental health and physiological well-being. Falsification of Type is a growing term in the field of organisational psychology that measures occupational stress when working in a job that does not match one's, natural leader.

AIM: The present work aims at determining the prevalence of falsification of type and associated socio-demographic and work-related factors.

METHODS: The study sample consists of 150 researchers working at the National Research Centre of Egypt. Participants were asked to complete a self-report Falsification of Type Questionnaire, Andrews and Withey scale for Job Satisfaction, in addition to socio-demographic and work-related variables. Statistics included descriptive and comparative analyses. A regression model was built with falsification of the type as the dependent variable.

RESULTS: Facilities showed the highest rate of dissatisfaction in the Job Satisfaction Questionnaire. The most prominent manifestations of falsification were fatigue and irritability, and its predictors were the position, interpersonal relationships, facilities and sex according to the regression model. Falsification of type could seriously contribute to occupational stress. Job satisfaction is highly about falsification.

CONCLUSION: More research on the Falsification of Type at work is recommended with the greater attention of employers to the importance of the concept of person-job fit.

Introduction

Occupational-stress is a prevalent, costly problem in today's workplace. It is reported to cause psychological and physiological dysfunctions for the workforce and to decrease motivation in excelling in their position (Colligan et al., 2006) [1]. It is defined as the response people may have when presented with work demands and pressures that do not match their knowledge and abilities (WHO 2015) [2].

Occupational-stress was reported to be highly attributed to the surrounding working environment (Tabatabaei and Hashemi 2014) [3]. Job-satisfaction is a widely investigated job attitude that is highly associated with poor health outcomes due to occupational-stress (Khamisa et al., 2015) [4]. Gender, age, education level, years of experience and other psychosocial and work-related factors have also shown association with occupational stress (Ali et al., 2016; Jain et al., 2015) [5] [6]. Qualitative and quantitative assessments of occupational-stress prevalence, its most predominant signs and predisposing factors are of crucial importance Kok and Muula 2013) [7].

A recent approach relates occupational-stress to what is called *Falsification of Type*. Falsification of Type is a concept first introduced by the famous psychologist Carl Jung (1923) [8]. In his book, Jung stated that "…*whenever such a falsification of type takes place as a result of external influence, the individual becomes neurotic later, and a cure can successfully be sought only in a development of that attitude which corresponds with the individual's natural way*." (Jung, 1921: pp. 415-416) [9]. Extended work on falsification by Katherine Benziger (2013)

[10], highlighted the intimate relationship between pathological signs of stress and working in a job that does not match one's natural talents and interests. Studies on the physiological foundations for Falsification of Type over the past two decades found that the short-term consequences of Falsification of Type were increased irritability, headaches, and difficulty in mastering new tasks. Long-term sequelae of falsification included exhaustion, depression, lack of joy, homeostatic imbalance, premature ageing of the brain, and a vulnerability to illness.

Although the idea is appealing, yet, very little is known about Falsification of Type at work. The present study is an attempt to assess Falsification of Type among a pilot sample of researchers at the National Research Centre (NRC) of Egypt and the factors that may influence such falsification.

Subjects and Methods

A random sample of 150 researchers (16% males and 84% females) working at the NRC of Egypt voluntarily participated in the study. Participants were asked to complete a questionnaire for socio-demographic data and work-related factors. Socio-demographic data included age, gender, marital status, income and presence of chronic diseases. Work-related factors encompassed daily working hours (≤ 5hrs or > 5hrs), total working years (< 10years or ≥ 10years) and job satisfaction.

Job satisfaction was measured using Andrews and Withey (1976) [11] Job Satisfaction Questionnaire. Respondents were asked to indicate how they feel about their job on a 7-point Likert scale where 1 is most satisfied, and 7 is least satisfied. The scale consists of five subscales about position, interpersonal relationships, job nature, job demands and facilities. The average score of the five subscales was used to represent overall job satisfaction.

Falsification of Type Scale is another self-report measure that was used to estimate falsification of type (Benziger 1996) [12]. It assesses stress resulting from a violation of one's natural preferences at work. The questionnaire targets some common signs of stress: laziness at work, irritability, loss of concentration, headache, loss of sense of humour, chronic fatigue and excess caffeine intake. It also asks about the feeling that time moves slowly at work, being bored at work, finding job demands non-interesting, feeling that one's work is underestimated, feeling that one's ideas are always offended at and feelings of disrespect of one's achievements. A three-point Likert scale with scores of zero, half and one represented the answering scheme. Average scores were then calculated for all the 14 items of the questionnaire to create a score of zero to one where one represented the greatest degree of falsification.

Analyses were performed using SPSS, version 23 (SPSS Inc., Chicago, IL, USA). Means, standard deviations and frequencies were calculated for all study variables. Multiple linear regressions were used to examine the influence of age, sex, marital status, monthly income, education level, chronic disease, working years and job satisfaction on falsification of type. Choice of the predictors above was based on our results from univariate analyses, in addition to the previously established impact on outcomes. All statistical tests were two-sided, and a p-value of 0.05 or less was considered significant.

Results

As shown in Table 1, the majority of the study population were female (84**%**), less than 40 years old (69%), married (79**%**), not suffering from chronic diseases (78%), have been working for more than 10 years (54%) and working for less than 5 hours per day (57**%**). Approximately 13% were dissatisfied with their job, and 11% suffered falsification of type.

The job satisfaction subscale showing highest rates of dissatisfaction was that pertaining to facilities (mean = 4.5, SD = 1.36). Fatigue (mean = 0.62, SD = .44) and irritability (mean = 0.54, SD = 0.46), were the most prevalent signs of falsification.

Table 1: Descriptive data of variables and measures as frequency percentage

Study Variables (N)	N (%)
Gender (1**50**)	
Male	24 (16%)
Female	125 (84%)
Age (1**38**)	
< 40	95 (69%)
≥ 40	43 (31%)
Social Status (95)	
Married	110 (79%)
Single	29 (21%)
Monthly income (133)	
< 5000LE	53 (40%)
≥ 5000LE	80 (60%)
Education (143)	
Post doctorate	65 (45%)
Postgraduate	78 (55%)
Daily Working Hours (129)	
≤ 5	73 (57%)
> 5	56 (43%)
Working Years (144)	
≤ 10	66 (46%)
> 10	78 (54%)
Chronic Diseases (144)	
Yes	32 (22%)
No	112 (78%)
Falsification of type (150)	
≤ 0.5(non-falsified)	133 (89%)
> 0.5(falsified)	17 (11%)
Job Satisfaction	
≤ 4(satisfied)	130 (87%)
> 4(dissatisfied)	20 (13%)

As shown in Table 2, none of the demographic or work-related variables differed significantly between the falsified and non-falsified groups except for job satisfaction. The vast majority (90%) of participants were satisfied with their jobs

when non-falsified compared to 65% of the falsified group (chi sq. = 8.002, p = 0.013).

Table 2: Demographic and job characteristics: Comparison of falsified versus non-falsified participants

Study Variables (N)	Non falsified group ≤ 0.5 (N = 133) N(%)	Falsified group > 0.5 (17) N(%)	P value
Gender (150)			
Male	24 (18%)	0 (0.0%)	0.076
Female	108 (81%)	17 (100%)	
Age (**138**)			
< 40	81 (66%)	14 88%)	0.148
≥ 40	41 (34%)	2(12%)	
Marital Status (95)			
Married	97 (79%)	13 (81%)	1.000
Single	26 (21%)	3 (19%)	
Monthly income (133)			
< 5000LE	49 (41%)	4 (31%)	0.563
≥ 5000LE	71 (59%)	9 (69%)	
Education (143)			
Post doctorate	61 (48%)	4 (27%)	0.172
Postgraduate	67 (52%)	11 (73%)	
Daily Working Hours (129)			
≤ 5	67 (56%)	6 (55%)	1.000
> 5	51 (43%)	5 (45%)	
Working Years (144)			
≤ 10	57(44%)	9 (60%)	0.282
> 10	72 (56%)	6 (40%)	
Chronic Diseases (144)			
Yes	27 (21%)	5 (33%)	0.325
No	102 (79%)	10 (67%)	
Job Satisfaction			
≤ 4(satisfied)	119 (90%)	11 (65%)	0.013
>(dissatisfied)	14(10%)	6(35%)	

The group suffering from falsification of type showed significantly higher job dissatisfaction overall and in all domains, namely; position, interpersonal relationship and job nature and facilities at the working environment with the only exception being job demands (Table 3).

Table 3: Comparing job satisfaction scores between falsified and non-falsified participants

Job Satisfaction scale and its items	Non-falsified group ≤ 0.5 N=133	Falsified group > 0.5 N=17	P value
Job Satisfaction total score	3.2 ± 0.8**	4.1 ± 0.7**	< 0.001
Satisfaction with position	2. 9 ± 0.8**	3.9 ± 1.2**	< 0.001
Satisfaction with interpersonal relationships	2.8 ± 0.91**	3.8 ± 1.3**	0.006
Satisfaction with job nature	2.8 ± 1.0**	3.8 ± 1.3**	< 0.001
Satisfaction with job demands	3.5 ± 1.2	3.9 ± 1.2	0.169
Satisfaction with facilities	4.4 ± 1.4*	5.2 ± 1.3*	0.029

*significant at p < 0.05, ** significant at p < 0.001.

Multiple linear regression was performed with falsification of type as the dependent variable and age, sex, marital status, income, educational level, chronic diseases, working years and Job Satisfaction total score as the independent variables (Model 1a, Table 4).The model was significant (F (8, 99) = 6.100, p < 0.001, adjusted R2 = 0.276). The job satisfaction total score was the only significant predictor for falsification of type.

Table 4: Linear regression predicting falsification of Type (Model 1a)

	Falsification of type				
	Beta	SE	Standardised beta	T	P
Age	-0.016	0.050	-0.037	-0.308	0.759
Sex	0.081	0.042	0.164	1.918	0.058
Marital status	-0.061	0.046	-0.120	-1.328	0.187
Monthly income	0.005	0.039	0.014	0.137	0.891
Education level	0.079	0.048	0.203	1.638	0.105
Chronic diseases	-0.068	0.040	-0.147	-1.709	0.091
Working years	-0.018	0.043	-0.046	-0.413	0.680
Job Satisfaction	0.105	0.022	0.422	4.810	<.001

Replacing job satisfaction total score with its five subscales; position, interpersonal relationship, job nature, job demands and facilities produced Model 1b, Table 5 that was still significant (F (12, 95) = 6.784, p < 0.001, adjusted R2 = 0.393). Significant predictors for Falsification of Type were position, p = 0.002, interpersonal relationships, p = 0.004, facilities, p = 0.004 and sex, p = 0.027.

Table 5: Linear regression predicting falsification of Type (Model 1b)

	Falsification of type				
	Beta	SE	Standardised beta	T	P
Age	-0.043	0.048	-0.102	-0.892	0.374
Sex	0.088	0.039	0.179	2.245	0.027
Marital status	-0.026	0.044	-0.052	-0.599	0.551
Monthly income	0.019	0.036	0.047	0.518	0.606
Education level	0.041	0.047	0.106	0.880	0.381
Chronic diseases	-0.043	0.037	-0.094	-1.176	0.242
Working years	-0.022	0.041	-0.056	-0.527	0.599
Position	0.061	0.020	0.286	3.107	0.002
Interpersonal	0.049	0.017	0.255	2.917	0.004
relationship	0.005	0.017	0.027	0.289	0.773
Job nature	0.014	0.014	0.084	0.971	0.334
Job demands	0.039	0.013	0.269	2.970	0.004
Facilities					

Discussion

In a sample of Egyptian researchers, we have found that the majority (87%) are satisfied with their work and are not suffering falsification of type (89%). However, a minority of researchers (11%) do show signs of falsification, and 35% of them are dissatisfied with their work.

According to our regression analysis and in agreement with the literature (Saleh et al., 2016; Lindholm and Szelényi 2013; Pillay 2009; Piko 2006; Kalliath and Morris 2002) [13-17], job satisfaction was the main predictor of Falsification of Type among the study population; less job satisfaction was associated with higher levels of falsification of type. When the same regression analysis was repeated with Job Satisfaction total score being replaced by scores of its subscales, position, facilities and inter-personal relationships were the subscales significantly influencing Falsification of Type together with gender.

The less job-satisfaction with the availability of facilities among the falsified group in the present study is consistent with the findings stated by Graham and his colleagues (2011) [18] and emphasises how lack of resources represent a prominent stressor at work generally and for researchers specifically. Another predictor of falsification was employee's position or post that could be attributed to the imbalance between effort and reward as stated by Mark and Smith (2012) [19]. Researchers, rather than other occupations, are reported to suffer from chronic fatigue and anxiety due to the nature of their work and the various other challenges they are exposed to (Holleman et al., 2015) [20]. As for the role of interpersonal relationships in predicting work stress, conflict with

peers (Malinauskienė et al., 2009) [21] could be one explanation. Brown et al., (2015) [22], on the other hand, found a link between employees' relation with managers, concerning the issue of trust, and their overall performance.

Strikingly all subjects experiencing falsification of the type were females. This is not unusual in the Egyptian working environment where Ali et al., (2016) [5] found significantly higher Allostatic Load Index (ALI) of primary mediators -that predicted stress at its early stage- for females (2.0) than males (1.1). They also found that all the population group with ALI exceeding the normal limit (12.9%) were females.

One similar study conducted by Amer et al., (2016) [23] on researchers working at NRC in Egypt showed that those who didn't receive their PhD/MD had a significantly higher score on Falsification of Type scale compared to PhD/MD holders. Falsification of type score also showed a significant negative correlation with income among researchers in the same study. Similarly, but non-significantly, in the present work, 73% of the group suffering falsification of the type were postgraduates, yet, with no impact of income on falsification of any sort.

Results of this study together with reports from the literature suggest that attempts to improving working conditions and hence the level of job satisfaction among workers are needed. Enhanced Job Satisfaction is related to better performance, better mental, psychological and physical health, better coping with stressors and creates positive emotions in the working environment (Choo and Bowley 2007; Luthans 2006) [24] [25]. This study provides evidence for the suffering of Falsification of Type by some researchers in Egypt. More research, both theoretical and empirical is needed to further understand this phenomenon and better match peoples' jobs to their interests and abilities.

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Public Health

Knowledge and Concerns of Parents Regarding Childhood Fever at a Public Health Clinic in Kuching, East Malaysia

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Abstract

BACKGROUND: Parental anxiety regarding fever may be unwarranted as most cases are owing to self-limiting causes.

AIM: To assess the level of knowledge and concerns regarding childhood fever among parents with young children in a public health clinic in Kuching, East Malaysia.

METHODS: This cross-sectional study was conducted among parents recruited from a maternal and child health clinic, with children aged 6 months to 6 years. The participants completed a self-administered questionnaire regarding their knowledge and concerns about childhood fever. Descriptive statistical analyses were performed, and associations between dependent and independent variables were determined.

RESULTS: Only 26.1% of participants were found to have good knowledge. Knowledge regarding childhood fever was significantly associated with parent's ethnicity, education level, and household income. About 72% of parents were always worried about their child's illness. Three major reasons for their concerns were persistently rising temperature; discomfort caused by the fever, and feared complications of fever.

CONCLUSION: Excessive parental anxiety due to poor knowledge and misconceptions about fever may lead to poor quality of life and inappropriate management of fever. Healthcare providers may help by educating parents about fever and serious signs that indicate the need to seek healthcare advice.

Introduction

Children, particularly of preschool age, commonly present to primary health care clinics with fever. However, fever in children at the primary care setting is largely caused by self-limiting conditions with a low prevalence of serious infections [1] [2]. Parental anxiety can lead to increased healthcare service utilisation including consultation with doctors after office hours, leading to an increased burden to healthcare providers and inappropriate requests for antibiotics [3] [4]. Fever is thought to have a beneficial physiological effect in combating illness [5] and may indicate the presence of serious conditions. However, excessive anxiety among parents regarding their child's fever, also known as 'fever phobia', may lead to unnecessary medication and over-management at home [6] [7], which may pose safety issues for the child.

The term 'fever phobia' was first coined by Schmitt (1980) [7], who defined it as 'an unrealistic fear of fever expressed by parents', and has been observed in different countries [8] [9] [10]. Parents worry about the feared complications of fever such as febrile convulsion, dehydration, brain damage with subsequent intellectual impairment, and death [4] [11]. However, most parents lack the knowledge to assess the severity of their children's illness [6] [12] [13] and some parents believe fever to be a disease in itself

[14]. Parents may experience negative emotions such as helplessness and guilt if they do not act to reduce their child's temperature [12] [13] [15]. Parents' misconceptions about fever increase their anxiety and eventually influence their management strategy [6] [15].

Many parents define normal temperature and fever incorrectly [16] [17] [18]. Past studies conducted worldwide used core temperatures of 38°C and 39°C to define fever and high fever, respectively [6] [16] [17] [18]. Varying proportions of parents interpret normal body temperature (less than 38°C) as fever [9] [19] [20] [21]. About 24.8% to 63.9% of parents administer antipyretics to their child with a temperature of 37.8°C [9] [19] leading to a risk of over-medication. Parents also have misconceptions regarding antipyretics, believing that they can prevent febrile convulsion and brain damage [22] despite understanding that excessive antipyretics can be dangerous and lethal [23]. Some parents have unrealistic expectations for the fever to resolve within 1 to 2 days. When the duration of fever exceeds their expectations, they bring their children to a doctor [24].

With improved healthcare education, 'fever phobia' has generally reduced from 12–43% in the 1980s [7] [25] to 2–18% in the 2000s [6] [26]. However, it is still prevalent in Asian countries such as Taiwan and Singapore, where 68.8–77.7% of parents believe that fever causes brain damage, compared to only 14.4–21% in developed countries like the USA and Australia [9] [19] [20] [21]. This reflects lower health literacy levels among Asian parents regarding fever.

Most studies conducted in Malaysia regarding health-seeking behaviour are related to antibiotic use for common minor illness. One study on the predictors of health-seeking behaviour in upper respiratory tract infection among children found that ethnicity and low-income level were associated with early visits to seek medical advice [27]. Another study on parental knowledge focused on over-the-counter medications usage and found similar results in insufficient knowledge among parents [28]. To the best of our knowledge, there are no published studies on parental knowledge regarding childhood fever in Malaysia.

Thus, this study aimed to assess the knowledge and concerns of parents of young children regarding childhood fever in this country.

Methods

This cross-sectional study was conducted at the Maternal and Child Health Clinic (MCH) of an urban public primary care clinic in Kuching, in Sarawak, a state in East Malaysia with an ethnically diverse population. Parents of young children aged 6 months to 6 years who visited the clinic were approached to participate in the study. Eligible respondents consisted of adult parents with children aged 6 months to 6 years, who were literate in Malay or English. If both parents were present, the parent who was primarily involved in managing the child with a fever was chosen as the participant. We excluded parents of children with serious chronic medical diseases, such as immunosuppression, congenital heart disease, and neurological or oncological conditions, as well as parents who were not involved in caring for their sick child.

The sample size was calculated to determine the population mean based on the variance derived from Chang's study [21]. A minimum sample size of 135 respondents was required to achieve a 95% confidence interval and 0.5% precision. However, this was increased to 169 participants to allow for the possibility of a 20% non-response rate. Data were collected from June to August 2017 using convenience sampling. Parents were approached in the waiting area of the MCH and screened according to the inclusion and exclusion criteria. Parents who agreed to participate provided written consent before the study materials were administered with researcher assistance.

This study received ethical approval from both the Ministry of Health Medical Research Ethics Committee (Approval number: NMRR-16-1337-31628) as well as the Medical Research Ethics Committee (Project code number FF-2016-385). Permission was also obtained from the local District Health Office.

The study instrument comprised 3 main sections: sociodemographic data of parents, knowledge regarding childhood fever, and parental concerns regarding fever. The section regarding knowledge and concerns regarding childhood fever was adapted from a Taiwanese study with the permission of the original authors [21]. The questionnaire underwent back-to-back translation from the original Chinese version into English and Malay. The translated versions were pre-tested with five parents to determine face validity and comprehensibility. The questionnaire items were reviewed for content validity by an expert panel comprising a consultant paediatrician, two family medicine specialists, and a clinical psychologist. The expert panel also evaluated the correct answers for each item. The questionnaire was modified and adjusted accordingly. Finally, a pilot study was conducted on 17 participants to test the questionnaire. Results showed satisfactory internal consistency reliability with a Cronbach's alpha of 0.7. The mean knowledge score obtained from the pilot study was 13, which was arbitrarily selected as the cut-off point for good knowledge.

The first 18 questionnaire items were true-false questions regarding parents' understanding of fever. For this part, parents were awarded 1 point for

each correct answer, while no points were given for other responses. This was followed by 5 open-ended questions about temperature. We adopted the World Health Organization (WHO) definitions for fever for this study: normal body temperature was defined as an axillary temperature of 36.2–37.4˚C, fever as 37.5–41˚C, and high fever as 38.5–41˚C [29]. A body temperature exceeding 39˚C was considered as a fever that might cause harm to children. Again, a score of 1 point was awarded for correct temperatures written by parents. The scale for knowledge ranged from 0 to 23 points.

Parental concerns were assessed using a self-completed close-ended data collection form regarding parents' concerns in certain situations and their common perceptions regarding fever and its outcomes. Samples with missing data on the knowledge scale were excluded from the analysis.

Data were analysed using Statistical Package for the Social Sciences (SPSS) version 24 (IBM). Descriptive analysis included frequencies, percentages, means, and standard deviations. Associations between dependent and independent variables were determined using a t-test, Pearson's correlation, and a one-way ANOVA. Missing data were excluded via pairwise analysis. Level of significance was set at $p < 0.05$.

Results

A total of 157 participants were included, providing a response rate of 94.7%. The majority of participants were young mothers (81.5%), with a mean age of 30.4 ± 6 years. Participants were distributed almost evenly across different ethnicities. About 37.5% of the participants were first-time parents. Most of them had completed secondary education (70.6%) and had a low household income level (80.9%) (Table 1).

Table 1: Baseline characteristics of respondents

Sociodemographic characteristics	Mean (SD)	n (%)
Age (years)	30.39 (6.07)	
Less than 30 years		53 (33.8)
30 years and above		89 (56.7)
Not stated		15 (9.5)
Relationship with child		
Father		28 (17.8)
Mother		128 (81.5)
Not stated		1 (0.7)
Ethnic race		
Malay		40 (25.5)
Chinese		43 (27.4)
Iban		36 (22.9)
Others		31 (19.7)
Not stated		7 (4.5)
Level of education		
Primary or non-formal education		12 (7.6)
Secondary		99 (63.0)
Tertiary		45 (28.7)
Not stated		1 (0.7)
Household income		
Less than RM4000		127 (80.9)
RM4000 to RM7999		23 (14.6)
RM8000 and above		7 (4.5)
Number of children		
One		59 (37.5)
Two		53 (33.8)
Three or more		43 (27.4)
Not stated		2 (1.3)

The knowledge score of parents was normally distributed, with a mean score of 10.03 ± 3.6 (Table 2).

Table 2: Parental knowledge regarding childhood fever

	Mean (SD)	n (%)
Parental knowledge regarding childhood fever	10.03 (3.62)	
Good knowledge (score ≥13)		41 (26.1)
Poor knowledge (score < 13)		116 (73.9)

Only 26.1% of parents had good knowledge regarding the management of childhood fever. A large proportion of parents (71.3%) had the misconception that fever causes diseases (Item 17) (Table 3). Almost all parents believed that fever could cause harm to children (Item 23, 93.6%) and indicated that they would administer fever medication to treat feared fever complications (Item 22, 92.4%). Less than half of them knew the correct answer for normal body temperature (Item 8, 49.7%) and fever (Item 13, 39.5%).

Table 3: Items in the knowledge scale according to the percentage of correct answers

Items	Percentage of correct answers
Fever is a condition when the temperature rises above normal. (T)	91.1
Fever is an immune reaction. (T)	80.9
Fever is the consequence of bacterial or viral infection. (T)	80.3
Fever helps alert parents. (T)	79.0
Fever is the sign of a disease. (T)	75.8
Fever is the sign of a potential underlying disease. (T)	71.3
Temperature in high fever (38.5-41°C)	51.0
Normal body temperature (36.2-37.4°C)	49.7
It is necessary to treat fever regardless of body temperature. (F)	43.3
Fever is due to exposure to cold weather. (F)	40.8
Maintaining comfort is more important than bringing down the temperature. (T)	40.1
Fever helps to boost immunity. (T)	39.5
The temperature that indicates fever (37.5-41°C)	39.5
Fever helps to combat illness. (T)	36.3
The temperature that is harmful (>39°C)	36.3
Possible temperature if fever medication is not given (41°C)	34.4
Fever causes disease. (F)	28.7
It is reasonable to wait 3 days before seeing a doctor. (T)	24.2
Fever results from disease. (F)	19.1
Temperature would keep rising if fever medications are not given. (F)	15.3
Fever is due to an imbalance of heat and cold in the body. (F)	14.0
Fever medication can treat complications arising from fever (F)	7.6
Fever will cause harm to children. (F)	6.4

Chinese participants were found to have a significantly better knowledge score compared to other ethnicities (F (3,146) = 8.584, $p < 0.001$) (Table 4).

Table 4: Association between parental knowledge regarding childhood fever and sociodemographic characteristics of the parent

Sociodemographic characteristics	Knowledge score Mean (SD)	Statistical tests	*P*
Age		Spearman's correlation R = 0.020	0.847
Relationship with the child		Student's t-test	0.628
Father	10.32 (3.84)	T = 0.485	
Mother	9.95 (3.60)		
Ethnicity		One way ANOVA	< 0.001
Malay	9.18 (2.54)	F (3, 146) = 8.584	
Chinese	12.12 (3.92)		
Iban	8.61 (3.45)		
Others	9.87 (3.31)		
Level of education		One-way ANOVA	< 0.001
Primary or non-formal education	6.75 (2.53)	F (2, 153) = 22.209	
Secondary	9.27 (3.18)		
Tertiary	12.49 (3.43)		
Household income		One-way ANOVA	< 0.001
Less than RM4000	9.34 (3.34)	F (2, 154) = 17.825	
RM4000 to RM7999	12.09 (3.07)		
RM8000 and above	15.71 (2.75)		
Number of children		One-way ANOVA	0.190
One	9.37 (3.43)	F (2,152) = 1.677	
Two	10.23 (3.75)		
Three or more	10.65 (3.73)		

This was confirmed by Bonferroni post-hoc tests (Table 5), which revealed that differences in

knowledge scores were statistically significant between Chinese and other ethnicities.

Parents with tertiary education had better knowledge compared to those with lower educational levels (F (2,153) = 22.209, $p < 0.001$). Finally, parents from the high-income group had better knowledge compared to those from other income categories (F (2,143) = 17.823, $p < 0.001$). These results were also confirmed by post-hoc tests.

Table 5: Bonferroni post-hoc analysis

Categories (I vs J)	Mean difference	Standard error	*p*-value
Ethnicity			
Chinese vs Malay	2.941	0.737	< 0.001
Chinese vs Iban	3.505	0.758	< 0.001
Chinese vs Bidayuh and others	2.245	0.790	0.031
Level of education			
Tertiary vs secondary	3.216	0.517	< 0.001
Tertiary vs primary/non-formal education	5.739	1.043	< 0.001
Secondary vs primary/non-formal education	2.523	0.981	0.033
Household income			
≥ RM8000 vs RM4000-7999	3.627	1.417	0.036
≥ RM8000 vs <RM4000	6.438	1.286	< 0.001
RM4000-7999 vs <RM4000	2.811	0.754	0.001

About 72% of participants reported always being worried when their child had a fever. The three main reasons for parental concern were the discomfort of the child during fever (68.8%), persistently rising body temperature (68.2%), and feared harms of fever (63.7%). The feared harms of fever that worried the parents the most were a seizure (67.5%), brain damage (52.2%), mental incapacity (44.6%), and death (38.9%). Participants reported that their concerns were mainly influenced by their own or a family member's previous experience with child fever (59.9% and 42.0% respectively), not knowing the cause of the fever (39.5%), and doctor's advice upon consultation (35.7%).

Discussion

This study showed that the knowledge level of parents regarding childhood fever was alarmingly deficient. Many parents did not know the correct normal body temperature, and that considered as fever. They were confused with the causal relationship between fever and disease and believed that fever itself is harmful to their child. Parental knowledge concerning the purpose of antipyretics was also incorrect.

In the present study, only 39.5% and 51% of participants knew the correct temperature to define fever and high fever, respectively. This was comparable to previous studies conducted in Australia, the United States, and the United Arab Emirates [19] [20] [26]. Incorrect understanding of fever would subsequently lead to the more frequent use of antipyretics [3] [30]. This suggests that poor knowledge regarding the temperature considered as fever may be a worldwide phenomenon. Efforts to improve health education regarding fever should be considered for the general public, as the ability to correctly identify fever would protect against inappropriate management.

The association between knowledge and ethnicity, level of education, and income were not unexpected. A previous study found that Chinese parents were less likely than Malay or Indian parents to see a medical professional for upper respiratory tract infection, suggesting they were more comfortable managing the condition themselves possibly because of better health literacy [27]. Education and income are known to influence factors on health literacy [31]. Parents with lower levels of education were found to be more likely to believe that fever is dangerous [8]. Therefore, parents' sociodemographic characteristics could influence their knowledge regarding childhood fever.

There were a large proportion of parents in this study who reported high levels of worry when their child had a fever. Other similar studies in Taiwan, the United Kingdom, and Singapore also had similar findings [9] [21] [32]. Appropriate levels of anxiety or concern are important to promote protective parental behaviours including increasing the fluid intake and being more attentive towards the child [32]. However, excessive or inappropriate concerns should be addressed to avoid negative emotional outcomes in parents.

Common misconceptions regarding complications of fever such as seizures, brain damage, mental incapacity, and death were also reported in other studies [11] [26] [32]. In particular, more Asian parents reported concern regarding possible brain damage (35.9–77.7%) compared to Western parents (7.7–15%) [9] [11] [20] [21] [26] [32]. Soon et al., (2003) [9] postulated that this phenomenon could be due to Asian parents' emphasis on educational attainment. The current study also showed a larger proportion of parents who were worried about fever leading to death (38.9%) compared to other countries (3.8–18%) [11] [19] [32]. The concept of fever among this population could be further explored in future studies.

Teaching parents what to do when their child develops a fever can help to improve parental knowledge and parental satisfaction and reduce inappropriate healthcare visits [33]. However, the method of delivering this education needs to be suited to the population, particularly among those with lower health literacy [33]. Important educational points should include the definition of fever, the role of fever in childhood illnesses, what to assess during febrile episodes, and when a healthcare visit is required [33] [34]. Before administering antipyretics, simple

measures such as tepid sponging and making sure that the child's clothing or bedcovers are not too heavy should be recommended.

This is the first local study on parental knowledge and concerns regarding childhood fever in Malaysia. However, its generalizability is limited by convenience sampling and recruitment from a single public health clinic. There are also different definitions of temperature according to the site of thermometer measurement, which may affect their knowledge scores. We recommend that this study is replicated at multiple sites to gain a better understanding of Malaysian parents' knowledge and concerns regarding childhood fever. The current study has shown glaring deficiencies in the knowledge of this population, which would warrant a health education intervention for parents who bring their children to the clinic for consultation. Developing local self-care guidance for parents may help them to identify the signs of serious conditions, as well as minimise unnecessary healthcare service utilisation. This guidance should also include information regarding correct antipyretic medication administration.

Parents with young children attending Batu Kawa Health Clinic in Kuching, East Malaysia do not have sufficient knowledge about childhood fever and may have excessive concerns about fever. Thus, it is important to provide health education to parents about childhood fever, including management guidelines, and the ability to identify signs indicating the need to seek medical attention.

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Review Article

Advances in the Diagnosis of GERD Using the Esophageal pH Monitoring, Gastro-Esophageal Impedance-pH Monitoring, And Pitfalls

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Abstract

PH monitoring is not capable of detecting all types of reflux, especially when the amount of acid is very low or not at all in the refluxate. Multichannel intraluminal impedance-pH monitoring (MII-pH) is used as a new method to assess bolus transport. The types of reflexes including acid, weak acid and weak alkaline MII-pH is capable of distinguishing more reflux episodes based upon use of physical and chemical parameters of the refluxate, leads to a diagnosis of normal acid reflux from abnormal nonacidic reflux. 24-h oesophagal pH monitoring can be effectively used to assess the potential relationship between symptoms and refluxes. MII-pH is capable of distinguishing more reflux episodes based upon use of physical and chemical parameters of the refluxate, leads to a diagnosis of normal acid reflux from abnormal nonacidic reflux. It can be used to confirm gastro-oesophagal reflux episodes, where has a sensitivity and specificity for diagnosing GERD in comparison with endoscopy or pH-metry.

Introduction

Gastro-oesophagal reflux disease (GERD) is a digestive disorder, which is associated with the flowing back of acid and gastric contents to the oesophagus. GERD occurs when gastroesophageal reflux causes symptoms and/or unpleasant complications, and it is described as the most common chronic upper gastrointestinal disease.

A variety of GERD clinical symptoms are commonly seen in this condition include heartburn, regurgitation, nausea, vomiting, belching, heavy stomach feelings, and epigastric pain, while atypical symptoms are associated with hoarse voice, coughing, sore throat or ear pain [1]. It is noteworthy that typical GERD has been described to be troublesome heartburn with/without regurgitation [2] [3]

Proton-pump inhibitors are commonly used as a treatment option for patients with typical symptoms of the disease, which is more than 80% effective in treating esophagitis and heartburn [2] [4]. Empirical treatment with PPIs is a method that initially leads to an assessment of more individuals with persistent symptoms despite the use of repressive therapies [5]. The most common cause of failure is the misdiagnosis of GERD with various functional disorders [6]. It has

been revealed that weakly acidic reflux episodes are other causative factors involved in the symptoms of heartburn and regurgitation [7] [8].

Diagnostic evaluations of patients with PPI-refractory heartburn and uninvestigated PPI-responsive cases are required in the absence of alarm manifestation [2] [4]. Patients suffering from GERD are generally divided into two groups of non-erosive reflux disease and erosive esophagitis [9]. Methods such as diet and lifestyle changes and protein pump inhibitors (PPIs) are recommended for the treatment of NERD [1].

It has been reported that endoscopy revealed a small percentage of patients with erosive reflux disease, whereas most patients with endoscopy-negative heartburn are considered as non-erosive reflux disease. The criteria for this attitude is described as abnormal results in pH or impedance-pH monitoring, while the normal results and unfavourable response to a PPI are categorised as functional heartburn [2] [10] [11].

Functional heartburn (FH) treatment is most commonly performed with an individual approach and is mostly an experimental therapy because the poor response to the treatment of acid suppression is abundant in which the psycho-pathological component is also present. Nevertheless, it can be said that monitoring heartburn in patients who are diagnosed with non-erosive reflux disease will be an important factor in distinguishing these individuals from those who have true FH [12] [13]. Other methods other than endoscopy should be used for monitoring of gastro-oesophagal reflux. Gastro-oesophageal refluxate, independent of mucosal lesions, is initially performed using PH monitoring in the distal oesophagus. This method is routinely used as a gold standard for diagnosis and monitoring of treatment interventions [5]. PH monitoring is not capable of detecting all types of reflux, especially when the amount of acid is very low or not at all in the refluxate. Multichannel intraluminal impedance-pH monitoring (MII-pH) is used as a new method to assess bolus transport and types of reflexes including acid, weak acid and weak alkaline [14]. The current paper was aimed to discuss the technical aspects in implementing PH monitoring and impedance-pH monitoring techniques for the detection of reflux.

Methods

We have collected all documents using a curated medical database such as PubMed, Scopus Embase, MEDLINE, Web of Science Core Collection, Google Scholar, etc.

Results and Discussion

A 24-h oesophagal pH Monitoring can be effectively used to assess the potential relationship between symptoms and refluxes. Oesophagal pH monitoring is routinely applied using catheter-based systems (Single sensor or Dual sensor) and recently without pH catheter (wireless Bravo pH capsule or OMOM PH capsule).

This method is performed in cases which do not respond to medication, where there are common GERD symptoms, such as heartburn and regurgitation. In terms of atypical GERD symptoms such as chest pain, cough, hoarseness, wheezing, and a sore throat or ear pain, if symptoms are caused by gastro-oesophageal reflux, monitoring of PH can be occasionally applied for determining therapeutic drug *effectiveness* against GERD, where it is effective in determining the association of times of reflux with atypical symptoms. This test is usually performed as part of procedures before performing an *antireflux operation* [15] [16].

A cutoff pH 4.0 is usually accepted by the most specialist for diagnosis of acid reflux episodes in both catheter-based and catheter-free devices due to the decreased pepsin's proteolytic activity in solutions with a pH higher than 4.0 and the reporting of symptoms of common reflux in intraesophageal pH below 4.0 [5] [17]. However, a pH of fewer than 4.0 units may be associated with the acid swallowing, and oesophagal exposure is likely to be overestimated. Furthermore, it has recognised that the proteolytic activity of pepsins is mainly needed for oesophagal mucosa damage [2] [18]. It should be into consideration that pepsin's proteolytic activity can be sustained up to pH 6.0 [19]. Moreover, healing of mucosal damage is achieved through reparative processes, whereas stopped at pH 6.5 [2] [20].

DeMeester score has been previously provided to quantify the *exposure* of the *distal oesophagus* to the acid based upon the use of six parameters where a DeMeester score > 14.72 shows reflux [5] [21], (Figure 1).

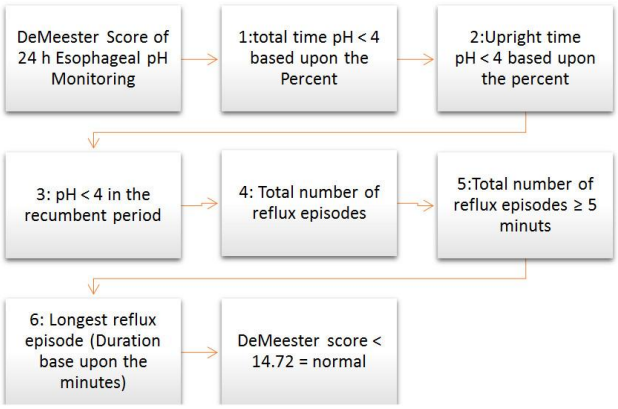

Figure 1: DeMeester Score of 24 h Esophageal pH Monitoring

The acid exposure time (percentage time pH < 4) has been applied as the most appropriate character to distinguish physiologic from pathologic reflux [22].

Both catheter and wireless-based pH monitoring is performed based on the use of the high acid concentration for the diagnosis of oesophagal reflux. This can quantitatively examine the exposure of distal oesophagal and the relationship of symptoms with acid reflux episodes. The extensive and prolonged use of this technique has made it as the gold standard in the diagnosis of GERD [5]. It should be noted, that cutoff of 4.0 has been rejected by some experts, and they believe that a cutoff value of 5 can be more effective in distinguishing healthy individuals from patients with reflux. Moreover, some studies have stated that the range of PH between 3 and 6 is better than determining just one threshold for detection [23] [24].

The normal values of the acid exposure time in different centres have been widely computed from 3.2 to 7.2 per cent. Additionally, the normative values of the acid exposure time have been reported in more than 30% of patients with reflux esophagitis [2] [22]. Accordingly, the acid exposure time is associated with limitation, therefore symptom–reflux correlation indexes have been provided to determine the association of reflux episodes with symptoms. Two important tools are available about this issue including symptom index (SI), symptom sensitivity index (SSI) and symptom association probability (SAP). The SI is described as the percentage of reflux associated symptom episodes which considered as positive when > 50%, representing at least half of the symptoms caused by GERD [5]. The SI is calculated by the following formula:

$$\frac{\text{Number of symptom episodes associated with pH} < 4}{\text{Total number of symptom episodes}} \times 100$$

This index is not able to assess the total number of cases of reflux in its calculation. (Hog et al., 2009). Moreover, there is a potential probability for false positive correlation results with an increase in the number of refluxes and a reduction in the number of symptoms. The symptom sensitivity index (SSI) has been developed as the percentage of symptom linked reflux episode based upon use of following formula [25] [26].

$$\frac{\text{Number of symptom episodes associated with pH} < 4}{\text{Total number of reflux episodes}} \times 100$$

SSI has increased by more than 10% the association of symptoms with reflux. The SSI and SI differ in their arbitrary cut off points and are based on the simultaneous frequency of reflux and symptoms, whereas the frequency of non-related reflux and episodes of symptoms are ignored in them [25] [27]. To eliminate these shortcomings, SAP can assess whether there are changes in the distribution of reflux episodes and symptoms during the monitoring by using statistical analysis, suggesting the meaningful probability of symptom–reflux correlation.

A SAP > 95 can be determined as positive (Figure 2), [5] [28] [29]. Studies have revealed that the evaluation of SAP and SI for non-acid reflux could provide a diagnostic value of between 16% and 33% in patients evaluated in the treatment of PPI [2] [30] [31]. Furthermore, off-PPI SAP positivity for non-acidic reflux events is only 10 to 12% of significant diagnostic value [2] [11] [32].

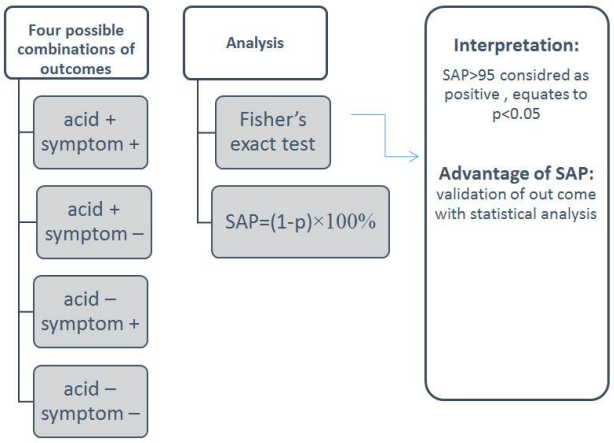

Figure 2: The analysis of contingency diagram of four possible combinations of reflux and symptoms for each segment (SAP)

The SI index is not able to assess the total number of cases of reflux in its calculation. A high amount of SI may be related to many parts of the patient's reflux episodes, and in this case, there is a great deal of chance. The superiority of the SAP is linked to its suitable statistical analysis [33].

The severity and clinical effect of the symptoms are not measured by SAP and SI, so nocturnal heartburn with unpleasant symptoms may be negative by using SAP and SI due to the calculation of these indicators based on the total 24-hour monitoring period.

Also, in low-reflux rate, the positive clinical value of SAP and SI is doubtful because the positive results in this case (low levels of reflux) are completely related to chance [2]. A study has suggested that SI and SAP are likely to be relevant in a patient with moderate to severe reflux. However, in patients with mild reflux, SI or SAP is not recommended for clinical decision making, such as whether the surgery should be performed [33] (Figure 2).

Despite the remarkable progress in the methods above, the lack of factual and reliable gold standard tests remains a problem, to which mentioned indicators are comparable [25] [34].

Two types of multichannel intraluminal

impedance (MII) are recommended for clinical evaluation that is a combination of MII with pH monitoring (MII-pH) and another MII conjugated with oesophagal manometry. MII has been initially provided to assess the movement of liquid, solid, and gas in the oesophagus without pH measurements [35].

MII-pH is another tool, which is developed with a combination of pH with impedance monitoring. This method is capable of distinguishing more reflux episodes based upon use of physical and chemical parameters of the refluxate, in which higher sensitivity and specificity for the diagnosis of GERD when comparing with other methods such as endoscopy or PH Monitoring. This method can be effective in determining acid reflux from non-acid reflux. Therefore, MII-pH indicates a situation that could rarely be affected by PPI treatment [36]. The advantages and disadvantages of MII-pH monitoring are summarised in Figure 3 [5] [37] [38]. As shown in the figure, this technique can analyse the symptoms associated with reflux in the event of acid suppression. As a result, negative results from MII-pH monitoring are very important for eliminating reflux compared to pH monitoring method [38] [39].

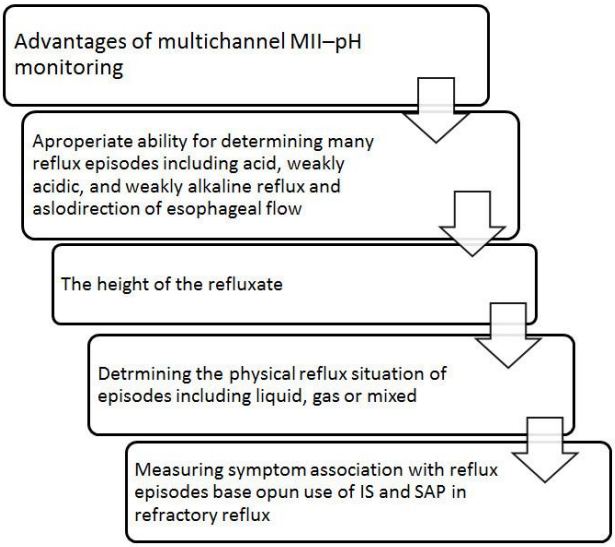

Figure 3: Advantages of multichannel intraluminal impedance (MII)

Impedance-PH Monitoring increases the diagnostic value of the GERD by 15–20% [40] [41]. Compared to a pH monitoring test, the most important feature of this test is the ability to evaluate patients with persistent symptoms, despite the use of PPIs, Impedance-PH Monitoring can also be effectively applied to the clinical evaluation of patients suffering from NERD and extra-oesophageal reflux symptoms (Figure 3) [41] [42] [42] [44].

To help grasp the gastroesophageal reflux disease, MII-pH may be a very suitable technique for diagnosis of non-acid reflux. However, the clinical practice of this method has some limitations that have been previously explained by different studies controversial results can be found in the clinical study:

1) Diagnosis of reflux episodes may be interrupted by lowering the impedance of MII-pH [45].

2) A catheter-based MII-pH monitoring will cause the patient's poor tolerance for monitoring pH-impedance [45].

3) Dietary changes during the MI-pH evaluation may lead to a poor predictor of gastroesophageal reflux [45].

4) Studies have reported a different prevalence of week- acid reflux. In the terms of PH impedance analysis, studies have shown that moderate and severe esophagitis exhibit similar or slightly higher levels of weakly acidic reflux than healthy subjects [46] [47]. On the other hand, very similar finding has been achieved in terms of distal esophageal exposure to weakly acidic reflux ate in patients with a non-erosive reflux disease (NERD) and esophagitis [48]. Regarding the available residence, the division of patients with weak acid reflux and physiological acid reflux as reflex, can raise one question, which Alkaline reflux is rarely seen [46] [49].

5) In context of esophageal bolus transit, measurement of bolus clearance with internal impedance does not determine the abnormal functioning of the esophagus, especially in terms of minor malformations [45].

6) The existing tool does not determine the reliability between small and large volumetric storage by with failed bolus transit, where it limits the clinical interpretation and physiological diagnosis of pathologic fluid from pathologic fluid [45].

7) There are other limitations that make the diagnosis even more difficult such as pathologic changes in the esophageal mucosa (esophagitis), which may reduce the baseline impedance values, and consequently the diagnosis of bolus movement in the esophagus will be complicated [16] [50]. Moreover, mucosal changes in the esophagus may also contribute to the disruption of the esophagus and transition of esophagus material, where eventually lead to the maintenance of liquids in the esophagus [50].

8) In patients with impotence syndrome, it is possible that the level of esophageal acid exposure time and reflux be increased using MII-pH, which may lead to problems with the diagnosis of GERD. The flow of gas or air in the MII-pH monitoring method can be performed manually due to the lack of automatic tools. Applying a meal to manometry has been revealed to be time consuming for diagnosis and treatment strategies in terms of rumination, where lead to dissatisfaction with the patient's intubation [45] [51] [52].

9) MII-pH is not appropriate to predict response to treatment. Based on available studies,

MII parameters for non-acid reflux are not predictive of patients with GER in response to PPIs [8] [53] [54]. However, the base impedance is mentioned that could be a good predictor for the response to treatment with PPI [45]. A stud indicated that showed that base impedance might have the necessary efficacy in predicting therapeutic outcomes in patients who suffer from sensitivity of the esophagus and functional heartburn [45] [55] [56].

10) MII-pH monitoring was not effective in the postoperative period of antireflux surgery, in addition, false-positive results of the MII-PH monitoring (50%) was reported that makes the test clinically meaningless in asymptomatic individuals with negative PH monitoring [46] [57] [58].

11) There are other limitations that make the diagnosis even more difficult such as pathologic changes in the esophageal mucosa (esophagitis), which may reduce the baseline impedance values, and consequently the diagnosis of bolus movement in the esophagus will be complicated [16]. Moreover, mucosal changes in the esophagus may also contribute to the disruption of the esophagus and transition of esophagus material, where eventually lead to the maintenance of liquids in the esophagus [50].

12) The base impedance on the MSI-pH has been clinically showed to have the utility of measuring at sleeping period when there is no swallow [45].

13) High cost than pH monitoring (about 4-fold).

14) Lack of normative data among children

Conclusion

A 24-h oesophagal pH Monitoring can be effectively used to assess the potential relationship between symptoms and refluxes. Both catheter and wireless-based pH monitoring have been associated with the high acid concentration for the diagnosis of oesophagal reflux. Leading quantitative examined the exposure of distal oesophagal and the association of symptoms with acid reflux episodes.

The wireless oesophagal pH test is associated with the patient's comfort and mobility, where have a significant effect for measuring over long periods of time.

MII-pH is capable of distinguishing more reflux episodes based upon use of physical and chemical parameters of the refluxate, leads to a diagnosis of normal acid reflux from abnormal nonacidic reflux. It can be used to confirm gastro-oesophagal reflux episodes, where has a sensitivity and specificity for diagnosing GERD in comparison with endoscopy or pH-metry. Evidence suggests that bolus clearance does not have the ability to diagnose effectively symptomatic patients from asymptomatic patients using intraluminal impedance, and consequently may be relatively limited in patients with minor manometric abnormalities [45].

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