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Basic Science

The Influence of Wharton Jelly Mesenchymal Stem Cell toward Matrix *Metalloproteinase-13* and *RELA* Synoviocyte Gene Expression on Osteoarthritis

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Keywords: Matrix Metalloproteinase-13; RELA; Mesenchymal Stem Cell Wharton Jelly; Co-culture

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Abstract

BACKGROUND: Therapy for osteoarthritis (OA) with satisfactory results has not been found to date. In OA pathogenesis, *RELA* gene involved in cartilage degradation and MMP-13 in degrade cartilage, as a member family of NF-κβ genes, *RELA* serves to modulate inflammatory responses and activates pro-inflammatory cytokines.

AIM: This study aims to identify the influence of Wharton Jelly Mesenchymal Stem Cell (MSC-WJ) on *MMP-13* and *RELA* expression gene in synoviocyte by in vitro.

MATERIAL AND METHODS: This research is pure experimental research. The sample used derived from synovial tissue of OA patients who underwent Total Knee Replacement (TKR) surgery. This study was divided into six groups treated with 4 replications. Group I and II (control groups) were synoviocyte of OA incubated for 24 and 48 hours, respectively. Group III and IV were MSC-WJ incubated for 24 and 48 hours, respectively. Group V and VI were Synoviocyte-MSC-WJ co-culture group incubated for 24 and 48 hours, respectively. Identification of *MMP-13* and *RELA* gene expression in each group was performed by using qPCR.

RESULT: The results showed that MSC-WJ reduced *MMP-13* gene expression after co-culture for 24 and 48 hours in OA synoviocyte. The highest gene expression of MMP-13 was in Group I and II (1.00 ng/μl), followed by Group III (0.41 ng/μl), Group IV (0.24 ng/μl), Group V (0.13 ng/μl), and Group VI (0.04 ng/μl). MSC-WJ administration also decreased *RELA* gene expression. The highest gene expression of *RELA* gene was in Group I and II (1.00 ng/μl), Group V (0.67 ng/μl), Group III (0.58 ng/μl), Group IV (0.16 ng/μl), and Group VI (0.16 ng/μl).

CONCLUSION: This study concluded that MSC-WJ in OA synoviocyte significantly reduced the expression of MMP-13 and *RELA* gene ($p < 0.05$).

Introduction

Osteoarthritis (OA) is a local disease, caused by primary and secondary degenerative disorders due to "wear and tear" and ageing process [1]. According to the World Health Organization (2004), the prevalence of OA in the world reached 151.4 million people, and about 27.4 million people were in the Southeast Asia region. In Indonesia, 8.1% of the total population experienced OA [2].

At the molecular level, the imbalance between catabolic and anabolic in the joint cartilage causes OA [3]. The expression of several genes involved in inflammatory responses and cartilage degradation, such as IL-1 and TNF-α, is regulated predominantly by Nuclear Factor Kappa Beta (NF-κβ). NF-κβ stimulates TNF-α and IL-1β cytokines which contribute to the inflammatory process in OA. NF-κβ is also important in the transcription process of MMP-13 gene [4], [5]. *RELA* is a subunit of the NF-κβ p65 gene which plays an important role in the pathogenesis of OA.

In the last decade, stem cell research has

shown rapid progress. The stem cells were employed to identify growth and development process of human body tissues, and the pathogenesis of diseases suffered.

This study aims to investigate the influence of Wharton Jelly Mesenchymal Stem Cell (MSC-WJ) on Matrix Metalloproteinase-13 and *RELA* gene expression in synoviocyte by *in vitro*.

Material and Method

A pure experimental study was divided into six treatment groups with four replications. Group I and II (control groups) were synoviocyte of OA incubated for 24 and 48 hours, respectively. Group III and IV were Mesenchymal Stem Cell-Wharton Jelly incubated for 24 and 48 hours, respectively. Group V and VI were synoviocyte- Mesenchymal Stem Cell-Wharton Jelly co-culture group incubated for 24 and 48 hours, respectively. Each treatment group consisted of 10^5 cells, each for synoviocyte and Mesenchymal Stem Cell-Wharton Jelly cells. Mesenchymal Stem Cell-Wharton Jelly derived from IMERI (Indonesian Medical Education and Research Institute) Faculty of Medicine, University of Indonesia. Synoviocyte is derived from synovial tissue of OA grade IV underwent Total Knee Replacement (TKR) surgery at Public Hospital Dr M. Djamil Padang, Indonesia. The experimental synoviocyte were the results of the 3rd passage cell culture. The samples taken did not use informed consent because the samples used were stored biologically after post-knee joint surgery Osteoarthritis Grade IV. Samples were taken from six patients with male sex aged 40-70 years.

Isolation of OA primary cells

Synovial tissue and search are obtained from OA patients after Total Knee Replacement (TKR). Ten samples were used for experiments. Synovial tissue is planted in the well plate with 10% Fetal Bovine Serum (FBS), 1% penicillin/streptomycin and 1% fungizone in Dulbecco's Modified Eagle's Medium (DMEM, Life Technologies) which is planted with an explant planting system. Cells were sub-cultured three times, and and the result of 3rd sub-culture was used for treatment. Each experiment was repeated for three times.

Coculture of stem cells with OA primary cells

OA primary cells were cultured with 50–60% confluence, then cultured together with mesenchymal stem cells from Wharton Jelly. The cells were observed for 24 and 48 hours and calculated with Haemocytometer with 10^5 cells/well.

Table 1: Primer Design

No. Primer Nucleotide Sequence NM Amplicon	NCBI Accession Size	Number Gene
1. MMP-13F 5'-CACTTTATGCTTACTGATGACG-3'	NM_002427.3	154 bp
2. MMP-13R 5'-TCCTCGGAGACTGGTAATGG-3'	NM_002427.3	154 bp
3. RELA F 5'-CGCATCCAGACCAACAACAA-3'	NM_001243984.1	154 bp
4. RELA R 5'-AGATGGGATGAGAAAGGACAGG-3'	NM_001243984.1	154 bp
5. HPRT1 5'-CCTGGCGTCGTGATTAGTGAT-3'	NM_000194.2	158 bp
6. HPRT1 5'-CCCATCTCCTTCATCACATCTC-3'.	NM_000194.2	158 bp

RNA extraction and cDNA synthesis

RNA was extracted from the isolates of synovial tissue grade IV from OA patients with TRIzol® (Invitrogen, USA) according to the manufacturer's protocol. The quantity of RNA was calculated by NanoDrop. Synthesis of cDNA was performed by using iScript cDNA Synthesis Kit (BioRad, USA) on thermal cycler C1000 (BioRad, USA) Reverse Transcriptase PCR (RT-PCR) devices. The reaction of cDNA synthesis was 5 µg total RNA, 1 x RT buffer, 20 pmol oligodT, 4 mM dNTP, 10 mM DTT, 40 U TMII RTase and H_2O-DEPC SuperScript enzymes with a total volume of 20 µl. The cDNA synthesis was performed at 52°C for 50 min according to the manual kit protocol (Biorad, USA).

PCR Gradient Amplification

DNA was amplified with SYBR Green amplification kits. The PCR program was 95°C pre-denaturation for 30 sec, followed by 5-sec denaturation, gradient annealing at 55°C for 5 sec for 50 cycles, additional melting curve 65-95°C with an increase of 0.5°C every 5 sec.

Measurement of gene concentration

The measurement of gene concentration in this study was the relative quantification method (Livak-Schmittgen, 2001) [5].

$$\Delta C_{T\ experiment} = C_{T\ experiment\ target} - experiment\ housekeeping$$

$$\Delta C_{T\ control} = C_{T\ control\ target} - control\ housekeeping$$

$$\Delta\Delta C_{T\ experiment} = \Delta C_{T\ experiment} - \Delta C_{Tcontrol}$$

The comparison of gene expression levels = $2^{\Delta\Delta CT}$. The measurement of gene concentration was by using LightCycler® software program referred to Livak formula (concentration in picogram size). HPRT1 gene was a housekeeping gene, and calibrator gene was from the control group.

Research Ethics

This study was already passed the ethics clearance and has been approved by the Ethics Committee of the Faculty of Medicine, Andalas University, Padang with registration number: 550/KEP/FK/2017 (Attached).

Statistical analysis

Normality test for MMP-13 and *RELA* gene expression was performed using Saphiro Wilk Test and homogeneity test with Levene test. The data is normally distributed if p-value > 0.05 and homogenous if p-value > 0.05. For *MMP-13* gene expression, normally distributed and homogeneous data were analysed with ANOVA Test and Tukey's HSD Post hoc Test. For *RELA* gene expression, the data were not normally distributed, and homogeneous were analysed with a non-parametric test (Kruskal Wallis test) then followed by Mann Whitney Test) [6].

Results

Sample Characteristics

The result of synoviocyte isolation from synovial tissue was fibroblast-shaped cells, cultured in a plate. The morphology of synoviocyte is presented in Figure 1.

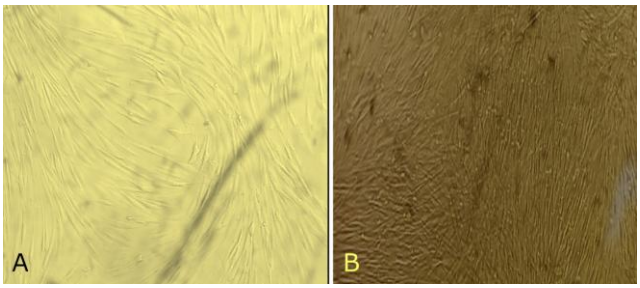

Figure 1: Morphology cells (A) Cell synoviocyte and (B) MSC-WJ

In each passage, cell morphology is like a fibroblast cell, a nucleus located in the middle of the cell and attaches to the base of the flask containing a complete medium.

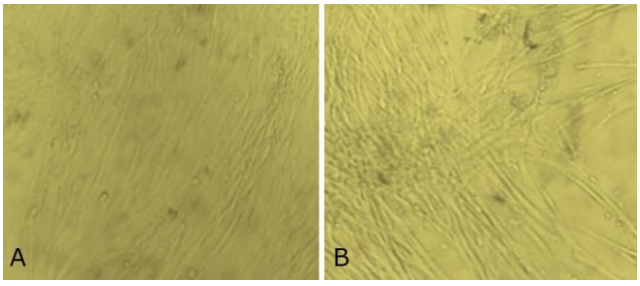

Figure 2: Morphology of synoviocyte co-culture MSC-WJ (A) Co-culture 24 hour and (B) Co-culture 48 hour

The optimisation of RT-PCR was presented in Figure 4. The *MMP-13* target gene is well-amplified (Figure 4A). The melting peak graph (Figure 4B) of *MMP-13* primer optimisation was sharp and homogeneous peaks.

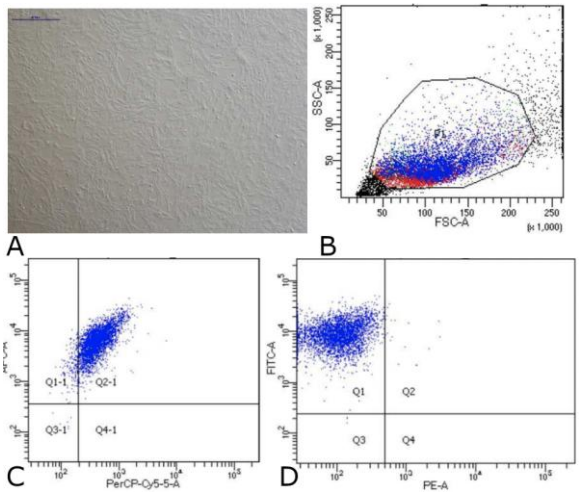

Figure 3: Data on Characteristics of Mesenchymal Stem Cells Wharton Jelly. (A) Cells MSC-WJ reach confluence. Scale bar: 500 µM. Photographs of cells taken using a Nikon Ti-S microscope. (B) Data flow cytometry. Forward scatter (FCS) plot & side scatter (SSC) plot. Population gated events (P1): 20,000. (C) Cell surface markers expression: CD73-APC 99.8% and CD105- PerCP-Cy5.5 95%. (D) Cell surface markers expression: CD90-FITC 99.9% and Lin (-) - PE 0.4%

Electrophoresis was performed to confirm the result of primer optimisation in Figure 5. The results of RT- PCR optimisation for *RELA* genes and *HPRT1* as the housekeeping gene, as shown in Figure 6 and confirmed with electrophoresis on agarose gel 0.5% in Figure 7.

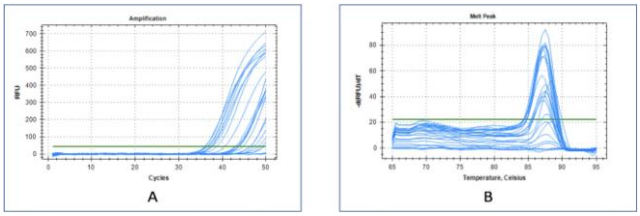

Figure 4: qPCR primer optimisation results of MMP-13 gene (A) Amplification curve results on qPCR and (B) Homogeneous melting peak graphs from the results of qPCR

MMP-13 gene expression

A preliminary analysis (normality and homogeneity tests) was performed before the ANOVA test. Normality test with Saphiro Wilk Test showed a significant result (≥ 0.05, data was normally distributed) in all treatment group.

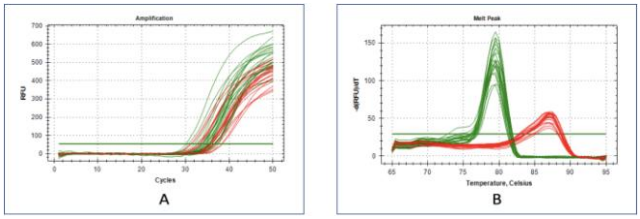

Figure 5: qPCR primer optimisation results of RELA gene (A) Amplification curve results on qPCR and (B) Homogeneous melting peak graphs from the results of qPCR

Descriptive analysis with Skewness ratio < 2 in all treatment groups. Homogeneity test with the

Levene test was 0.18 ≥ 0.05, showed that data of *MMP-13* have the same (homogeneous) variant.

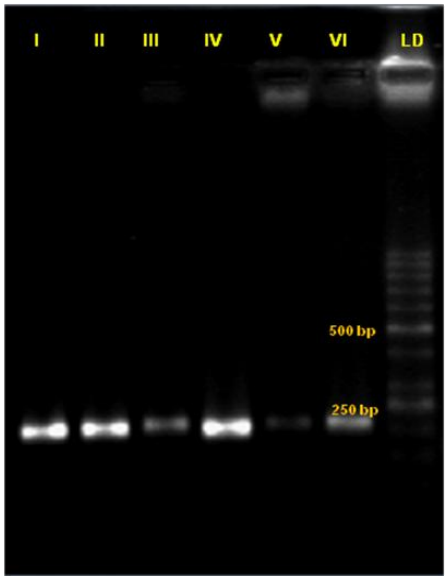

Figure 6: Results of the primer optimisation on electrophoresis of the MMP-113 gene

The analysis was continued to ANOVA test and then Post Hoc Tukey's HSD Test. Anova test for *MMP-13* gene expression was F = 5.963 with a significance value (0.002 ≤ 0.05). There are significant differences between treatment groups. Based on the results, analysis of Tukey's HSD Post Hoc Test was continued to find the differences between groups.

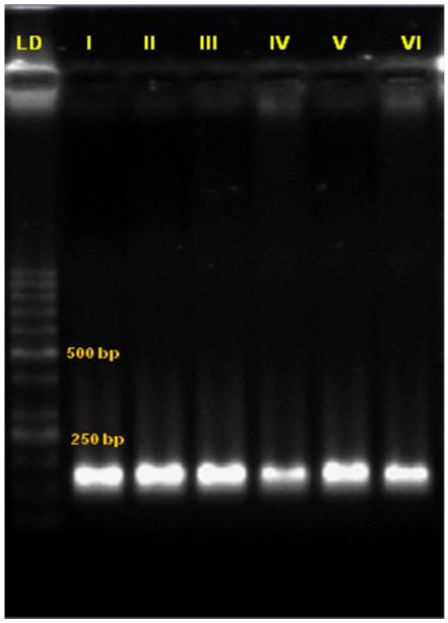

Figure 7: Results of the primer optimisation on electrophoresis of the RELA gene

Table 1 summarises the results of the ANOVA test and the differences between treatment groups summarised in Table 2.

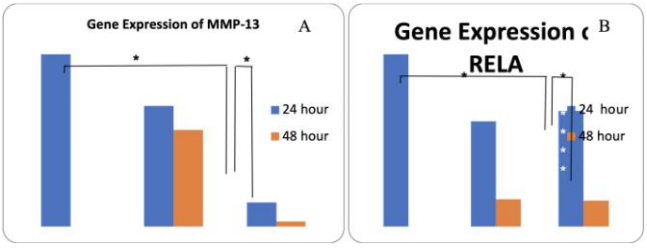

Figure 8: Gene Expression (A) MMP-13 and (B) RELA

The highest gene expression of *MMP-13* was in Group I and II (1.00 ng/µl), followed by Group III (0.41 ng/µl), Group IV (0.24 ng/µl), Group V (0.13 ng/µl), and Group VI (0.04 ng/µl).

Table 1: *MMP-13* gene expression level toward several treatment groups in OA

Groups	*MMP-13* gene expression (ng/µl)	
	Average	*p*-value
Group I	1.00 ± 0.00	0.002
Group II	1.00 ± 0.00	
Group III	0.41 ± 0.13	
Group IV	0.24 ± 0.03	
Group V	0.13 ± 0.04	
Group VI	0.04 ± 0.01	

Description: Group I = Synoviocyte control incubated for 24 hours; Group II = Synoviocyte control incubated for 48 hours; Group III = Mesenchymal Stem Cell Wharton Jelly (MSC-WJ) incubated for 24 hours; Group IV = Mesenchymal Stem Cell Wharton Jelly (MSC-WJ) incubated for 48 hours; Group V = Synoviocyte MSC-WJ co-culture incubated for 24 hours; Group VI = Synoviocyte MSC-WJ co-culture incubated for 48 hours.

The treatment group shows significant differences in *MMP-13* gene expression between groups I, III, IV, V and VI, whereas there was no significant difference between groups I and II ($p < 0.05$).

Table 2: Analysis of the effect of MSC-WJ administration toward *MMP-13* gene expression (ng/µl)

Groups	*MMP-13* gene expression (ng/µl)					
	I	II	III	IV	V	VI
I	-	1.00	0.00	0.00	0.00	0.00
II	1.00	-	0.00	0.00	0.00	0.00
III	0.00	0.00	-	0.11	0.00	0.00
IV	0.00	0.00	0.11	-	0.06	0.00
V	0.00	0.00	0.00	0.06	-	0.00
VI	0.00	0.00	0.00	0.06	0.00	-

*) Significantly different ($p < 0.05$).

RELA Gene Expression

A normality and homogeneity tests were performed before the ANOVA test. Normality test with Saphiro Wilk Test showed a significant result (≥ 0.05, data was normally distributed) in all treatment group. Descriptive analysis with Skewness ratio < 2 in all treatment groups. Homogeneity test with Levene test was 0.013 ≥ 0.05, showed that data of *MMP-13* were not homogeneous. The analysis was continued to non-parametric test (Kruskal Wallis test), Mann Whitney test was done if the different was significant.

The highest gene expression of *RELA* gene was in Group I and II (1.00 ng/µl), Group V (0.67 ng/µl), Group III (0.58 ng/µl), Group IV (0.16 ng/µl), and Group VI (0.16 ng/µl) (Table 3). Analysis of MSC-

WJ administration toward *RELA* gene expression among treatment groups was presented in Table 3.

Table 3: Analysis of the effect of MSC-WJ administration toward *RELA* gene expression (ng/µl) with Mann Whitney test

Groups	*RELA* gene expression (ng/µl)					
	I	II	III	IV	V	VI
I	-	1.00	0.01	0.01	0.01	0.01
II	1.00	-	0.01	0.01	0.01	0.01
III	0.01	0.01	-	0.02	0.25	0.02
IV	0.01	0.01	0.02	-	0.02	1.00
V	0.01	0.01	0.25	0.02	-	0.02
VI	0.01	0.01	0.02	1.00	0.02	-

*) Significantly different ($p < 0.05$)

There were significant different in *RELA* gene expression between groups I with groups III, IV, V and VI ($p < 0.05$), whereas there was no significant different ($p > 0.05$) between groups I and II (MSC-WJ administration no affected *RELA* gene expression in 24 and 48-hour synoviocyte group ($p > 0.05$). The expression of *RELA* gene between group II and group III, IV, V, VI was significantly different ($p < 0.05$) [6] *RELA* gene expression in group II was higher than group III, IV, V and VI as shown in Table 4.

Table 4: Analysis of the effect of MSC-WJ administration toward *MMP-13* gene expression (ng/µl)

Groups	*RELA* gene expression (ng/µl) (ng/µl)	
	Average	*p*-value
Group I	1.00 ± 0.00	0.001
Group II	1.00 ± 0.00	
Group III	0.58 ± 0.04	
Group IV	0.16 ± 0.04	
Group V	0.67 ± 0.10	
Group VI	0.16 ± 0.01	

Description: Group I = Synoviocyte control incubated for 24 hours; Group II = Synoviocyte control incubated for 48 hours; Group III = Mesenchymal Stem Cell Wharton Jelly (MSC-WJ) incubated for 24 hours; Group IV = Mesenchymal Stem Cell Wharton Jelly (MSC-WJ) incubated for 48 hours; Group V = Synoviocyte MSC-WJ co-culture incubated for 24 hours; Group VI = Synoviocyte MSC-WJ co-culture incubated for 48 hours.

The expression of *RELA* gene between group III and group V was not significantly different ($p > 0.005$), whereas between group III and groups I, II, IV and VI, the expression of *RELA* was significantly different ($p < 0.05$). The expression of *RELA* gene in group III was higher than groups IV and VI, but lower than groups I, II and V.

There was a significantly different ($p < 0.05$) between group IV with groups I, II, III and V. *RELA* gene expression between group IV and group VI was not significant ($p > 0.05$), expression level in group IV was lower than group I, II, III and V, but similar to group VI (Table 4). *RELA* gene expression between group V with groups I, II, IV and VI was significantly different ($p < 0.05$), but not for group III ($p > 0.05$). *RELA* gene expression level in group V was higher than groups III, IV and VI, but lower than groups I and II. RELA gene expression between group VI and groups I, II, III and V were statistically different ($p < 0.05$). The gene expression in group VI was lower than groups I, II, III and V, while group IV was not significantly different ($p > 0.05$). The expression in group VI was lower than groups I, II, III and V. The expression level of RELA genes in groups IV and VI was similar.

Discussion

In the present study, the lowest MMP-13 gene expression was shown in group VI (48 hours synoviocyte MSC-WJ co-culture group). The results of this study showed that MSC-WJ culture in synoviocyte OA after 48 hours reduced MMP-13 gene expression by 0.04 times compared to the control group, whereas in group V (24 hours MSC-WJ cell culture) synoviocyte culture) reduced gene expression MMP-13 in OA synoviocyte cells was 0.13 times compared to the control group ($p < 0.05$).

From the results of Tukey's Test, HSD in Table 1, there were not significantly different between 24 and 48 hours synoviocyte control groups. MMP-13 is released in synoviocyte of OA when inflammation occurs. According to Li, et al., 2011 it is said that when compared with other types of MMP, *MMP-13* is an important target gene during the development of OA because *MMP-13* gene expression is specifically found in cartilages and no *MMP-13* expression is found in normal patient cartilages [7].

In MSC-WJ culture group for 24 and 48 hours, the *MMP-13* gene was expressed (the result of electrophoresis). Almaki and Agrawal (2016) revealed that MMP plays an important role in the process of proliferation, migration, angiogenesis and differentiation of mesenchymal stem cells. *MMP-13* gene expression increases in the process of chondrogenic and osteogenic differentiation of Mesenchymal Stem Cell [8]. The previous study conducted by Mannello *et a.,l* (2006) investigated the role and function of MMP in the process of differentiation and characterisation of Mesenchymal Stem Cell, MMP-13 is also involved in the initial phase of the differentiation process from MSC [9]. In the differentiation process of MSC into chondrocytes, MMP-13 gene expression increases, but the specific mechanism is still unknown [10].

The results of the Tukey test for 24- and 48-hour synoviocyte-MSC-WJ co-culture groups showed significantly different compared to the control of synoviocyte. Weiss *et al.,* (2017) found that meniscus cells co-cultured with MSC significantly reduce *MMP-13* gene expression. The results of the study found that *MMP-13* gene expression in 48 hours synoviocyte MSC-WJ co-culture group was lower than 48 hours synoviocyte MSC-WJ co-culture group [11].

RELA gene expression

In the current study, the lowest gene expression of *RELA* was in group VI (a treatment group of synoviocyte and MSC-WJ co-culture for 48 hours). The result was by the initial hypothesis that MSC-WJ decreases the expression of *RELA* gene. *RELA* gene expression in 48-hour synoviocyte and MSC-WJ co-culture group decreased 0.16 times

relatively lower compared to the control group, whereas in group V which was the co-culture treatment group of synoviocyte and MSC-WJ cells during 24 hours decreased by 0.67 times relatively lower than the control group.

In the initial inflammatory process, *RELA* gene as a sub-family of NF-κβ is involved in the expression of several genes that play a role in the inflammatory response. The transcription of NF-κβ is stimulated by pro-inflammatory cytokines and chemokines. Activation of NF-κβ triggers the expression of genes to induce articular joint damage resulting in osteoarthritis. In line with Tortatore et al., (2012) reported that low value of ΔCq in synoviocyte OA control group increases *RELA* gene expression [12]. NFKβ activity will be high during the initial formation of new bones, including cartilage, but will decrease after the bones become mature [13]. High expression levels of *RELA* gene in 24-hour MSC-WJ co-culture group showed that NF-κβ plays a role in the differentiation and self-renewal processes of MSC-WJ [14]. Most of the pro-inflammatory effects of interferon γ and TNF-α are induced through NF-κβ translocation; the pathway is also modulated by MSC. Wen et al., (2014) stated that the expression of *NF-κβ p-65 gene* (*RELA*) increased significantly in the first 24 hours and 48 hours [15]. NF-κβ activity is high during the initial formation of new bone, including cartilage, but will decrease after the bone is mature [13].

The relative expression of *RELA* gene in 48-hour synoviocyte MSC-WJ co-culture group was significantly lower than the 24-hour synoviocyte MSC-WJ co-culture group. The result was due to the effect of MSC-WJ immunomodulatory which has begun to work on synoviocyte OA to control NF-κβ gene. Wen et al. (2014) reported that bone marrow-derived Mesenchymal Stem Cells modulate the effects of pro-inflammatory cytokines on human corneal epithelial cells. This study explained that the influence of MSC-WJ on synoviocyte of OA toward the parameters of MMP-13 and *RELA* genes expression as a subfamily of NF-κβ gene sub-unit p65. In general, MSC-WJ can reduce the expression of MMP-13 and *RELA* genes which are pro-inflammatory cytokines in osteoarthritis. The results of the study are useful as a reference for the use of stem cells, especially for MSC-WJ as a promising OA therapy in the future [15].

This study concluded that MSC-WJ in OA synoviocyte significantly reduced the expression of *MMP-13* and *RELA* gene ($p < 0.05$).

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Basic Science

Phenotypic Identification and Molecular Characterization of *Malassezia Spp.* Isolated from Pityriasis Versicolor Patients with Special Emphasis to Risk Factors in Diyala Province, Iraq

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Keywords: Pityriasis versicolor; *Malassezia spp.*; Molecular diagnosis; Risk factors; Iraq

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Abstract

AIM: The main objective is isolation and molecular characterisation of *Malassezia spp.* from pityriasis versicolor (PV) patients with special emphasis to risk factors in Diyala province, Iraq.

METHODS: Fifty patients (32 males and 18 females) presented with PV, the age ranged (15-45) years were included. Direct wet mount using KOH 10%, culture of skin scraping and PCR were used for confirmatory diagnosis.

RESULTS: *Malassezia spp*. was isolated from (54%) of skin scraping; *M. furfur* (32%); *M. pachydermatis* (8%) and *M. globosa* (14%). The age group (15-22) years were frequently exposed to Malassezia infection. A significant inverse correlation was reported between age and exposure to *Malassezia spp.* Infection. Males were frequently exposed to Malassezia infection, (40%). A significant correlation was reported between gender and exposure to Malassezia spp. Infection. Females were at risk of getting Malassezia infection (2.619) time than males. Patient resident in the urban area frequently exposed to Malassezia infection, (34%). Patients resident in the rural area appears to be at risk of getting Malassezia infection (1.093) time than those in an urban area. Patient with good economic status was frequently exposed to Malassezia infection, (36%). Patients with middle economic status appear to be at risk of getting Malassezia infection (0.42) time than those with good economic status. Patients with primary education were frequently exposed to Malassezia infection, (22%). A significant correlation was reported between education level and exposure to *Malassezia spp.* Infection. No significant correlation was reported between economic status; type of job; source of water; contact with dogs and birds and *Malassezia spp.* Infection.

CONCLUSION: *M. furfur, M. pachydermatis* and *M. globosa* represent the most common *Malassezia spp.* causing PV. Using of PCR is very critical to confirm the diagnosis of *Malassezia spp*. Malassezia infection inversely correlated with age and positively correlated with females gender and education. The residency in a rural area and middle economic status increase the possibility of infection. Infection was not affected by the source of water; job and contact with dogs and birds.

Introduction

The family *Malassezia* involves a gathering of superficial dimorphic fungi occurring as normal skin biota on the human body; however, they can also cause infection or are related to certain skin ailments. Rarely, they can become invasive, to cause opportunistic systemic infection in the presence of certain predisposing factors [1]. However, the complexity of the interaction of a unicellular eukaryotic creature (*Malassezia*) with a tissue of a multicellular living being (skin) makes understanding the communications and advancement of infection a complex process [2].

Malassezia yeasts inhabit various body sites including scalp, forehead, shoulder, abdomen, lower axilla, groin and forearm, due to an increased thickness of sebaceous organs at these locales [3]. It

has been reported that *Malassezia* yeasts are related to dermatoses such as pityriasis versicolor, seborrheic dermatitis and *Malassezia* folliculitis, recently, reported is the implication of *Malassezia* yeasts in atopic dermatitis and psoriasis [4]. Pityriasis versicolor (PV) is a superficial fungal infection, characterized by changes in skin pigment due to colonization of the stratum corneum by a dimorphic lipophilic fungus of the normal flora of the skin, and its characteristic by multiple macules and/or patches of variable appearance (hypopigmented, hyperpigmented, dark brown or erythematous) surrounded by normal skin [5]. Pityriasis versicolor is a worldwide in distribution, particularly in tropical areas. Infection by pityriasis versicolor has varied according to age and gender. It usually infects adults due to increased sebum secretion after puberty.

The main objective of this research is isolation and molecular characterisation of *Malassezia spp.* from pityriasis versicolor (PV) patients with special emphasis to risk factors in Diyala province, Iraq

Material and Methods

Study Area and Study Population

This study was performed in Baqubah city - Diyala province 33°45'34.71" N; 44°36'23.97" E, Northeast. The study included 50 pityriasis versicolor patients, age ranged (15-45) years; (32 males and 18 females) attended to the dermatology outpatient's clinic, Baqubah teaching hospital from 14 of October 2017 to 13 of January 2018.

Collection of Samples

Samples used were hair and skin scrapings. Forceps and surgical blades were used for collecting hair and skin samples. The scales were collected in sterile empty Petri dishes. [6].

Wet mount KOH

Scales specimens were subjected to direct examination by placing on a clean slide mounted with a drop of 10% KOH, covered with a coverslip. The slide was warmed gently and examined under a microscope (40X). Characteristic spaghetti and meatballs appearance (shortly curved hyphae and round yeasts) confirmed the diagnosis of PV [7].

Culture

Scales were inoculated onto Sabouraud's dextrose agar (SDA), containing 0.05 gm/L chloramphenicol, Penicillin at a concentration of 0.4 ml/L and Streptomycin at a concentration of 2 ml/L with olive oil and without olive oil. The vials were incubated at 37°C for 1-2 weeks. All the slants examined on days 3, 7 and then at weekly intervals up to three weeks for any developing colonies [8].

Staining

For examination, 5 ml of sterile distilled water was mixed with a loopful of actively growing yeast, and the concentration was adjusted to about 10^5 cell/ml Smears from the colonies were stained with Gram's stain and examined under a microscope for identification of Malassezia [9]. Lactophenol cotton blue stain used for microscopic observation of yeast cells, the suspension of yeast cells were prepared, loopful of culture were stained with lactophenol cotton blue on a sterile glass slide.

Catalase

Catalase test was applied by using a drop of 3% hydrogen peroxide, and the production of gas bubbles was considered a positive reaction [10].

Tween assimilation tests

According to the method reported by [11], yeast cells of (2 x 10 to 3 x 10 CFU/ml) was suspended in 1ml sterile distilled water and poured into plate containing SDA with 0.05 gm/L chloramphenicol, Penicillin at concentration of 0.4 ml/L and Streptomycin at concentration of 2 ml/L cooled at about 50ºC. The inoculum was then spread evenly. After solidification, four holes were made using a 2 mm diameter punch and filled with 5 µl of Tween 20, 40, 60 and 80, respectively. The plates were incubated for 1 week at 32ºC. The utilisation of Tween was assessed by the degree of growth and/or reaction (precipitation) of the lipophilic yeasts around the wells [11].

Assimilation of Castor oil

Assimilation of castor oil was examined using the agar diffusion test. Ten ml sterile Brain Heart Agar (BHI), was melted and allowed to cool to about 50ºC. Yeast cells of (2 x 10 to 3 x 10 CFU/ml) were suspended in 1ml sterilised distilled water and poured into a plate containing the medium was then spread evenly. Once the medium had solidified, one hole was made using a sterile 6mm punch and filled with 50µl of castor oil. The plates were incubated at 32ºC for 10 days and assessed for growth around the individual wells after 2, 4, 6, 8 and 10 days [6].

Splitting of Esculin

Glucosidase activity was assayed by using

the esculin agar tube. Using a loop, the yeast inoculum was deeply inoculated into the agar and incubated at 32ºC for 5 days. The splitting of esculin into esculetin and glucose is revealed by the darkening of the medium with the liberation of soluble ferric salt incorporated in the medium [10].

Tryptophan test (Pigment Induction medium)

Yeast cells were cultured on m Dixons medium which was prepared earlier addition of 0.6% of tryptophan instead of peptone to the original medium. After sterilisation and cooling at room temperature, the suspension was smeared on the agar medium using a sterile swab. The plates were incubated at 32ºC for 2 to 4 weeks [12].

Preservation of the Malassezia isolates

Malassezia spp. isolates were grown in the universal bottle containing 5ml of SDA used as slants, and they were enveloped with parafilm and stocked at 4ºC for 3 months [13].

DNA Extraction

DNA was extracted from *Malassezia* spp. By using the QIAamp DNA Mini Kit (Qiagen, Germany) according to the protocol stated by the kit manufacturer [14].

Concentration and purity of DNA

DNA was extracted from a hundred isolated of *Malassezia* spp*.* And they were concentrated in one tube. The concentration and the purity of the DNA samples were determined by Quantus Fluorometer at (9.9 ng/µl and 57 ng/µl) was used to detect the concentration of extracted DNA to detect the goodness of samples for downstream applications. For 1 µl of DNA, 199 µl of diluted QuantyFlour Dye was mixed. After 5min incubation at room temperature, DNA concentration values were detected, according to the protocol stated by the kit manufacturer (Promega, U.S.A).

Primers selection and preparation

Universal primers ITS3 (GCATCGATGAAGAACGCAGC) and ITS4 (TCC TCC GCT TATTGA TAT GC). The 5.8S rDNA and the it's 2 region, amplified from type, neotype, reference and clinical isolates of Malassezia species by using the ITS 3 and 4 primers (Gaitanis, G et al., 2002) [2]. We're synthesised by (QIAGEN, Germany)**.**

PCR working solution

Optimisation of PCR was accomplished after several trials. Thus the following mixture was adopted amplification reactions were produced in the 25µl final volume containing 12.5 µl Go Taq® master mix (Promega, USA), 2µl of the primers and 2µl DNA template and complete the volume by 8.2 ul nuclease-free water

Programmable thermal controller

Program for amplifying the 5.8S rDNA and the it's 2 region, amplified from the type of ITS3 and ITS4 for *Malassezia* spp. For identification of *Malassezia* spp.*,*an initial denaturation step at 95°C for five minutes was followed by thirty cycles of denaturation at 95°C for thirty seconds, annealing at 55°C for thirty seconds , and extension at 72°C for thirty seconds, with a final extension step of 72°C for seven minutes [15].

Agarose Gel Electrophoresis

After PCR amplification, agarose gel electrophoresis was adopted to confirm the presence of amplification. PCR was completely dependable on the extracted DNA criteria, according to the protocol stated by the kit manufacturer (Promega, U.S.A).

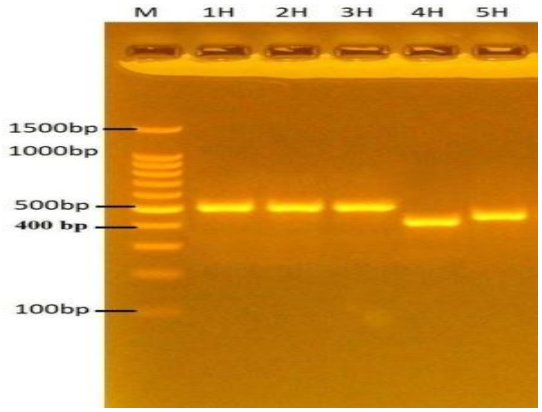

Figure 1: Agarose gel electrophoresis (2%) for 100 v/mAmp for 80 min of Malassezia spp. DNA products generated through ITS 3 (GCATCGATGAAGAACGCAGC), and ITS4 (TCCTCCGCTTATTGATATGC) primers, stained with Ethidium bromide; M: Molecular marker (100bp); lanes 1H, 2H, 3H: M. furfur (509bp); lanes H4: M. globosa (430bp); lanes H5: M. pachydermatis (483bp)

Statistical Data Analysis

Patients demography and cross tabulation were calculated by Statistical Package for the Social Sciences for Windows version 17 (SPSS, Armonk, NY: IBM Corp.). Pearson's chi-square and Pearson's correlation coefficient were used for the correlation between the variables of the two tests. A p value of ≤ 0.05 and ≤ 0.01 (two-tailed) was set to be statistically significant

Results

Table 1 shows the morphological characteristics and physiological characteristics of Malassezia spp. Isolated from human samples. According to these features, the total number of *Malassezia spp.* Isolated from a human was 27/50, (54%), *M. furfur* isolated from 16/50, (32%); *M. pachydermatis* 4/50, (8%) and *M. globosa were isolated from* 7/50, (14%). These results were confirmed by PCR as shown in Figure 1.

Table 1: Diagnosis of *Malassezia spp.* from human samples by morphological and physiological characteristics compared with conventional PCR using ITS3&ITS4 primers

Characteristics		*Malassezia Isolates*		
		M. furfur	*M. pachydermatis*	*M. globosa*
Morphological characteristics	Colony morphology	Mat, dull, smooth, umbonate or slightly folded with convex elevation	Mat, convex, umbonate (sometimes)	Raised, folded, rough
	Pigmentation	+	+	+
	Cell shape	Oval, cylindrical spherical	Oval	Spherical
Physiological characteristics	Growth on SDA			
	Growth on SDA-castor oil	+	+	+
	Growth on Esculin agar	+	+	+
	Growth on mDA 32°C	+	+	+
	Growth on mDA 37°C	+	+	+
	Growth on mDA 41°C	+	+	+
	Catalase reaction	+	+	+
	Utilization of Tween 20	+	+	+
	Utilization of Tween 40	+	+	+
	Utilization of Tween 60	+	+	+
	Utilization of Tween 80	+	+	+
Total number of *Malassezia isolates*		16 (32%)	4 (8%)	7 (14%)
Diagnosis by Conventional PCR using ITS3&ITS4 primers		16 (32%)	4 (8%)	7 (14%)

As shown in Table 2, the age group (15-22) years was more frequently exposed to Malassezia infection, followed by (23-30) year's age group. The age group (39-42) years was the minimally exposed to infection compared with other groups. *M. furfur* was the predominant species recovered from PV cases while *M. pachydermatis* was the least once. The isolation rate was drastically decreased while the age of patients approximates to forties. No significant difference was reported between age groups exposed to Malassezia *spp.* Infection. An inverse significant correlation was reported between age and exposure to Malassezia *spp.* Infection.

As shown in Table 3, males were more frequently exposed to Malassezia infection, 20/50, (40%). *M. furfur* was the predominant species recovered from males with PV cases, 11/50, (22%) while *M. globosa* recovered from 5/50, (10%) and *M. pachydermatis* was the least once, isolated from 4/50, (8%).

Table 2: Age as a possible risk factor associated with Malassezia infection in human

Descriptive statistic of patient's age					
Minimum (years)	15				
Maximum (years)	45				
Mean ± Std. Deviation	27.82 ± 8.39				
Age group (years)	Malassezia *spp.* infection				
	Positive No. (%)			Total No. (%) of positive cases	Negative No. (%)
	M. furfur	*M. pachydermatis*	*M. globosa*		
15-22	7 (10%)	1 (2%)	3 (6%)	11 (22%)	5 (10%)
23-30	4 (8%)	2 (4%)	3 (6%)	9 (18%)	7 (14%)
31-38	3 (6%)	1 (2%)	0 (0%)	4 (8%)	8 (16%)
39-42	1 (2%)	0 (0%)	0 (0%)	1 (2%)	2 (4%)
43-46	1 (2%)	0 (0%)	1 (2%)	2 (4%)	1 (2%)
Total No. (%)	16 (32%)	4 (8%)	7 (14%)	27 (54%)	23 (46%)
$\chi 2$	27.523				
P value	0.154				
R	-0.284				
P value	0.046				

No significant difference was reported between genders exposed to Malassezia *spp.* Infection. A significant correlation was reported between gender and exposure to Malassezia *spp.* Infection. Females appear to be at risk of getting Malassezia infection at (2.619) time than males.

Table 3: Gender as a possible risk factor associated with Malassezia infection human

Gender	Positive No.(%)			Total No. of positive cases	Negative No.(%)
	M. furfur	*M. pachydermatis*	*M. globosa*		
Male	11 (22%)	4 (8%)	5 (10%)	20 (40%)	12 (24%)
Female	5 (10%)	0 (0%)	2 (4%)	7 (14%)	11 (22%)
Total No. (%)	16 (32%)	4 (8%)	7 (14%)	27 (54%)	23 (46%)
χ 2	3.970				
P value	0.265				
R	0.270				
P value	0.05				
Risk Estimate	95% Confidence Interval				
Odds Ratio for gender (female / male)	Value	Lower	Upper		
	2.619	0.799	8.588		

As shown in Table 4, patient resident in urban area were more frequently exposed to malassezia infection, 17/50, (34%). *M. furfur* was the predominant species recovered from patients with PV cases, 11/50, (22%) while *M. globosa* recovered from 4/50, (8%) and *M. pachydermatis* was the least once, isolated from 2/50, (4%).

Neither significant difference nor correlation was reported between residency and exposure to malassezia *spp.* infection. Patients residency in rural area appear to be at risk of getting malassezia infection at (1.093) time than those in urban area.

Table 4: Residence as a possible risk factor associated with Malassezia infection in human

Residence	Positive NO.(%)				Negative No.(%)
	M. furfur	*M. pachydermatis*	*M. globosa*	Total No. of positive cases	
Rural	5 (10%)	2 (4%)	3 (6%)	10 (20%)	9 (18%)
Urban	11 (22%)	2 (4%)	4 (8%)	17 (34%)	14 (28%)
Total No.(%)	16 (32%)	4 (8%)	7 (14%)	27 (54%)	23 (46%)
χ 2	0.023				
P value	0.879				
R	-0.021				
P value	0.882				
Risk Estimate	95% Confidence Interval				
Odds Ratio for Residence (rural / urban)	Value	Lower	Upper		
	1.093	0.348	3.435		

As shown in Table 5, patient with good economic status were more frequently exposed to malassezia infection, 18/50, (36%). *M. furfur* was the

predominant species recovered from patients with PV cases, 6/50, (12%) while *M. globosa* recovered from 4/50, (8%). Neither significant difference nor correlation was reported between economic status and exposure to Malassezia *spp.* Infection. Patients with middle economic status appear to be at risk of getting Malassezia infection at (0.42) time than those with good economic status.

Table 5: Family economic status as a possible risk factor associated with Malassezia infection in human

Economic Status	Positive No.(%)				Negative NO.(%)
	M. furfur	*M. pachydermatis*	*M. globosa*	Total No. of positive cases	
Middle	6 (12%)	0 (0%)	3 (6%)	9 (18%)	4 (8%)
Good	10 (20%)	4 (8%)	4 (8%)	18 (36%)	18 (36%)
Very good	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)
Total No.(%)	16 (32%)	4 (8%)	7 (14%)	27 (54%)	23 (46%)
$\chi 2$	5.424				
P value	0.491				
R	-0.086				
Risk Estimate	95% Confidence Interval				
Odds Ratio for economic status (middle/good)	Value 0.421		Lower 0.110		Upper 1.612

As shown in Table 6, patients with Primary education were more frequently exposed to Malassezia infection, 11/50, (22%). *M. furfur* was the predominant species recovered from patients with PV cases, 8/50, (16%) while *M. globosa* recovered from 3/50, (6%). Illiterate patients were less frequently exposed to Malassezia infection, 2/50, (4%). *M. pachydermatis* was the predominant species recovered from patients with PV cases, 2/50, (4%).

No significant differences were reported between education status and Malassezia s*pp.* Significant correlation was reported between education level and exposure to Malassezia *spp.* Infection.

Table 6: Patients education status as possible risk factors associated with Malassezia infection in human

Education level	Malassezia spp				*Total No. (%) of negative cases*
	M. furfur	*M. pachydermatis*	*M. globosa*	*Total No. (%) of positive cases*	
Illiterate	0 (0%)	2 (4%)	0 (0%)	2 (4%)	4 (8%)
Primary	8 (16%)	0 (0%)	3 (6%)	11 (22%)	12 (24%)
Secondary	6 (12%)	1 (2%)	2 (4%)	9 (18%)	6 (12%)
Higher Education	2 (4%)	1 (2%)	2 (4%)	5 (10%)	1 (2%)
Total	16 (32%)	4 (8%)	7 (14%)	27 (54%)	23 (46%)
$\chi 2$	3.785				
P value	0.286				
R	0.280				
P value	0.05				

As shown in Table 7, Students were more frequently exposed to Malassezia infection, 10/50, (20%). *M. furfur* was the predominant species recovered from patients with PV cases, 8/50, (16%) while *M. globosa* and *M. pachydermatis* recovered from 1/50, (2%). Patients with the academic job were less frequently exposed to Malassezia infection, 2/50, (4%). *M. pachydermatis* and *M. globosa* were the predominant species recovered from patients with PV cases, 2/50, (4%). Neither significant difference nor correlations were reported between the type of job and *Malassezia spp.* Infection.

Table 7: Patient's job as possible risk factors associated with Malassezia infection in human

Job	Malassezia spp			*Total No. (%) of positive cases*	*Total No. (%) of negative cases*
	M. furfur	*M. pachydermatis*	*M. globosa*		
Free Work (Self-employer)	2 (4%)	0 (0%)	2 (4%)	4 (8%)	3 (6%)
Farmer	2 (4%)	2 (4%)	1 (2%)	5 (10%)	3 (6%)
Housewife	2 (4%)	0 (0%)	1 (2%)	3 (6%)	7 (14%)
Military	2 (4%)	0 (0%)	1 (2%)	3 (6%)	6 (12%)
Student	8 (16%)	1 (2%)	1 (2%)	10 (20%)	3 (6%)
Education	0 (0%)	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Total	16 (32%)	4 (8%)	7 (14%)	27 (54%)	23 (46%)
$\chi 2$	18.981				
P value	0.215				
R	0.063				
P value	0.663				

As shown in Table 8, patient utilizing filtrated water were more frequently exposed to Malassezia infection, 21/50, (42%). *M. furfur* was the predominant species recovered from patients with PV cases, 14/50, (28%) while *M. globosa* 4/50, (8%) *and M. pachydermatis* recovered from 3/50, (6%). patients utilizing river water were less frequently exposed to malassezia infection, mainly *M. furfur,* 1/50, (2%). Neither significant difference nor correlations were reported between source of water and *Malassezia spp.* infection.

Table 8: Source of water as possible risk factors associated with Malassezia infection in human

Source of water	Malassezia spp			*Total No. (%) of positive cases*	*Total No. (%) of negative cases*
	M. furfur	*M. pachydermatis*	*M. globosa*		
Tap Water	1 (2%)	1 (2%)	3 (6%)	5 (10%)	2 (4%)
Filtrated	14 (28%)	3 (6%)	4 (8%)	21 (42%)	21 (42%)
River Water	1 (2%)	0 (0%)	0 (0%)	1 (2%)	0 (0%)
Total	16 (32%)	4 (8%)	7 (14%)	27 (54%)	23 (46%)
$\chi 2$	8.592				
P value	0.198				
R	-0.219				
P value	0.126				

As shown in Table 9, patients who do not have any type of contact with animals were more frequently exposed to malassezia infection, 15/50, (30%). *M. furfur* was the predominant species recovered from patients with PV cases, 10/50, (20%) while *M. globosa* 4/50, (8%) and *M. pachydermatis* recovered from 1/50, (2%). patients who have contact with dogs only were exposed equally to *M. furfur* 1 /50, (2 %) and *M. pachydermatis* recovered from 1/50, (2%) infection. Neither significant difference nor correlations were reported between contact with animals and *Malassezia* spp. infection.

Table 9: contact with animals as a possible risk factors associated with Malassezia infection in human

contact with Animals	Malassezia spp			*Total No. (%) of positive cases*	*Total No. (%) of negative cases*
	M. furfur	*M. pachydermatis*	*M. globosa*		
No contact	10 (20%)	1 (2%)	4 (8%)	*15 (30%)*	13 (26%)
bird	3 (6%)	1 (2%)	1 (2%)	*5 (10%)*	6 (12%)
dogs	1 (2%)	1 (2%)	0 (0%)	*2 (4%)*	2 (4%)
Birds and dogs	2 (4%)	1 (2%)	2 (4%)	5 (10%)	2 (4%)
Total	16 (32%)	4 (8%)	7 (14%)	27 (54%)	23 (46%)
$\chi 2$	5.243				
P value	0.813				
R	0.185				
P value	0.199				

Discussion

Malassezia species are part of the resident skin flora of humans and other warm-blooded vertebrates that are discovered in 75-80 % of healthy subjects [16]**.** These yeasts are associated with a wide spectrum of clinical manifestations from benign skin conditions, such as pityriasis versicolor to fungemia in the immunocompromised host [17]. A molecular analysis-based nonculture method appears to be the most reliable and appropriate method by which to analyse *Malassezia* spp since the isolation media and procedures used does not influence such a method [18]. Therefore molecular methods have revolutionised the study of diseases caused by Malassezia species because the organisms are fastidious and difficult to identify. Therefore molecular methods overcome the limits of the conventional methods [19].

The current study showed that *M.furfur* was isolated from (32%) which was more common than *M. globosa* which isolated from (14%) and *M. pachydermatis* which was isolated from (8%).

This study comes in line with that reported by Al-ammari *et al.,* [20], in Baghdad, with different clinical presentations including steroids acne, folliculitis, seborrheic dermatitis and atopic dermatitis. In another study, in Diyala province-Iraq; Al-Ezzy *et al*., [21], found that Malassezia was isolated from (61.7%) of patients with PV specimens with similar age group distribution during 2017 in Baqubah teaching hospital. Current result comes by Shah in India [17], who recovered *Malassezia* spp from (50.35%) of PV patients. Current result disagrees with Shah and Chaudhary et al. from India [17], [22], reported the recovery of *M. globosa* from the majority of PV cases and stated that *M. globosa* is more pathogenic than other *Malassezia* species as it has greater enzymatic activity, involving lipase and esterase production.

Current isolation rate come in contrary with that reported by Chaudhary *et al.* and Archana *et al.* from India [22], [23], who recorded that the isolation rate of *Malassezia* species in Indian patients with PV was (70% and 96.66%) respectively. Also, current result comes in contrary with that reported by Shokohi from Iran [8], who recorded (88.4%) as isolation rate from PV cases.

The current study revealed that the age group (15-22) years was frequently exposed to Malassezia infection, followed by (23-30) years age group.The age group (39-42) years was the minimally exposed to infection compared with other groups. This result come in accordance with that reported in India and Iraq by [17], [23], [24], [25], [26], [27], [28]. *M. furfur* was the predominant species recovered from PV cases while *M. pachydermatis* was the least once. The isolation rate was drastically decreased while the age of patients approximates to forties which come in line with Al-ammari from Iraq and Akaza from Japan [20], [28] and disagree with Shah from India, [17] who stated that *M. globosa* was the predominant yeast recovered from PV cases. Also disagree with Archana from India, [23] stated that *M. furfur* represent the second cause of PV after *M. sympodialis*. An inverse significant correlation was reported between age and exposure to Malassezia *spp.* Infection, which agrees with Al-ammari from Iraq and Thayikkannu et al., from India [20], [25].

The current study revealed that males were more frequently exposed to malassezia infection, (40%) . *M. furfur* was the predominant species recovered from males with PV cases, (22%) which come in accordance with studies from Iraq and India [20], [21], [23], [24], [26], [27], [29]. While *M. globosa* recovered from, (10%) and *M. pachydermatis* was the least once, isolated from, (8%). Significant correlation was reported between gender and exposure to malassezia *spp* infection which come in accordance with Al-Ezzy from Iraq [21]. On the other hand, Hasan *et al.,* [24], stated that military personnel, athletes, and those doing hard works that usually associated with hyper sweating were more vulnerable to pityriasis versicolor infection. Furthermore, sebaceous glands secretion is increased in males 15 years and older compared to females, and that may also promote pityriasis versicolor infection in males.

Females appear to be at risk of getting malassezia infection at (2.619) time than males which come in accordance with Al-Ammari from Iraq [20], stated that many factors play role in Malassezia pathogenicity such as increased sebum production , hormonal fluctuations, stress, illness, infrequent shampooing, food allergies, vitamin B deficiency, hair curlers and blow dryers, cold weather (winter), use of hair sprays, gels and hair coloring chemicals which more probably resented in females and hence they become more susceptible to Malassezia infections than males.

Current study revealed that patient resident in urban area were more frequently exposed to malassezia infection, (34%) which come in line with Devendrappa and Javed from India [26]. *M. furfur* was the predominant species, (22%) while *M. globosa* recovered from, (8%) and *M. pachydermatis* was the least once, isolated from, (4%). This result disagree with Hasan et al., from Iraq, [24], who reported no significant correlation. On the other hand current study revealed that patients residency in rural area appear to be at risk of getting malassezia infection at (1.093) time than those in urban area. This result come in accordance with Sharma from India [30], stated that geographical variations have been observed in the densities of different Malassezia species on the skin. Malassezia infections more commonly reported in the warm and humid tropical and subtropical climates which is more suited for its growth. The higher incidence in urban people was mainly due to the

location of the hospital in the city. As the health facilities are also available in rural areas, the attendance of the patients from these areas to this hospital was reduced; probably this could be the reason for the low incidence from rural areas. Another factor could be that the rural population is not bothered about the cosmetic aspect of this disease.

The current study revealed that no correlation was reported between economic status and exposure to Malassezia spp. Infection. This may be attributed to factors other than economy play a vital role in infection such as psychological and immunological stress.

The current study revealed that patients with primary education as well as students were more frequently exposed to Malassezia infection. Illiterate patients and patients with an academic job were less frequently exposed to Malassezia infection, (4%). A significant correlation was reported between education level and exposure to Malassezia *spp* while Malassezia *spp.* Infection does not correlate with the type of job*.* This result may be attributed to the limited number of illiterate patients seeking medical consultations as well as investigations related to skin infections possibly due to they consider this is a type of luxury checkup. On the other hand, the limited number of patients with higher education reflects their knowledge about the complications of cutaneous infections as well as their attention to check any skin abnormalities such as hyperpigmentation or hypopigmentation or even scales. Type of patient's job may facilitate the flaring of infection indirectly by acting as a stress factor which acts with other genetic, hormonal, and environmental factors such as humidity and temperature to establishing a clinical presentation of PV.

The current study revealed those patients utilising filtrated water were more frequently exposed to Malassezia infection. *M. furfur* was the predominant species recovered from patients with PV. Patients utilizing river water were less frequently exposed to Malassezia infection, mainly *M. furfur,* (2%) although, no significant correlation was reported between the source of water and Malassezia *spp.* Infection. Current result comes in agreement with Babic *et al.,* from Slovenia [31], who stated that fungi including genus Malassezia can form fungal biofilms within 48 h in tap systems in private homes, hospitals or industrial network was confirmed for opportunistic and pathogenic species from the genera Malassezia. Once established, biofilms are difficult to be fully removed from the pipe system, which on the long-term leads to altered taste and odour of water, production of allergenic or irritating compounds, and mycotoxins with an effect on human health [31].

The current study revealed that patients who do not have any type of contact with animals were more frequently exposed to Malassezia infection and *M. furfur* was the predominant species which explain that the source of infection not restricted to birds or dogs. Patients who have contact with dogs only were equally exposed to *M. furfur,* and *M. pachydermatis* which reflect that dogs not only the source of infection but also other factors such as this may be attributed to several factors includes but not limited to, humidity and high temperature, hyperhidrosis, familial susceptibility, and immunosuppression [32]. A range of skin microenvironmental factors, such as the bacterial microbiota present, pH, salts, immune responses, cutaneous biochemistry, and physiology, may play a role in adherence and growth of Malassezia species, favouring distinct genotypes depending on the geographical area and/or the skin sites [33]. No significant correlation was reported between contact with animals and *Malassezia* spp — infection which comes in line with Al-Ezzy from Iraq [21].

In conclusion, *M. furfur, M. pachydermatis* and *M. globosa* represent the most common Malassezia spp. causing PV*.* Using of PCR is very critical to confirm the diagnosis of *Malassezia spp*. Malassezia infection inversely correlated with age and positively correlated with females gender and education. The residency in a rural area and middle economic status increase the possibility of infection. Infection was not affected by the source of water; job and contact with dogs and birds.

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Basic Science

Association between Glycated Hemoglobin with the Levels of Serum Proinflammatory Cytokines and Antioxidants in Patients with Type 2 Diabetes Mellitus in Universitas Sumatera Utara Hospital

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Abstract

BACKGROUND: Hyperglycemia condition in diabetes mellitus (DM) influences proinflammatory cytokine levels and disrupts antioxidant balances. Glycated Hemoglobin is used as a biomarker of glycemic control in DM.

AIM: This study aimed to analyse the association between glycated Hemoglobin with the levels of serum proinflammatory cytokines (interleukin (IL)-6) and antioxidants (glutathione peroxidase (GPx) and glutathione (GSH)) in type 2 diabetes mellitus (T2DM) patients in Universitas Sumatera Utara (USU) Hospital.

METHODS: A total of eighty-nine T2DM patients were recruited at USU Hospital. Glycated Hemoglobin levels were measured using routine laboratory tests at USU Hospital. The IL-6, GPx, and GSH levels were measured using enzyme-linked immunosorbent (ELISA) method. The statistical significance was determined using the Kruskal Wallis test, followed by Mann-Whitney test ($p < 0.05$).

RESULTS: The mean of glycated hemoglobin (%), IL-6 (pg/ml), GPx (ng/ml), and GSH (ng/ml) levels in T2DM patients were 8.96 ± 2.28, 59.27 ± 16.04, 32.13 ± 12.10, and 7.42 ± 3.50, respectively. Regarding the glycated Hemoglobin levels, 28.09% of patients had controlled diabetes, 24.72% of patients had poorly controlled diabetes, and 47.19% of patients had uncontrolled diabetes. The IL-6 levels of the three study groups based on glycated Hemoglobin levels were related significantly ($p < 0.05$), but there was no statistically significant difference observed between the GPx and GSH levels ($p > 0.05$).

CONCLUSION: The present study suggests that the glycated Hemoglobin was associated with the levels of serum IL-6 levels but not GPx and GSH levels in T2DM patients in USU Hospital.

Introduction

Diabetes mellitus (DM) is metabolic syndromes caused by the failure of the pancreas to produce sufficient amount of insulin, or the body cannot use insulin effectively which will increase blood glucose levels or hyperglycemia [1]. The prevalence of diabetic patients in the world in 2015 was 415 million cases, and the number of diabetic patients is estimated to increase to 642 million in 2040 [2]. According to the data of the International Diabetes Federation in 2015, Indonesia ranked as the seven highest number of diabetic patients in the world with an estimated of 10 million patients [3].

Based on the data of the Indonesian Ministry of Health in 2013, the number of diabetic patients in North Sumatra was around 205 thousand people. Hence, North Sumatra ranked 8th in terms of the number of diabetic patients in Indonesia [4].

Low levels of insulin in diabetic patients will

cause an increase in blood glucose levels (BGLs)/hyperglycemia. In hyperglycemia condition, oxidation to the glucose triggers the formation of reactive oxygen species (ROS). The accumulated ROS is the central point to an increase in oxidative stress [5], [6]. Continuous oxidative stress leads to inflammatory conditions in the cells which will ultimately increase the formation of proinflammatory cytokines. Proinflammatory cytokines, such as tumour necrosis factor (TNF)-α, interleukin (IL)-1, and interleukin (IL)-6, can induce insulin resistance by interfering insulin signalling. It will worsen the function of insulin [7]. Previous studies showed a significant relationship between increased risk of type 2 diabetes mellitus (T2DM) and increased levels of IL-6 [8], [9]. T2DM is the most common type of DM because nearly 90% of all DM cases are this type of DM [3]. IL-6 is a pleiotropic cytokine known to be involved in the amplification and protection against inflammation [10].

Also, oxidative stress in T2DM patients disrupts antioxidant balances in cells, such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), and glutathione (GSH) [6]. Past research reported that T2DM patients had lower levels of total antioxidants status (TAS), GPx, and GSH than the healthy group [11], [12], [13]. GPx is an antioxidant enzyme that has several functions such as detoxifying lipid peroxide and hydrogen peroxide (H2O2), eliminating ROS, preventing the formation of new free radicals, and converting free radicals that have been formed into less reactive molecules [12]. The activity of GPx requires GSH as a direct scavenger and co-substrate for the peroxidase enzyme. GSH is the main buffer in the reduction process of intracellular oxidation. The GPx and GSH antioxidants are believed to have useful benefits for the body, such as to prevent and repair damage caused by free radicals [14], [15].

Optimum glycemic control is required to control DM and prevent worsening DM disease so that the increased proinflammatory cytokines and antioxidant imbalances due to oxidative stress can be controlled. HbA1c (glycated Hemoglobin) is used as a biomarker of glycemic control in DM condition because it describes blood glucose levels in the last 60-90 days. Glycemic control in T2DM used glycated Hemoglobin measurement which is described as follows: < 7.5% of glycated Hemoglobin describes controlled diabetes, 7-9% of glycated Hemoglobin describes poor diabetes control, and > 9% of glycated Hemoglobin describes uncontrolled diabetes [1], [16], [17]. Previous studies found that glycated Hemoglobin levels were positively correlated with cytokine levels [18] but negatively correlated with antioxidant levels [19]. Therefore, the present study aimed to analyse the association between glycated Hemoglobin with serum proinflammatory cytokine and antioxidant levels in patients with T2DM in Universitas Sumatera Utara (USU) Hospital.

Methods

Research participants

A total of eighty-nine T2DM patients were recruited at USU Hospital from March to September 2018. The diagnosis was made according to the criteria of Indonesian Endocrinology Society by endocrine specialists, namely: (1) blood glucose level was more or equal to 200 mg/dl; (2) fasting blood glucose level was more or equal to 126 mg/dl; (3) blood glucose level was more or equal to 200 mg/dl at 2 hours after administered 75 gram of glucose in the glucose tolerance test; and (4) glycated hemoglobin was > 6.5% [16].

Before participating in the research, patients were asked to sign an informed consent form explaining the research purposes, procedures, risks, and benefits of the study. The procedures have been approved by the ethics committee of the Faculty of Medicine, University of Sumatera Utara (No.227/TGL/KEPK FK USU-RSUP HAM/2018) following the Second Declaration of Helsinki. The characteristics of patients including age, medication, duration of diabetes, smoking, pregnant, and other chronic illnesses were asked using questionnaires. Patients who had one of these characteristics were excluded from the study: pregnant, took vitamin supplements, infections (e.g. HIV, tuberculosis, hepatitis, malignant diseases), anemia, hemoglobinopathy, history of blood transfusion in the last 2-3 months, other conditions that might affect the age of erythrocytes, or impaired kidney function.

The patients were divided into three groups based on the glycated Hemoglobin levels: < 7% as controlled T2DM patients, 7-9% as poorly controlled T2DM patients, and > 9% as uncontrolled T2DM patients.

Preparation of samples

Blood samples (5 ml) were collected for serum separation using standard venipuncture after fasting for at least 8-12 hours. The blood samples were centrifuged at 3000 rpm for 10 minutes. Fasting blood glucose (FBG) levels were measured within 2 hours after being taken while glycated Hemoglobin was measured in the whole blood using the routine laboratory tests (Cobas 6000 analyser with hexokinase and immunoturbidimetric method, Roche Diagnostics, Switzerland) at USU Hospital. Furthermore, postprandial blood glucose (PBG) levels were measured 2 hours after the patients had meals.

Serum samples were stored at -80° C for interleukin-6 (IL-6), glutathione peroxidase (GPx), and glutathione (GSH) measurements. IL-6, GPx, and GSH were measured using enzyme-linked immunosorbent assay (ELISA) with commercial kits

from BioLegend, USA. The levels of proinflammatory cytokines and antioxidants were read with a microplate reader at a wavelength of 450 nm. All measurement process of proinflammatory cytokine and antioxidant levels were performed at the Integrated Laboratory of Faculty of Medicine, USU.

Statistical analysis

Statistical Package for the Social Sciences (SPSS) version 22 was used for the statistical analysis of the data. Kolmogorov-Smirnov test was used to analyse the data normality. The mean comparison of IL-6, GPx, and GSH levels in the study groups based on glycated Hemoglobin was performed using the Kruskal Wallis test, followed with Mann-Whitney test if the test results showed a significance level. The level of significance in the study was a p-value of < 0.05.

Results

The characteristics of T2DM patients are shown in Table 1.

Table 1: Clinical and biochemical characteristics of T2DM patients

Characteristics	N = 89
Age (years), mean ± SD	57.77 ± 10.43
Gender (male/ female), N (%)	52 (58.4)/37 (41.6)
Duration of ill (years), mean ± SD	8.60 ± 3.66
Smoker N (%)	39 (44)
FBG (mg/dl), mean ± SD	220.83 ± 31.13
PBG (mg/dl), mean ± SD	301.57 ± 52.90
Glycated Hemoglobin (%)	8.96 ± 2.28
Interleukin-6 (pg/ml), mean ± SD	59.27 ± 16.04
Glutathione peroxidase (ng/ml), mean ± SD	32.13 ± 12.10
Glutathione (ng/ml), mean ± SD	7.42 ± 3.50

The average age of T2DM patients was 57.77 ± 10.43 years old. Based on the gender of T2DM patients in the study, there were more male patients than female patients (58.4% vs 41.6%). The average duration of diabetes in the study was 8.60 ± 3.66 years. This study also found that 44% of T2DM patients were smokers. The average FBG (mg/dl), PBG (mg/dl), glycated hemoglobin (%), IL-6 (pg/ml), GPx (ng/ml), and GSH (ng/ml) levels in T2DM patients were 220.83 ± 31.13, 301.57 ± 52.90, 8.96 ± 2.28, 59.27 ± 16.04, 32.13 ± 12.10, and 7.42 ± 3.50, respectively.

The distribution of glycated Hemoglobin of T2DM patients can be seen in Table 2.

Table 2: Distribution of glycated Hemoglobin of T2DM patients

T2DM patients N = 89	N (%)	Glycated Hemoglobin (%) Mean, SD
Controlled	25 (28.09)	6.38 ± 0.11
Poorly controlled	22 (24.72)	8.25 ±0.09
Uncontrolled	42 (47.19)	10.90 ±0.21

In Table 2, it can be seen that regarding the glycated hemoglobin levels of T2DM patients, 28.09% of the patients were categorized as controlled (mean/SD = 6.38 ± 0.11), 24.72% of the patients were categorized as poorly controlled (mean/SD = 8.25 ± 0.09), and 47.19% of the patients were categorized as uncontrolled (mean/SD = 10.90 ± 0.21).

The comparison of age, duration of illness, FBG, PBG, GPx, GSH, IL-6 levels between T2DM patients following glycated Hemoglobin groups can be seen in Table 3.

Table 3: Comparison of biochemical levels between glycated Hemoglobin groups in T2DM patients (Kruskal-Wallis test)

Characteristics (Mean Rank)	T2DM patients			p*
	Controlled (N = 25)	Poorly controlled (N = 22)	Uncontrolled (N = 42)	
Age (years)	58.28 ± 1.07	50.57 ± 1.75	63.18 ± 0.96	0.206
FBG (mg/dl)	25.00	39.00	60.00	0.001*
PBG (mg/dl)	25.22	41.57	58.57	0.001*
Interleukin-6 (pg/ml)	20.76	54.50	54.55	0.001*
Glutathione peroxidase (ng/ml)	44.32	54.36	40.50	0.124
Glutathione (ng/ml)	44.54	54.09	40.51	0.135

p* < 0.05.

The FBG, PBG, and IL-6 levels in the uncontrolled T2DM patients were higher than the poorly controlled and controlled T2DM patients. The Kruskal-Wallis test also showed significant differences between the study groups (p < 0.05). Also, the GPx and GSH levels (ng/ml) in the controlled T2DM patients were lower than the poorly controlled T2DM patients but higher than the uncontrolled T2DM patients. Based on the Kruskal Wallis test, the association between the GPx and GSH levels with the three groups of T2DM patients based on glycated Hemoglobin levels was not significant (p > 0.05).

The comparison of biochemical levels between glycated Hemoglobin groups in T2DM patients can be seen in Table 4.

Table 4: Comparison of biochemical levels between glycated Hemoglobin groups in T2DM patients (Mann-Whitney test)

Characteristics (mean rank)	T2DM patients (N = 89)			P*		
	Controlled[a] (N = 25)	Poorly controlled[b] (N = 22)	Uncontrolled[c] (N = 42)	Ab	Ac	Bc
FBG (mg/dl)	25.00	39.00	60.00	0.022*	0.001*	0.001*
PBG (mg/dl)	25.22	41.57	58.57	0.013*	0.001*	0.007*
Interleukin-6 (pg/ml)	20.76	54.50	54.55	0.001*	0.001*	0.899

p* < 0.05.

In Table 4, it can be seen that the Mann-Whitney test showed significant differences in the FBG, PBG, and IL-6 levels between the controlled and poorly controlled T2DM patients (p < 0.05). Similar results were also found between the controlled and uncontrolled T2DM patients. The FBG and PBG levels of the two study groups (poorly controlled and uncontrolled T2DM) were related significantly (p < 0.05), whereas the IL-6 levels of the poorly controlled and uncontrolled T2DM patients showed the opposite result (p > 0.05).

Discussion

Diabetes mellitus (DM) is metabolic syndrome conditions with an increasing prevalence of patients every year in the world. Chronic DM can cause complications such as hypertension, cardiovascular disease, kidney problems, and death. The American Diabetes Association (ADA) recommends the examination of HbA1c (glycated Hemoglobin) as the long-term glycemic control in DM patients [1]. Glycated Hemoglobin describes the history of blood glucose control in the previous 60-90 days. The glycated Hemoglobin test is recognised as the gold standard in assessing the development of DM [20].

In this study, the average glycated Hemoglobin (%) of T2DM patients was 8.96 ± 2.28 whereas the average duration of DM in the study was 8.60 ± 3.66 years. A long duration of DM disease may cause a high average of glycated Hemoglobin in the study. This is because chronic hyperglycemia can increase glycated Hemoglobin levels by about 2-3 times [21].

In addition to describing a history of blood glucose control in DM, glycated Hemoglobin is also used as an indicator of prognosis in the development of DM complications [20].

The highest percentage was found in the uncontrolled T2DM patients with 47.19%, and the average glycated Hemoglobin (%) in this group was 10.90 ± 0.21. Similar results were also found in Jordanian diabetic patients [22]. Several factors might cause uncontrolled DM, such as education level, type of DM drugs consumed, duration of DM, and weight gain [22], [23]. Moreover, age, gender, and occupational factors may also affect glycated Hemoglobin levels in patients with DM [24]. Other factors that can also affect controlled diabetes regarding glycated Hemoglobin are adherence in diet, physical activity, and family history of DM [1].

Continuously increasing blood sugar levels can increase reactive oxygen compounds (ROS) through enzymatic processes, namely through oxidation, phosphorylation reactions, and ADPH-Oxidase reactions. ROS compounds formed in hyperglycemia will trigger complications in DM patients through several mechanisms. ROS tends to be oxidised which produces some free radical molecules, such as anion superoxide ($O2^-$), hydroxyl radicals (OH), and hydrogen peroxide (H_2O_2) [25], [26].

The increased free radicals that exceed the ability of cells in overcoming them will cause damage to various cell components. This condition leads to the damage of DNA, proteins, carbohydrates, lipids, and other macromolecules. It will also result in the fragmentation of proteins and lipids peroxidation and dysfunction of cell membranes and enzymes which will eventually cause cell death. The earliest known mechanism of cell or tissue damage due to attack by free radicals is lipid peroxidation. Malondialdehyde (MDA) is one of the results of lipid peroxidation formed by free radicals under hyperglycemia conditions [6], [27]. Previous studies have shown that there is a relationship between blood glucose and HbA1c levels with an increase in MDA [28], [29].

Another complication due to the increased ROS is the activation mechanism of Nuclear Factor-κB (NF-κB). NF-kB is transcription factors which stimulate the production of proinflammatory cytokines, such as TNF-α and IL-1. These proinflammatory cytokines have autocrine and paracrine effects which can trigger the production of other proinflammatory cytokines, such as IL-6. IL-6 is secreted by T cells and macrophages to stimulate the body's immune response. A systemic proinflammatory cascade process occurs. IL-6 is an intermediate cytokine which is known to have dual functions. First, in an inflammatory state, the production of IL-6 will increase as proinflammatory cytokines. The second function is that IL-6 can activate other macrophage and neutrophil cells to produce anti-inflammatory cytokines. Genes that encode IL-6 (IL6, MIM # 147620) are found on chromosomes (Chr) 7p21 in humans [30], [31], [32].

In the present study, IL-6 levels in the uncontrolled diabetic patients were found to be higher than those of the poorly controlled and controlled diabetic patients ($p < 0.05$). This study also found that there were significant differences in the IL-6 levels between the controlled and poorly controlled group, and between the controlled and uncontrolled group. In contrast, the IL-6 levels did not differ significantly between the poorly controlled and uncontrolled group.

A previous study has reported a significant difference in the IL-6 levels between a group of diabetic patients and a group of healthy people [8]. Furthermore, there was a significant difference found in the IL-6 levels of the controlled and uncontrolled DM patients [33].

If the formation of ROS in diabetic patients exceeds the ability of cells to overcome free radicals, it can cause oxidative stress in the cells. Continuous oxidative stress leads to a decrease in antioxidant capacity [27]. Antioxidants are compounds that can neutralise free radicals produced when ROS is oxidised. Antioxidants have a molecular structure that can give its electron compounds to free radical molecules and can break chain reactions from free radicals. The body can produce antioxidants in the form of enzymes, such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), and glutathione (GSH) [6].

GPx levels of the controlled T2DM patients in this study were lower than the poorly controlled T2DM patients but higher than the uncontrolled T2DM patients although the difference was not statistically significant. In line with the results of this study, a past

study found that there was no relationship between the levels of GPx and glycated Hemoglobin in T2DM patients. The past study also reported that the GPx levels (U/ml) of controlled DM were lower than those of the uncontrolled DM (3696.25 ± 1199.61 vs 3914.13 ± 1219.36) [34]. Similarly, another previous study showed no relationship between blood glucose levels and glycated Hemoglobin levels with GPx levels even though adjustments have been made to age and gender [35]. In contrast, a study conducted by Wong et al. ,(2018) found that there was a relationship between GPx levels in controlled DM with chronic kidney disease (CDK) and uncontrolled DM in CDK [36]. GPx is an enzymatic antioxidant which is capable of detoxifying hydrogen peroxide and lipid hydroperoxide, preventing the formation of new free radicals, or converting free radicals that have been formed into less reactive molecules. This process occurs using other antioxidants, such as glutathione (GSH) [5], [14].

GSH plays an important role in the maintenance of cell survival, DNA replication, protein synthesis, enzyme catalysis, transport of membrane transduction, receptor action, metabolism and maturation of cells, and regulation of immune cell function. Both GSH and GPx can catalyse the process of reducing fatty hydroperoxide to alcohol and hydrogen peroxide to water. When catalysing the process, disulfide bonds from GSH will bind to form oxidised glutathione (GSSG), and the glutathione reductase (GRx) enzyme can recycle GSSG into GSH again by oxidising NADPH. When cells are exposed to oxidation stress, there will be a cumulation of GSSG, and the GSH/GSSG ratio will decrease [6], [15].

The results showed that GSH levels were higher in the poorly controlled T2DM patients than in the controlled and uncontrolled T2DM patients, but it was not related significantly ($p > 0.05$).

The increase in GSH levels in the poorly controlled T2DM patients may be related to the increase in GPx in the poorly controlled T2DM patients. GSH levels were also seen to be in line with GPx levels in the controlled and uncontrolled T2DM patients. A previous study showed an increase in the GSH levels of T2DM patients compared to the healthy controlled people (6.77 ± 0.59 vs 9.78 ± 0.58 uM /mg of protein) [36] whereas another study showed different result [37]. This might occur because of different compensations of cells to increase the expression of GSH proteins to counteract oxidative stress/ROS [38].

Furthermore, the duration of DM disease can also determine the GSH levels of patients. In addition to the duration of the disease, GSH and GPx levels are also influenced by age. GSH and GPx levels will decrease in older patients as an ageing process. It causes older people to be more vulnerable to free radicals [12]. Several previous studies revealed a decrease in antioxidant status in plasma and serum samples compared to the controls based on age. The decrease in various antioxidants is related to the formation of marker compounds for oxidative stress; for example, an increase in lipid hydroperoxide. In T2DM patients aged 50-60 years, there was an increase in lipid peroxidation since the onset of diabetes [39]. This study found that the average age of the poorly controlled T2DM patients was 50.57 ± 1.75 years. This result was lower than the average age of the controlled and uncontrolled T2DM patients (58.28 ± 1.07, 63.18 ± 0.96, respectively). This difference might have a role in the higher antioxidant levels of GPx and GSH in the poorly controlled diabetic patients than the other two groups.

This research was the first study investigating the topic at USU Hospital with cross-sectional study design. Longitudinal design research needs to be done with a larger size of samples to assess all variables in the data using complete questionnaires. Data can also be obtained regarding the history of the drug used.

In conclusion, this study suggests that glycated Hemoglobin levels were associated with the levels of serum IL-6 but not GPx and GSH in T2DM patients in Universitas Sumatera Utara (USU) Hospital. Future studies should be conducted using a longitudinal design study and a larger size of samples to assess all variables measured in complete questionnaires.

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Basic Science

High Fetuin-A Level as a Protective Factor to Abdominal Aortic Calcification in Indonesian Regular Hemodialysis Patients

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Abstract

BACKGROUND: Although the prevalence of cardiovascular disease decreases in the general population, this pattern is not followed in hemodialysis patients. Hence cardiovascular events still occur in 50% of cases resulting in hemodialysis patients. One of the risk factors is vascular calcification. The pathogenesis is not yet fully understood, but recent years studies have shown that vascular calcification in chronic kidney disease (CKD) occurs as a result of the interaction of stimulatory and inhibitory factors. One of the inhibitory factors is Fetuin-A. Until now there has been no data on levels of Fetuin-A as a risk factor for abdominal aortic calcification in Indonesia.

AIM: To determine the effect of Fetuin-A levels on abdominal aortic calcification in regular hemodialysis patients.

METHODS: This study was a cross-sectional study on 76 regular hemodialysis patients at Rasyida Renal Hospital Medan. Fetuin-A level was examined by enzyme-linked immunosorbent assay (ELISA). Assessment of abdominal aortic calcification was done by lateral lumbar X-ray.

RESULTS: Most patients (68.4%) had abdominal aortic calcification, in both layers; intima and media (44.7%). Abdominal aortic calcification was associated with Fetuin-A level and age. Multivariate analysis showed that high Fetuin-A levels were significantly associated with abdominal aortic calcification.

CONCLUSION: High Fetuin-A level appeared to be a protective factor against abdominal aortic calcification in regular hemodialysis patients in Indonesia.

Introduction

The life expectancy of hemodialysis patients is still very low. According to the United State Renal Data System in 2010, the 5-year cumulative life expectancy for chronic hemodialysis patients in North America is around 25% compared to 2005 which was only 20% [1]. Muzasti in 2011 found that the 5 years of survival rate for regular hemodialysis patients in Medan, Indonesia, was only 37.8% [2]. A study by Sibarani et al., in Medan, Indonesia, showed that the 1-year survival rate of maintenance hemodialysis patients was 63.4% [3]. One of the main causes of this low survival in various countries is cardiovascular disease. Despite the declining prevalence in the general population, this pattern is not followed in hemodialysis patients. Hence cardiovascular events still occur in 50% of cases of mortality in hemodialysis patients [1].

One risk factor for high mortality from cardiovascular disease in hemodialysis patients is vascular calcification. The pathogenesis is very complex and not fully understood [4]. However, research in recent years has shown that vascular calcification in CKD occurs as a result of the interaction of stimulatory and inhibitory factors. One of the inhibitory factors is Fetuin-A. Research in dialysis

patients shows that the lower the level of Fetuin-A, the more extensive the vascular calcification that occurs [5]. The role of Fetuin-A in physiology is still under study. However, several reports have shown that Fetuin-A is a multifunctional protein that can work as a major vascular calcification inhibitor by inhibiting 50% calcium phosphate salts formation [6]. Until now in Indonesia, there are no data regarding the levels of Fetuin-A as a risk factor for vascular calcification in hemodialysis patients.

Therefore, this study was conducted to determine the association of Fetuin-A levels and abdominal aortic calcification in regular hemodialysis patients.

Methods

Study design

This study was a cross-sectional study on 76 regular HD patients conducted in April 2018 at Rasyida Renal Hospital Medan. Each medical record was reviewed to verify the diagnosis and to obtain all relevant demographic and clinical data. Inclusion criteria were outpatients with age ≥ 18 years, on hemodialysis for ≥ 36 months independently of aetiology, and willing to undergo a lateral examination with lumbar X-ray and were taken a blood sample for laboratory examination. Patients who refused to continue the research and with lack of medical records were excluded.

Assessments

Lateral lumbar X-rays interpretation was performed by a radiologist who did not know the patient's clinical condition. Grading of calcification was assessed using abdominal aortic calcification scores where the scores in the abdominal aortic segment both the anterior and posterior walls in front of the one to fourth lumbar vertebrae (L1-L4) were summed up. Patients were told to have no calcification if the score was 0, mild calcification if the score was 1-4, severe calcification if the score was above 4 [7]. Blood samples were taken 5-10 minutes before the hemodialysis procedure. Then the levels of Fetuin-A serum were examined by ELISA technique.

Statistical analysis

All data were analysed with statistical SPSS 22.0 software using univariate, bivariate and multivariate analysis. To assess numerical data distribution, the Kolmogorov-Smirnov test was used. Categorical and numerical variables were analysed using an independent T-test or Mann Whitney U test, and two categorical variables were analysed using the Chi-Square test or physic test. The multivariate analysis used logistic regression. A p-value < 0.05 is considered significant.

Results

From the 76 respondents, 47 patients (61.8%) were male, while the rest 29 (38.2%) were female. Patients aged between 25-78 years (mean 54.39 ± 11.32 years) with a mean BMI of 24.16 ± 4.49 kg/m^2 (range 16.73 to 42.67). Most patients underwent hemodialysis for 10 hours a week (71.1%) and have undergone hemodialysis for 73.24 ± 35.11 months.

The medical history showed that almost all patients had hypertension (75%). Only 14 patients (18.4%) had diabetes. A history of cardiovascular disease was known in only 15.8% of patients. Twenty-four patients (31.6%) reported previously or currently smoking.

On laboratory examination, the median (mean ± SD) levels of calcium, phosphate, calcium phosphate product and Fetuin-A were 9.80 (9.74 ± 0.73) mg/dL, 5.5 (5.47 ± 0.61) mg/dL, 52.91 (53.54 ± 8.59) mg^2/dL^2 and 235 (254.08 ± 112.50) pg/ml.

Examination of lateral lumbar images showed that vascular calcification was found in 52 patients (68.4%) with an average abdominal aortic calcification score of 6.67 ± 6.34. The highest incidence for calcification was found in both layers both intima and media (44.7%) followed by tunica media in only (15.8%), whereas the calcification of the tunica intima was found in 2 patients (2.6%). Based on the grading, it was found that the majority of respondents experienced severe vascular calcification (52.6%). Clinical characteristics of these study participants are presented in (Table 1).

Table 1: Demographic and clinical characteristics of the study participants

Variables	n (%), median (min-max), mean ± SD
Gender: Male vs female	47 (61.8%) vs 29 (38.2%)
Age (years):	57 (25-78), 54.39 ± 11.32
≤ 60 vs > 60	50 (65.7%) vs 26 (34.2%)
BMI (kg/m^2)	23.37 (16.73 – 42.67), 24.16 ± 4.49
< 23 vs ≥	34 (44.7%) vs 42 (55.3%)
Dialysis duration (months):	67 (36-231), 73.24 ± 35.11
≤ 60 vs > 60	35 (46.1%) vs 41 (53.9%)
Dialysis hours per week: 10 vs 12	54 (71.1%) vs 22 (28.9%)
Renal failure etiology:	
diabetic vs hipertension	14 (18.4%) vs 57 (75%)
History of cardiovascular disease: yes	12 (15.8%)
Tobacco-smokers: yes	24 (31.6%)
Abdominal aorta calcification scores	6 (0-19), 6.67 ± 6.34
The degree of vascular calcification:	
None vs mild vs severe	24 (31.6%) vs 12 (15.8%) vs 40 (52.6%)
Location of calcification:	
Intima vs media vs intima + media	2 (2.6%) vs 12 (15.8%) vs 34 (44.7%)
Calcium	9.80 (8 – 11), 9.74 ± 0.73
Phospate	5.5 (4.1 – 6.8), 5.47 ± 0.61
CaxP	52.91 (33.60 – 69.36), 53.54 ± 8.59
Fetuin-A	235 (78 – 756), 254.08 ± 112.50

To determine the relationship of various factors with abdominal aortic calcification, bivariate analysis was performed. Table 2 shows that, even though statistically not significant, male sex, higher BMI and longer dialysis vintage were associated with more vascular calcification. Patients who suffer from diabetes, hypertension, are smoking and have a history of the cardiovascular disease also, had more vascular calcifications than non-DM patients, normotensive patients, non-smokers and those with no history of cardiovascular disease, although the statistical differences were not significant ($p > 0.05$). The same appeared for higher calcium and phosphate levels; it was more likely to experience vascular calcification than lower calcium levels and phosphate levels.

Table 2: Association of demographic and clinical characteristics with vascular calcification

Variables	Calcification (+)	Calcification (-)	p
Gender; male	66%	34%	0.556
Age:	59.04 ± 9.68	52.25 ± 11.46	0.014*
BMI:	24.73 ± 4.99	22.94 ± 2.87	0.053
Diabetes: yes	71.4%	28.6%	0.789
Hypertension: yes	68.4%	31.6%	1.000
History of cardiovascular disease: yes	91.7%	8.3%	0.059
Tobacco-smokers: yes	75.0%	25.0%	0.402
Dialysis duration:	83.96 ± 42.47	68.29 ± 30.34	0.070
Calcium	9.85 ± 0.61	9.70 ± 0.78	0.366
Phospate	5.55 ± 0.61	5.44 ± 0.61	0.478
CaxP	54.72 ±7.82	53.0 ± 8.95	0.422
Fetuin-A	205.48 ± 41.81	359.38 ± 143.12	< 0.001*

The results of the statistical analysis in table 2 showed that the only significant variables for vascular calcifications were Fetuin-A levels ($p < 0.001$) and age ($p = 0.014$).

To determine the correlation and strength between Fetuin-A levels and abdominal aortic calcification scores, a correlation test was conducted. The Kruskal Wallis test in Figure 1 showed that there was a significant negative correlation between Fetuin-A levels and abdominal aortic calcification score ($p < 0.001$), meaning that the lower the level of Fetuin-A, the higher the abdominal aortic calcification score. The value of $r = -0.60$ indicates that the strength of the correlation between the two variables is moderate.

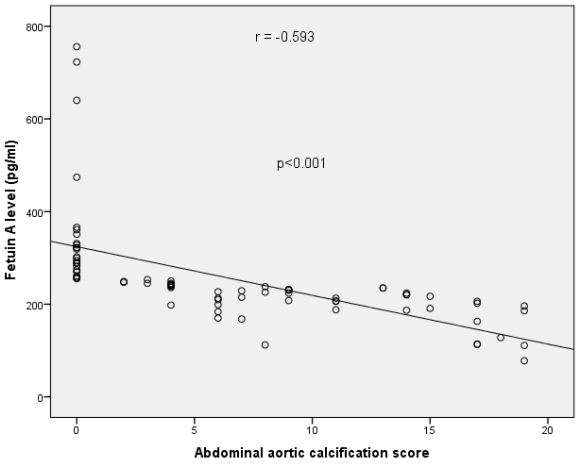

Figure 1: Serum Fetuin-A levels correlated negatively with abdominal aortic calcification score

Figure 2 shows that there is a significant negative correlation between the levels of Fetuin-A and the degree of calcification ($p < 0.001$), where the more severe the calcification, the lower the level of Fetuin-A.

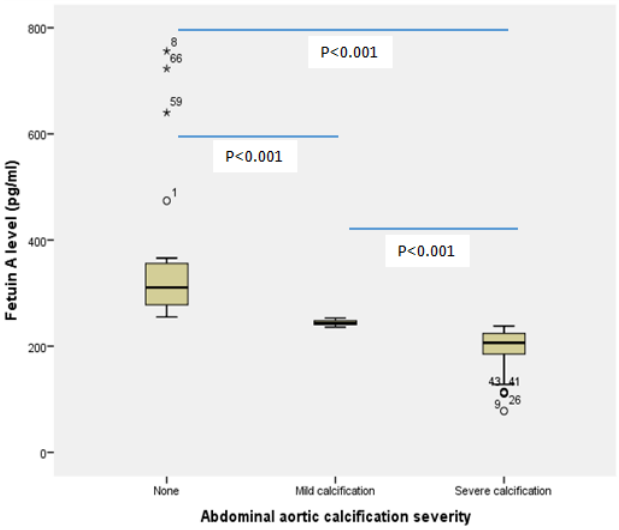

Figure 2: Serum Fetuin-A levels based on abdominal aortic calcification severity

To determine the most predominant variables associated with significant abdominal aortic calcification, a multivariate analysis was performed, including age, BMI, history of cardiovascular disease, dialysis vintage, and Fetuin-A levels

Statistical analysis showed that Fetuin-A levels were protective, and older age and higher BMI were risk factors for developing abdominal aortic calcification.

Table 3: Multivariate logistic regression analysis

Variable	OR	95% CI	P
Fetuin-A	0.98	0.96 – 0.99	0.001
History of cardiovascular disease: yes	6.13	0.97 – 38.92	0.054
Age	4.44	1.12 – 17.68	0.034
BMI	1.28	1.05 – 1.57	0.014

Discussion

Aetiology of CKD in Indonesia is different from other countries. In the United States, diabetes is the leading cause of CKD with a proportion reaching 37% followed by hypertension (24%), and the rest by other diseases. While in Indonesia, the main cause of CKD based on Indonesian Renal Registry (IRR) data is hypertension (44%), followed by diabetes (22%) and other diseases. The results of this study are consistent with the IRR data. The high rate of hypertension in this study is likely because patients

are late referrals, where secondary hypertension has occurred due to kidney failure, so it is difficult to recognise whether hypertension occurs as a cause or as a result of CKD [8], [9]. Limitation of this study is that there is no biopsy done in patients to determine the aetiology of CKD, only by history taking of hypertension and consumed anti-hypertension drugs.

In contrast to developed countries, all patients received dialysis services 3 times a week (4 hours/session), but in this study, most patients received dialysis services only twice a week (5 hours/session). This is because hemodialysis facilities in Indonesia are strongly influenced by the financing system, namely using national health insurance (JKN), which is generally done twice a week [8], [9].

This study proves that the prevalence of vascular calcification in chronic hemodialysis patients is quite high, i.e. 68% with a median (range) abdominal aortic calcification score of 5 (0-19). Publication in recent years shows that the prevalence of vascular calcification varies greatly from 60-100%, depending on the location of the examination and the diagnostic method used and in the area where the study was conducted [10]. London et al. reported 68% of 202 hemodialysis patients in France had arterial calcification determined by radiography and echocardiography [11]. An Australian study of 137 hemodialysis patients found that the prevalence of abdominal aortic vascular calcification was 90% [12] while international multicenter reports from America, Canada, Puerto Rico, Spain, and the United Kingdom showed that with the radiographic method, the prevalence of abdominal aortic calcification was 77.8% and with echocardiography, all patients (100%) had calcifications in the aortic and mitral valves [13].

Variation in this prevalence of vascular calcification is strongly influenced by the characteristics of the sample study. When the results of this study were compared with the results of previous studies, all of them found that older age was associated with vascular calcification [7], [14]. Reaven and Sack's study of 245 participants using EBCT found that patients over 61 years experienced severe aortic calcification [15]. This study also shows the same thing, that is the older the patient, the more severe the degree of vascular calcification. This is because the process of atherosclerosis has started since childhood, and continues to form atheroma with various lesions that form calcification. So, age is considered to be an important determinant of the presence of calcification [15].

Several studies have shown that abdominal aortic calcification is more common in patients undergoing long-term dialysis. For example, the Goldsmith et al., the study of 38 patients who underwent hemodialysis for 10-25 years, had radiographically increased the prevalence of vascular calcification from 39% at the start of dialysis to 92% after 16 years of dialysis with an average onset of 9.7 years after starting dialysis [16]. Kawaguchi et al. found that the average duration of dialysis in patients with grade 1 aortic abdominal calcification was 41 months, while patients with grade 3 calcification, had been on dialysis for 68 months [15].

This study shows that Fetuin-A levels are associated with vascular calcification, where the higher the level of Fetuin-A, the lower the degree of vascular calcification. These results are supported by previous studies which proved that low levels of Fetuin-A in hemodialysis patients were independently associated with the extent of vascular calcification [17].

Several limitations of our study should be emphasized. First, our study population is a single centre with a lower prevalence of diabetes, so it has not described the condition of all hemodialysis patients in Indonesia. Second, our study method is cross-sectional and observational, so that it cannot describe the causal relationship between various variables with vascular calcification and thirdly; fetuin-A levels were measured once at the inclusion.

Even so, for the best of our knowledge from the current body of evidence, this study was the first study in Indonesia to prove that high levels of Fetuin-A are a protective factor against vascular calcification. Further research is needed to extend the role of Fetuin-A in preventing the occurrence of vascular calcification.

In conclusion, high serum fetuin-A levels are shown to be protective factors and are associated with low severity of abdominal aortic calcifications in regular hemodialysis patients in Indonesia.

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Basic Science

Cell Cycle Inhibition of Ethylacetate Fraction of *Zanthoxylum Acanthopodium DC.* Fruit against T47D Cells

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Keywords: *Cytotoxic; Ethylacetate; Cell cycle inhibition; Zanthoxylum acanthopodium*

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Abstract

BACKGROUND: The use of medicinal plants is increasing in several decades for relief many diseases. Indonesia consists of thousands of islands with various plants and the manners of the community using plants as a treatment for every disease traditionally.

AIM: Cytotoxic activity of ethyl acetate fraction (EAF) of *Zanthoxylum acanthopodium* fruit was tested towards T47D breast cancer cells.

METHODS: The in vitro cytotoxic activity was performed by MTT assay, and the result was expressed as the IC50 (Inhibitory Concentration), and cell cycle inhibition was investigated by flow cytometry to assess the inhibition in every phase of cell cycle, and the role of expression cyclin D1 and p53 in cell cycle inhibition were performed by immunocytochemistry.

RESULTS: EAF was showed to have high activity with a value of IC_{50} 48.94 ± 0.32 µg/mL. EAF of 25 µg/mL caused cell accumulation at G0/G1 (60.48%) and in a control cell (51.69%) and decreased expression of cyclin D1 and increased expression of p53.

CONCLUSION: The results obtained in this study provided scientific support for further investigation on compounds in *Z. acanthopodium* fruit which in the future could be used for medication.

Introduction

The world health organisation (WHO) reported that breast cancer is one of the leading causes of death and the most common cancer type amongst women worldwide in 2012 [1]. Moreover, breast cancer ranks as the fifth cause of death from cancer overall (522,000 deaths), is the most frequent cause of cancer death in women in less developed countries (324,000 deaths, 14.3% of total), and the second cause of cancer death in developed countries (198,000 deaths, 15.4%) after lung cancer [2]. The diversity of medicinal plants in Indonesia is one of the chances in the potential development of Indonesia in the globalisation era [3], [4]. The use of medicinal plant extracts for the treatment of human disease is an ancient practice and thus has greatly increased in recent years.

Traditionally, andaliman fruits (*Zanthoxylum acanthopodium* DC.) has been used as aromaticum substances, tonicum, and treat dysentery. Indian people have used andaliman to treat paralyzed and skin diseases such as abscess and leprosy. Andaliman has been used as spices at North Sumatera especially at North Tapanuli [5], [6], [7]. The plants from *Zanthoxylum* genus contain many compounds such as phenol hydroquinones, flavonoids, steroids/ triterpenoids, tannins, glycosides, volatile oils, alkaloids, coumarines, lignans, amides

and terpenes [8], [9], [10], [11], [12], [13], [14], [15]. Ethyl acetate extract of andaliman fruits (EEA) was showed to have cytotoxicity effect against MCF-7 and T47D cell lines. EEA was found to have a synergistic effect when combined with doxorubicin. EEA was showed to have anticancer activity towards mice induced with benzo(a)pyrene, having a cardioprotective effect and active on T47D resistance cells [16], [17], [18].

This study was aimed to determine cytotoxic activity and cell cycle inhibition activity of ethyl acetate fraction of *Zanthoxylum acanthopodium* DC. fruits on T47D cells.

Material and Methods

Plant and Chemicals

Fresh fruits of *Zanthoxylum acanthopodium* DC. was collected from Onan Rungu village, Samosir regency, Sumatera Utara Province, Indonesia. *Zanthoxylum acanthopodium* DC. was identified in Research Centre for Biology, Indonesian Institute of Science, Bogor, and the voucher specimen was deposited in herbarium with a number of 332/IPH.1.01/If.07/II/2016, DMSO (Merck), [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide] (MTT) (Sigma), propidium iodide kit (Biolegend), chromogen 3,3-diaminobenzidin (DAB) (Novo Castra), monoclonal antibody cyclin D1 and p53 (Abcam).

Preparation of ethyl acetate fraction (EAF)

The air-dried and powdered fruits of *Zanthoxylum acanthopodium* DC. (1 kg) were repeatedly extracted by cold maceration with n-hexane (3 x 3 d, 7.5 L). The powder was dried in the air and extracted with ethyl acetate (3 x 3 d, 7.5 L) at room temperature on a shake. The filtrate was collected and then evaporated under reduced pressure to give a viscous extract and then freeze-dried to give a dried extract [4], [17], [18], [19].

Cytotoxicity assay

EAF was submitted for cytotoxicity test. In that way, T47D cell line was grown in RPMI 1640 medium containing 10% FetaL Bovine Serum (Gibco), 1% penicillin-streptomycin (Gibco), and fungizone 0.5% (Gibco) in a flask in a humidified atmosphere (5% CO_2) at 37°C. The inoculums seeded at 1 x 10^4 cells/mL at an optimal volume of 0.1 mL per well. After 24 h incubation, the medium was discharged and treated by EAF. After incubation for 24 h, the cells were incubated with 0.5 mg/mL MTT for 4 h at 37°C. Viable cells reacted with MTT to produce purple formazan crystals. After 4 h, SDS 10% as a stopper (Sigma) in 0.01N HCl (Merck) was added to dissolve the formazan crystals. The cells were incubated for 24 h in room temperature and protected from light. After incubation, the cells were shaken, and absorbance was measured using ELISA reader at λ 595 nm. The data which were absorbed from each well were converted to the percentage of viable cells [19] [20], [21]. The equation to determine the viability of cells:

$$Viability = \frac{Abs\ of\ treatment - Abs\ of\ medium}{Abs\ of\ control\ cells - Abs\ of\ medium}\ x\ 100\%$$

Cell cycle inhibition assay

T47D cells (1 x 10^6 cells/well) were seeded into 6-well plate and incubated for 24 h. After that, the cells were treated with EAF and then incubated for 24 h. Both floating and adherent cells were collected in a conical tube using trypsin 0.025%. The cells were washed thrice with cold PBS and centrifuged at 2500 rpm for 5 min. The supernatant was separated, while the sediment was collected and fixed in cold 70% ethanol in PBS at 4°C for 1 h. The cells were washed thrice with cold PBS and resuspended then centrifuged at 3000 rpm for 3 min, and PI kit (containing PI 40 µg/mL and RNAse 100 µg/mL) added to sediment and resuspended and incubated at 37°C for 30 min. The samples were analysed using FACScan flow cytometer. Based on DNA content, the percentage of cells in each of stage in the cell cycle (G1, S and G2/M) were calculated using ModFit Lt. 3.0.s [4], [17], [22].

Immunocytochemistry

T47D cells (1 x 10^5 cells/well) were seeded on coverslips in 24-well plate and incubated for 24 h. After that, the cells were treated with EAF and then incubated for 24 h. After incubation, the cells were washed with PBS and then fixed with cold methanol at 4°C for 10 min then the cells were washed with PBS and blocked in hydrogen peroxide blocking solution for 10 min at room temperature, incubated using primary antibody cyclin D1 and p53 for 1 h, then washed thrice with PBS, then incubated with secondary antibody for 10 min. The cells were washed with PBS, then incubated in 3,3-diaminobenzidine (DAB) solution for 10 min, and washed with distilled water. Afterwards, the cells were counterstained with Mayer-Haematoxylin for 5 min, and the coverslips were taken and washed with distilled water, and then immersed with xylol and ethanol 70% [4], [22], [23].

Statistical analysis

All data were analysed with regression analysis using SPSS 22.

Results

Cytotoxicity Effect of EAF

EAF was investigated for its cytotoxicity effect on T47D cell lines. The IC_{50} value of EAF was 48.94 ± 0.32 µg/mL. MTT method was used to determine cell viability after incubation for 24 h. In the treatment was showed the inhibition of cells growth.

Cell Cycle Arrest Activity

To investigate the effect of EAF on gained cell death by modulating cell cycle, we concentrated on it for further studies using flowcytometry method. The effect of EAF is given in Figure 1. Whereas, a single treatment with EAF on 25 µg/mL caused cell accumulation at G_0/G_1 (60.48%) and in control cells (51.69%). This fact was to indicate that EAF can inhibit cell grow at G_0/G_1 phase. In the cell cycle analysis, EAF was exhibited higher G_0-G_1 phase accumulation compared to control cells.

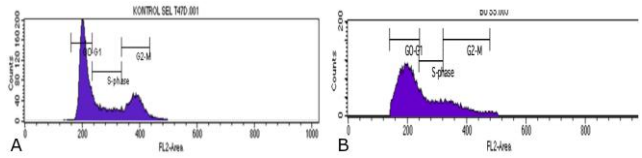

Figure 1: Cell cycle analysis using flow cytometry. T47D cells were treated by EAF for 24h and stained using propidium iodide; A) control cells; B) EAF 25 µg/mL

In the cell cycle analysis, EAF was exhibited higher G_0-G_1 phase accumulation compared to control. This analysis also showed cells underwent apoptosis, indicated by the occurrence of apoptosis during inhibition of cell cycle on the G_0-G_1 phase.

Cyclin D1 and p53 Expressions

Cyclin D1 and p53 (tumour suppressor gen) are proteins which play an important role in cell cycles process. In the study, the effect of EAF on cyclin D1 and p53 expressions were evaluated using immunocytochemistry.

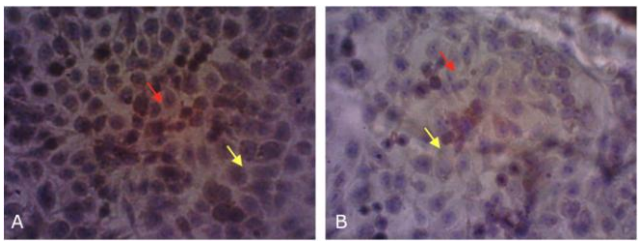

Figure 2: Expression of cyclin D1 on T47D cells using immunocytochemistry; A) control cells; B) EAF 25 µg/mL

Expression of cyclin D1 and p53 protein is positively characterised by brown stained nuclei in the cells (Figure 2 and Figure 3). In untreated cells (negative control) high intensity for cyclin D1 and low intensity for p53 was found. A single treatment of EAF was decreased on cyclin D1 and increased p53 expression.

Cells which express cyclin D1 Cells which not express cyclin D1. Inhibition of cyclin D1 protein expression strengthened the mechanism of modulating cell cycle especially in inhibition of cell cycle on the G_0-G_1 phase.

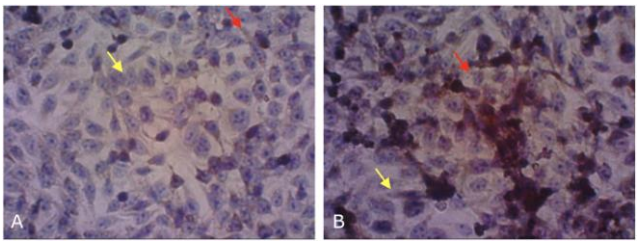

Figure 3: Expression of p53 on T47D cells using immunocytochemistry; A) control cells (Cells which express p53); B) EAF 25 µg/mL (Cells which not express p53)

Discussion

This research was aimed to investigate the efficacy of EAF in cell cycle inhibition. The cytotoxicity estimate of the natural product is related to the content of active compounds in these plants including *Zanthoxylum acanthopodium* DC. Flavonoids, alkaloids, saponins and tannins estimated as active compounds [17], [24].

This analysis also showed that cells underwent apoptosis, indicated by occurrence of apoptosis during inhibition of cell cycle on the G_0-G_1 phase [4]. Flavonoids and alkaloids could inhibit cell cycle progression [25], [26].

Cyclin D1 is a cyclin that role in G_0-G_1 phase with established complex with CDK-4 or CDK-6 to controlled G_1 to S phase transition [27]. Inhibition of cell cycle with combination EAE with doxorubicin reduced level of cyclin D1 which resulted in inhibition of pRb phosphorylation so that E2F can not apart from pRb and cells can not transcribe genes that needed in cell cycle process or cell proliferation [28], [29].

p53 plays an important function in the restriction of proliferation in abnormal cells. Functioning as a transcription factor p53 up-regulates expression of genes which contain a p53 binding element whose products contribute to cell cycle arrest [30], [31]. Activation of p53 can cause cell cycle arrest in different phases. In the majority of cases delay of G_1/S transition was previously observed [32]. The main key in p53 induced cell cycle arrest is played by $p21^{waf1}$ which binds to and inhibits a number of cyclin and Cdk (Cyclin-dependent kinase) complexes: cyclin

D1 – CDK4, cyclin E – CDK2, cyclin A – CDK2, and cyclin B – Cdc2 [30], [31], [32].

However, the molecular mechanism of cell cycle modulation by this EAF needs to be explored in more detail. Based on the results, it can be concluded that ethyl acetate fraction of *Zanthoxylum acanthopodium* DC. fruits have cytotoxicity activity through cell cycle arrest, decreases cyclin D1 and increase p53 protein expression. The extract is potential to be developed as a chemotherapeutic agent for breast cancer therapy.

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Superoxide Dismutase Levels and Polymorphism (*Ala16val*) In Tuberculosis Patients with Diabetes Mellitus in Medan City

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Abstract

BACKGROUND: Infectious diseases and metabolic disorders would result in oxidative stress in cells. Superoxide dismutase (SOD) is an antioxidant present inside cells that acts against oxidative stress. SOD gene polymorphism can affect the activity and levels of SOD.

AIM: This study aimed to analyse SOD levels and polymorphism of gene (ala16val) that regulated SOD in tuberculosis patients with diabetes mellitus in Medan city.

METHODS: A total of 40 tuberculosis patients with diabetes mellitus and 40 healthy subjects participated in the study. The levels of SOD were measured using enzyme-linked immunosorbent assay (ELISA). Analysis of SOD gene polymorphism (ala16val) was done using polymerase chain reaction-restriction fragment lengths polymorphisms (PCR-RFLP) with BsaW1 as the restriction enzyme. The statistical significance was determined using the Mann Whitney test, Fisher's exact test, and Kruskal Wallis test ($p < 0.05$).

RESULTS: The SOD levels of tuberculosis patients with diabetes mellitus were lower than those of the healthy subjects (102.474 ± 36.07 U/L vs 294.543 ± 58.75 U/L, $p < 0.05$). Patients of tuberculosis with diabetes mellitus tend to have more value/Val genotypes than the healthy group (57.5% vs 50%, $p > 0.05$). There was no association between SOD levels and SOD gene polymorphism (ala16val) in tuberculosis patients with diabetes mellitus.

CONCLUSION: In this study, there was an association between the levels of SOD and tuberculosis patients with diabetes mellitus, but not for the SOD gene polymorphism (ala16val). The SOD gene polymorphism (ala16val) was not the key role to influence the SOD levels in tuberculosis patients with diabetes mellitus in Medan city.

Introduction

Tuberculosis is an infectious disease which has been known to result in the highest mortality rate among bacterial infections in the world. Since 1992, the World Health Organization (WHO) has announced that tuberculosis infection is a global emergency. In Indonesia, tuberculosis infection is one of the major public health problems. Indonesia is one of five countries in the world which have the highest number of tuberculosis patients along with India, China, South Africa, and Nigeria [1].

To date, experts suggest that there is a disorder in the immune system of tuberculosis patients [2]. The cell's defence towards tuberculosis infection activates the immune system and causes oxidation-reduction (redox) imbalance. Antioxidants, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and others, can decrease this effect. However, previous studies showed that the activity or levels of SOD decreased in tuberculosis patients [3], [4].

In tuberculosis patients, the decrease of antioxidant levels can be worse in the presence of comorbidity conditions, such as metabolic disorder namely diabetes mellitus (DM). DM is an important risk factor for tuberculosis [5]. Eight of ten countries with the highest incidence of DM are also the countries with the highest number of tuberculosis

cases [6]. Several epidemiological studies have suggested the need to see the interaction between tuberculosis infections and DM [7].

DM is a chronic metabolic disorder that occurs when the pancreas is unable to produce insulin or when there is an occurrence of cell failure in using insulin effectively. This condition leads to metabolic disorders characterised by elevated blood glucose levels or hyperglycemia. Various complications of DM are related to increased oxidation of blood glucose. There is an abnormality in immunity and cell-mediated phagocyte function. The process will produce reactive oxygen species (ROS) which will increase oxidants and lipid peroxides [8]. A previous study showed an increase in lipid peroxides compounds, such as malondialdehyde in hyperglycemic conditions [9].

In DM, increased oxidants and lipid peroxides correlated with the decrease of antioxidant levels [10]. Previous studies exhibited a reduction of antioxidant levels in diabetic patients [11], [12]. SOD synthesis is regulated by specific SOD gene sequences. Single nucleotide polymorphism (SNP) or substitution of one nucleotide base in the SOD gene contributes to the expression of SOD. Differences in gene sequences determine the changes in gene expression and affect disease development [13]. SNP in the SOD gene was associated with the total antioxidant capacity in DM [14]. A study on diabetic patients by Flekac et al., (2008) found that SOD1 and SOD2 polymorphisms affect SOD activity [15].

Therefore, this study aimed to analyse the superoxide dismutase levels and SOD gene polymorphism (ala16val) in tuberculosis patients with diabetes mellitus in Medan city.

Methods

Subjects

This study was a cross-sectional design. Forty patients diagnosed with tuberculosis and diabetes mellitus were recruited at the health care facilities for pulmonary disease in Medan city. The diagnoses were made based on the criteria of Indonesian Lung Doctor Association [16] and the criteria of Indonesian Endocrinology Society [17]. The healthy subjects were recruited from a gym place in Medan city. The healthy subjects should not have a familial history of diabetes mellitus. Subjects who took vitamin supplements, who had infections such as HIV, hepatitis, or other malignant diseases, and who drank alcohol were excluded from this study.

Ethics

Each subject gave written informed consent after receiving a brief description of the purposes and benefits of the study. The procedure has been approved by the ethics committee of the Faculty of Medicine, Universitas Sumatera Utara (No. 327/2017) following the Second Declaration of Helsinki. The study was conducted from July 2017 to June 2018.

Sample collection

Blood sample (5 ml) was collected in plain and EDTA tube from the median cubital vein. The blood sample was centrifuged at 3000 rpm for 10 minutes. The blood glucose level was measured within 2 hours after being taken. The serum was stored at -80°C for further measurement of SOD levels.

DNA was extracted from the whole blood using the genomic DNA extraction kit (Promega, USA). The DNA template was then stored at a temperature of -80°C for the next stage of amputation. All process was carried out at the Integrated Laboratory of the Faculty of Medicine, Universitas Sumatera Utara.

Measurement of Blood Glucose Levels

The blood glucose levels (BGLs) were directly measured by the glucose oxidase-peroxidase (GOD-PAP) enzymatic colourimetric method and read with a spectrophotometer at a wavelength of 500 nm.

Measurement of Superoxide Dismutase (SOD) Levels

The SOD levels were measured using an enzyme-linked immunosorbent assay (ELISA) method with a commercial kit (Lab science, USA). The detection range was 3 to 900 U/L with a sensitivity of 1.52 U/L.

Amplification of SOD Gene

The DNA was amplified with the polymerase chain reaction (PCR) method. The amplification was performed using Forward primer 5'-CAG CCC AGC CTG CGG AGA CGG-3 'and Reverse primer 5'-CTT GGC CAA CGC CTC CTG GTA CTT-3'. Before performing the amplification process, the PCR solution (25 µl) was made by mixing master mix (12.5 µl), primers (1 µl each), DNA template (2 µl), and nuclease-free water (8.5 µl) (Promega, USA).

The amplification process started with initial denaturation of DNA at 95°C for 5 minutes, followed by (95°C for 45 s (melting), 54°C for 30 s (annealing), and 72°C for 30 s) for 30 cycles of amplification, and a final extension at 72°C for 5 minutes [18]. PCR products were analyzed on 2% agarose gel electrophoresis and staining with ethidium bromide.

Restriction Fragment Length Polymorphism (RFLP) of SOD gene polymorphism (ala16val)

The PCR reaction mixture (10 µl) was digested using restriction fragment length polymorphism (RFLP) method by the 0.2 µl of restriction endonuclease BsaW1 enzyme at 60°C in 10 minutes. The RFLP products were analysed on 4% agarose gel electrophoresis and staining with ethidium bromide.

Statistical Analysis

Statistical analysis was performed using SPSS version 22. Comparison of the mean of SOD levels in both groups was carried out using the Mann Whitney test. The Fisher's exact test was performed to assess the association of genotype and alleles in SOD gene polymorphism (ala16val) between both groups. In the group of patients, differences of the SOD levels on genotype variants were analysed using the Kruskal Wallis test whereas Hardy-Weinberg equilibrium (HWE) in both groups was analysed using the Chi-Square test.

Results

This study involved 40 tuberculosis patients with diabetes mellitus and 40 healthy subjects. The characteristics of the subject in both groups are shown in Table 1.

Table 1: Characteristics of studied groups

Characteristics	Patient (N = 40)	Healthy subject (N = 40)
Age (years), mean ± SD	53.26 ± 8.77	45.2 ± 10.90
Gender (male/female), N (%)	26 (65.0)/14 (35.0)	21 (52.5)/19 (47.5)
Duration of disease (years) mean ± SD	3.61 ± 1.38	-
Smokers N (%)	18 (45.0)	15 (37.5)
Blood glucose levels ad random (mg/dl) mean ± SD	295.51 ± 57.85	113 ± 19.8

The mean age of subjects in the patient group was 53.26 ± 8.77 years old, whereas the mean of subjects in the healthy group was 45.20 ± 10.90 years old. Based on gender, more male subjects were found than female subjects in the patient group (65% vs 35%). Similarly, there were more male subjects that female subjects in the healthy group (52.5% vs 47.5%). The mean duration of disease for 3.61 ± 1.38 years. 45% of the subjects in the patient group were smokers while 37.5% of the subjects in the healthy group were smokers. The mean BGLs in the patient group was 295.51 ± 57.85 mg/dl compared to 113 ± 19.8 mg/dl in the healthy group.

The mean levels of superoxide dismutase (SOD) both in patients and healthy control groups can be seen in Table 2.

Table 2: The mean levels of SOD in patient and control groups

	Patients group		Control group		p-value
	Mean	SD	Mean	SD	
SOD (U/L)	102.474	36.07	294.543	58.75	0.010

The SOD levels in the patient group were lower than the healthy group (102.474 ± 36.07 vs 294.543 ± 58.75), respectively. The Mann-Whitney test showed a significant difference between both groups ($p < 0.05$).

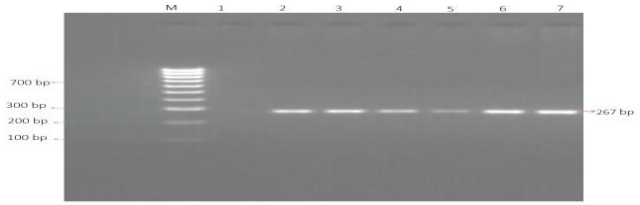

Figure 1: The PCR product of the SOD gene on 2% agarose gel electrophoresis. Lane M: DNA Ladder 100 bp; Lane 1: Negative Control; Lane 2, 3, 4, 5, 6, 7: PCR product of SOD gene at 267 bp

The PCR and the PCR-RFLP product of the SOD gene polymorphism (ala16val) can be seen in Figure 1 and Figure 2.

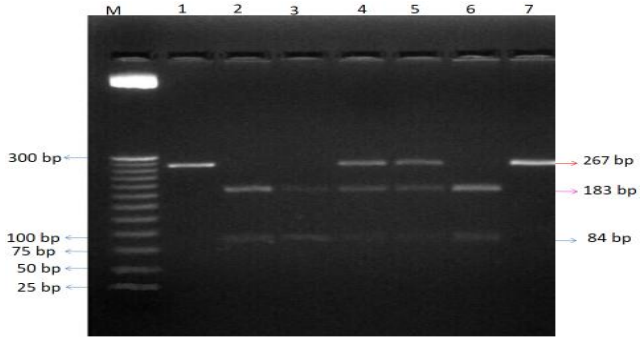

Figure 2: The PCR-RFLP product of the SOD gene Ala16val polymorphism on 4% agarose gel electrophoresis. Lane M: DNA Ladder 25 bp; Lane 1, 7: homozygous Ala/Ala without any cutting of PCR product; Lane 2, 3, 6: homozygous mutant Val/Val had two bands at 184 bp and 84 bp; Lane 4, 5: heterozygous Ala/Val had three bands at 267, 184 bp and 84 bp

The PCR product of the SOD gene was at 267 bp (Figure 1). After the digestion with BsaW1 restriction enzyme, the PCR product showed three different patterns. The ala/ala homozygous wild-type produced a band at 267 bp (without any cutting of PCR product). The ala/Val heterozygous showed three bands at 267 bp, 183 bp, and 84 bp. Whereas, the val/val homozygous mutant had two bands at 183 bp and 84 bp (Figure 2).

Frequencies of genotype and allele of SOD gene polymorphism (ala16val) in patients and healthy control groups can be seen in Table 3.

The ala/ala frequency in the patients and healthy subjects were similar (5%). The ala/val frequency in the healthy group was higher than the patient group, but the val/val frequency in the patient group was higher than the healthy group. However,

there was no statistical difference found in the genotypes and alleles between patients and healthy subjects ($p > 0.05$).

Table 3: Comparison in genotype and allele frequencies of SOD gene Ala16val polymorphism between the patients and healthy

Genotype	Patient (N = 40)	Healthy subject (N = 40)	P
Ala/ala	2 (5%)	2 (5%)	
Ala/val	15 (37.5%)	18 (45%)	0.863
Val/val	23 (57.5%)	20 (50%)	
Allele	Patient	Healthy subject	P
Ala	19 (23.75%)	22 (27.5%)	
Val	61 (76.25%)	58 (72.50%)	0.580

The association between levels of SOD and genotypes of SOD gene polymorphism (ala16val) in tuberculosis patients with diabetes mellitus shows in Table 4.

Table 4: Levels of SOD according to the genotype of SOD gene polymorphism (ala16val) in patients

	Genotype	Mean	SD	P
SOD levels (U/L)	Ala/ala (n = 2)	116.1092	6.68	0.051
	Ala/val (n = 15)	87.3318	12.47	
	Val/val (n = 23)	111.1639	24.20	

The SOD levels in the val/val genotype were lower than the ala/ala wild-type genotype. However, based on the Kruskal Wallis test, the association between genotypes of the SOD gene and SOD levels was not significant ($p > 0.05$).

Discussion

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis* (Mtb). The occurrence of oxidative stress in tuberculosis patients will cause an increase in ROS which is formed during the phagocytic actions on Mtb [18]. In metabolic disorders such as diabetes mellitus, the condition of hyperglycemia will trigger oxidative stress [19]. These conditions require antioxidant activities. Antioxidants are compounds which have complex and comprehensive functions against oxidative stress conditions [20].

The body can produce antioxidant compounds named endogenous antioxidants. SOD is one of the most important endogenous antioxidants and classified as the main antioxidant in homotetrameric enzyme group due to the ability in preventing cells from free radicals and oxidative stress [21]. SOD was first identified in 1938 by Mann and Keilin. SOD works as an antioxidant by preventing the initiation stage in the chain reactions of free radicals formation from ROS. SOD enzymes can degrade ROS superoxide anions into oxygen and hydrogen peroxide. However, in the condition of infection and metabolic disorders, the number of ROS significantly increases, resulting in more NADH and FADH2 electron donors enter the electron transport chain. The increase of ROS will increase the rate of lipid peroxidation which contributes to the production of free radicals. Oxidative modification, in turn, may inactivate SOD and decrease SOD levels [22], [23].

The present study found that serum superoxide dismutase (SOD) levels of tuberculosis patients with diabetes mellitus were significantly lower than the healthy subjects. Several previous studies have found that SOD levels were significantly lower in tuberculosis patients than the control group. The percentage of SOD inhibition in tuberculosis patients was lower than that of the healthy subjects [3], [4]. Other previous studies also showed that the SOD levels in the group of diabetic patients were lower than the group of healthy subjects [24], [25]. To the best of our knowledge, no study of SOD levels in tuberculosis patients with diabetes mellitus has been reported.

In the present study, the genotype frequency of SOD gene polymorphism (ala16val) in tuberculosis patients with diabetes mellitus and the healthy subjects was also compared. The result showed that val/val genotype as the mutant genotype was higher in the patient group than the healthy subject group although the difference was not statistically significant. This research was the first study which reported this finding. Several previous studies have only investigated the association between SOD gene polymorphisms (ala16val) and diabetes mellitus patients, but not SOD gene polymorphism (ala16val) in tuberculosis patients with diabetes mellitus. Past studies reported that val/val genotype was the most common in diabetic patients, and there was an association between SNP in SOD gene with diabetes mellitus [15], [26], [27].

SOD gene is located at the twenty-five point of the long arm of chromosome six which has five exons and four introns. The expression of SOD gene sequence influences SOD activity. Single nucleotide polymorphism (SNP) (i.e. the substitution of one of the nucleotide base in the SOD gene) contributes to the different expression of SOD. SOD gene polymorphism (ala16val) is a commonly known polymorphism. In this type of polymorphism, a single-nucleotide is substituted from C to T resulting in a change of the amino acid from ala/ala (GCT) to valine/val (GTT) at the 16th residue (ala16val) of the gene sequence. As a result, the SOD gene polymorphism (ala16val) has three genotypes, namely ala/ala genotype as wildtype genotype, and ala/val genotype and val/val genotype as mutant genotypes [13].

It has been reported that val/val genotype is associated with lower SOD activity than the ala/ala genotype. The substitution from ala to val reduces SOD activity and synthesis by 30 – 40% and decreases the transport efficiency of SOD into the mitochondria. This change is considered as an important pathophysiological mechanism that makes

people with val/val genotype have a risk factor for certain type of diseases. Furthermore, the val/val genotype of the SOD gene has been reported to be associated with a lower SOD efficiency against oxidative stress [28].

However, the present study did not find a significant association between the levels of SOD and genotypes of SOD gene polymorphism (ala16val) in tuberculosis patients with diabetes mellitus. Environmental factors such as nutrition intake and lifestyle were not assessed in this study. The activity and level of SOD can be influenced by environmental factors [29]. The SOD levels in this population might be more influenced by environmental factors, so there was no relationship between the level of SOD and the genotypes of SOD gene polymorphism (ala16val).

The other possibility of this finding might be due to the relatively small number of samples.

The distribution of SOD gene polymorphism (ala16val) in both groups was compared according to the Hardy – Weinberg Equilibrium (HWE) using the chi-square goodness-of-fit test. The results showed that SOD gene polymorphisms (ala16val) fulfilled the HWE. As the large population in Medan city is dominated by migrants, people commonly practice cross-ethnicity marriage. This condition results in a consistency of Hardy-Weinberg law in the population [30].

The present study also found that men were more likely to suffer from DM than women. This finding is consistent with a study in North India and North-west Nigeria [31]. Although gender is not a risk factor for infectious diseases including tuberculosis infection, it may be a risk factor for DM [32], [33].

In the present study, there was an association between SOD levels and tuberculosis patients with diabetes mellitus, but not for SOD gene polymorphism (ala16val). Moreover, SOD levels were not associated with val/val genotype in SOD gene polymorphism (ala16val) in the group of tuberculosis patients with diabetes mellitus in Medan city. These findings suggest that SOD gene polymorphism (ala16val) is not an important factor in influencing SOD levels in tuberculosis patients with diabetes mellitus in Medan city. Further study should be conducted using a large sample size, and the environmental factors of the research subjects should be analyzed.

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Basic Science

Differences in Brain-Derived Neurotrophic Factor and Matrix Metalloproteinase-9 between Appropriate Neonates between Normal Birth Weight and Intrauterine Growth Restriction

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Abstract

BACKGROUND: Intrauterine Growth Restriction (IUGR) was defined as the growth of the fetus less than its normal potential growth due to genetic and environmental factors. One of the most widely believed causes of IUGR was impaired uteroplacental mechanism from mother to fetus. Furthermore, factor which was thought to affect placental growth was due to the influence of Brain-Derived Neurotrophic Factor (BDNF) and Matrix Metalloproteinase (MMP-9) which play an important role in angiogenesis.

AIM: This study aims to determine differences in Brain-Derived Neurotrophic Factor (BDNF) and moderately mature Matrix Metalloproteinase (MMP-9) between normal birth weight and intrauterine growth restriction.

MATERIAL AND METHODS: The study design was a cross-sectional study at four hospitals in Padang city from August 2017-January 2018. The sample of this study was umbilical cord blood of appropriate gestational age neonate with normal birth weight (31 neonates) and IUGR (31 neonates) by consecutive sampling, samples taken from mothers who meet inclusion criteria. BDNF and MMP-9 levels were analysed by ELISA. The differences between normal birth weight and IUGR test were followed by unpaired T-test.

RESULTS: The results showed that BDNF levels in normal neonates was 1.58 ± 0.23 ng/ml and in IUGR neonates were 1.25 ± 0.35 ng/ml (p = 0.001). MMP-9 levels in normal neonates was 1.09 ± 0.20 ng/ml and in IUGR neonates were 1.25 ± 0.35 (p = 0.03).

CONCLUSION: The conclusion of this study was BDNF of moderately mature neonates was significantly higher in normal birth weight compared to intrauterine growth restriction, and the moderately high MMP-9 neonates were significantly higher in intrauterine growth restriction compared with normal birth weight.

Introduction

IUGR is defined as fetal growth that is less than normal potential growth due to genetic and environmental factors. IUGR is included in the category of low birth weight babies (LBW) [1]. IUGR is assessed by looking at the baby's growth chart. IUGR was diagnosed when the baby was born with a low birth weight (below the 10th percentile) with clinical signs of malnutrition [2]. If intrauterine growth disorders occur early in the pregnancy, it will have an impact on the growth of the brain and skeletons which are disrupted by the result associated with poor nerve development [3].

IUGR affects around 24% of newborns where around 30 million babies worldwide suffer from IUGR each year. One third (75%) occurs in Asia; the rest occurs in Africa (20%) and Latin America (5%). Indonesia ranks fourth for IUGR cases from all countries in Asia after Sri Lanka, Cambodia and Vietnam [2]. The cause of IUGR tends to be due to a disruption of the uteroplacental mechanism from mother to fetus. The placenta is an organ that facilitates the exchange of gas and nutrients between mother and fetus. If there are abnormalities in the placenta, this exchange will be disrupted; the fetus will not get enough nutrients needed to grow which will eventually lead to IUGR [4].

One of the factors thought to influence the

process of placental growth is due to the influence of Brain-Derived Neurotrophic Factor (BDNF) and Matrix Metalloproteinase (MMP-9). In a study showed that there were differences in BDNF levels in the placenta in pregnant women with preeclampsia where a higher BDNF level was found in patients with normotensive [5]. in another study found that the absence of MMP-9 in mice can cause severe abnormalities and lack of MMP-9 which causes disruption of trophoblast differentiation and the occurrence of defects in maternal blood vessels [6].

BDNF is one of the proteins needed for the growth of neurons. During the development period, BDNF plays a role in nerve growth, differentiation, repair, and survival of nerve cells [7]. Also, BDNF also shows an important role during the implantation period, placental development and fetal growth development in mice [8]. BDNF is known to have an important role in regulating angiogenesis needed for placental development [9]. Because of this role, BDNF deficiency will disrupt placental growth which in turn will cause fetal growth disorders or intrauterine growth restriction (IUGR) [8].

There are several factors that affect BDNF levels, including age, sex, weight, iron deficiency anaemia and depression. BDNF is inversely proportional to age and weight. Getting older and getting heavier, the BDNF decreases. Research showed that respondents aged 20-33 years have BDNF higher than respondents aged > 34 years. Women also tend to have low BDNF compared to men. Depressed pregnant women also have low BDNF concentrations [10], [11]. The umbilical cord BDNF levels are also influenced by maternal ferritin, where levels tend to be lower in women with iron-deficiency anaemia (< 12 ng/ml) than mothers with normal ferritin levels (≥ 12 ng/mL) [12].

Besides BDNF, another factor that affects placental growth is Matrix Metalloproteinase-9 (MMP-9). MMP-9 is believed to facilitate trophoblast invasion with its role as the destroyer of the extracellular matrix in the process of placentation. MMP is known as a mediator in tissue remodelling and angiogenesis. If this process is interrupted, the trophoblast will not be embedded properly in the uterus. As a result, the distribution of nutrients to the fetus will also be disrupted which will eventually lead to IUGR.

Based on the description above, the researcher wanted to conduct a study on the differences in Brain-Derived Neurotrophic Factor (BDNF) and the moderately mature Matrix Metalloproteinase (MMP-9) between normal birth weight and intrauterine growth restriction. This research is important to do with the hope that BDNF and MMP-9 placenta can be used as predictors to assess the occurrence of IUGR in pregnancy. The study was conducted in several hospitals in the city of Padang.

Material and Methods

This study was an observational study with cross-sectional [13]. This research was conducted from August 2017 to January 2018 at four hospitals in the city of Padang. Examination of BDNF and MMP-9 levels was carried out at the Biomedical Laboratory, Andalas University, Padang.

Population and Sample

The population in this study was all month-old neonates who were admitted to several hospitals in Padang city at the time of the study. The inclusion criteria in this study were those who were willing to be the subjects of the study, the age of mothers 20-33 years old, can remember HPHT or can show the results of TMG ultrasound examination 1, did not suffer from anemia (Hb11 gr/dl) and did not experience depression assessed from the questionnaire EPDS (Edinburgh Postnatal Depression Scale) (score > 13). Exclusion criteria are mothers with leukocytes > 13,000 mm^3 and blood glucose when > 200 mg/dl.

A total of 62 respondents as the study sample were taken using non-probability sampling methods by consecutive sampling. All subjects who came in sequence and fulfilled the inclusion criteria were included in the study until the required number of subjects was fulfilled [13].

Data normality test is done by using the Shapiro Wilk test. Data distribution is said to be normal if the significance value (sig) > 0.05). Data normality test was conducted to determine whether the data distribution of each variable. Data that is normally distributed is calculated on average and standard deviation. Bivariate analysis is used to determine the relationship between two variables. If it is normally distributed the unpaired t-test is used.

How to take samples

The method of sampling in this study is non-probability sampling that is by card consecutive sampling. All subjects who arrived sequentially and fulfilled the inclusion criteria were included in the study until the number of subjects needed was met [13].

How to determine sample size

Use the comparative numerical-numerical Lemeshow formula of two independent groups:

Sample formula

$$\mathrm{n}_1 = n_2 = 2\left(\frac{[\mathrm{Z}_\alpha + \mathrm{Z}_\beta]\mathrm{s}}{\mathrm{x}_1 - \mathrm{x}_2}\right)^2$$

n = Sample size, Zα = Error type 1 (α) of 5% = 1.96, Zβ = Error type 2 (β) of 10% = 1.28, S = Combined standard deviation, (x1-x2) = the smallest difference clinically important (Determined by researchers).

Because there are two variables examined by the researcher, then each sample size of the two variables will be searched for later compared to which sample size is greater.

a. BDNF

The standard deviation of the two groups is obtained based on the following calculations [14]:

$$(\mathrm{Sgab})^2 = \frac{s_1^2(n_1 - 1) + s_2^2(n_2 - 1)}{n_1 + n_2 - 2}$$

Sgab = Combined standard deviation

S1 = Standard deviation of group 1 = 3.153

n1 = Sample size of group 1 = 12

S2 = Standard deviation of group 2 = 1.718

n2 = Amount of sample group 2 = 34

Based on the formula above, the combined deviation is obtained as follows:

$$(\mathrm{Sgab})^2 = \frac{13.153^2\ (12 - 1) + 1.718^2\ (34 - 1)}{12 + 34 - 2}$$

Based on the formula above, the number of subjects is as follows:

$$n_1 = n_2 = 2\left(\frac{[1{,}96 + 01{,}28]2.167{,}72}{1.879}\right)^2 = 27{,}82 = 28$$

b. MMP-9

The standard deviation of the two groups is obtained based on the following calculations [15]:

$$(\mathrm{Sgab})^2 = \frac{s_1^2(n_1 - 1) + s_2^2(n_2 - 1)}{n_1 + n_2 - 2}$$

Sgab = Combined standard deviation, S1 = Standard deviation of group 1 = 87.4, n1 = Sample size of group 1 = 24, S2 = Standard deviation of group 2 = 53.37, n2 = Amount of sample group 2 = 38.

Based on the formula above, the combined deviation is obtained as follows:

$$(\mathrm{Sgab})^2 = \frac{87{,}4^2\ (24 - 1) + 53{,}37^2\ (38 - 1)}{24 + 38 - 2}$$

$$\mathrm{Sgab} = \sqrt{4.684{,}69} = 68{,}44$$

Based on the formula above, the number of subjects is as follows:

$$n_1 = n_2 = 2\left(\frac{[1{,}96 + 0{,}842]68{,}44}{104{,}5}\right)^2 = 8{,}48$$

Based on the calculation of the sample size of the two variables, the largest number of samples was taken, amounting to 28 people. To anticipate the subject who dropped out, the calculation was carried out as follows:

$$\boldsymbol{n = \frac{n}{1 - f}}$$

$$\boldsymbol{n = \frac{28}{1 - 0{,}1}}$$

$n = 31{,}1 = 31$, n = sample size = 28, f = estimated proportion of drop out = 10% = 0.1.

After anticipating the drop-out subjects, the sample size was 32, so that the total sample taken was 62 people (31 enough months with IUGR and 31 moderately-term neonates with normal birth weight).

Taking Blood Serum

Taking 3 ml umbilical cord blood sample using 3 ml syringe conducted by officers and researchers. The blood taken is taken by the researcher into a centrifuge tube (vacutainer) without anticoagulation using the syringe handle which is allowed to bleed itself from the syringe tube. Blood in the vacutainer is placed on the tube rack to avoid shocks and remain in position. The blood is allowed to stand for 15-20 minutes then centrifuged within 30 minutes at 3000 rpm for 10 minutes. Blood that has been centrifuged and then taken by the serum using a micropipette is then inserted into the 1½ ml cup serum that has been coded according to the identity of the respondent. Blood samples that have hemolysis are removed. The sample serum is then put into the refrigerator at a temperature of 40-60°C (a maximum of 24 hours) and then sent to the Biomedical laboratory, Faculty of Medicine of the Andalas University using a cool box containing ice gel (which has been frozen for at least 24 hours at -18°C) in time 4 hours. Deliveries will be made during laboratory working hours, namely at 08.00-16.00 WIB. Then the serum is stored in the refrigerator -80°C until the examination is done. After all the serum is fulfilled, then BDNF levels are examined using the Human BDNF ELISA Kit and MMP-9 levels using the Human MMP-9 ELISA Kit at the Biomedical Laboratory of the Faculty of Medicine, Andalas University.

***Examination of BDNF Levels* (*Work protocol based on Human BDNF ELISA Kit*)**

All reagents, samples and standards are prepared according to the instructions. All reagents and samples were left at room temperature 18-25°C before use. After that, 100 µL standard or sample is

added to each well then incubated for 2,5 hours at room temperature or overnight at 4°C. Then the prepared Streptavidin solution is then added to it for 45 minutes at room temperature. After that 100 µL of TMB One-Step Substrate Reagent was added to each well then incubated for 30 minutes at room temperature. Finally added 50 µL Stop Solution at each well then read immediately with a wavelength of 450 nm. Obtained the concentration value of the sample examined.

Examination of MMP-9 Levels (*Work protocol based on the Human MMP-9 ELISA* Kit)

All reagents, samples and standards are prepared according to the instructions. All reagents and samples were left at room temperature 18-25°C before use. After that, 100 µL standard or sample is added to each well then incubated for 2.5 hours at room temperature or overnight at 4°C. Then the prepared Streptavidin solution is then added to it for 45 minutes at room temperature. After that 100 µL of TMB One-Step Substrate Reagent was added to each well then incubated for 30 minutes at room temperature. Finally added 50 µL Stop Solution at each well then read immediately with a wavelength of 450 nm. Obtained the concentration value of the sample examined.

Data analysis

The data normality test was carried out using the Kolmogorov Smirovov test (sample ≥ 50). Data distribution is said to be normal if the significance value (sig) > 0.05, and if sig < 0.05, the data is not normally distributed. Data normality test was conducted to determine whether the data distribution of each variable. Data that is normally distributed is calculated on average and standard deviation. Data is abnormally distributed, calculated median values and maximum minimums. Categorical data is calculated by frequency distribution.

Research Ethics

This research has received ethical considerations and approval from the Research Ethics Committee Team Faculty of Medicine of Andalas University with registration number 236/KEP/FK/2017.

Results

The normality test was carried out in groups using the Shapiro Wilk test. The test results in Table 1 show that data are normally distributed (p > 0.05). Data with normal distribution will show the mean value. Then a comparative test of 2 unpaired groups was then carried out.

Table 1: The characteristics of the research subject

Characteristics	Normal	IUGR	P value
Age of mother (year)	27 (2-33)[b]	28 (20-33)[b]	0.86
IMT	22.61 ± 1.89[a]	22.05 ± 1.86[a]	0.25
Hb level (g/dl)	11 (11-12.6) [b]	11 (11-12.7) [b]	0.98
Level of GDS (g/dl)	93.52 ± 10.16[a]	94.65 ± 14.11[a]	0.72
Leukocytes (/mm^3)	9720.32 ± 1793.83[a]	10137.74 ± 19.09.06 [a]	0.38

It can be seen that there is not one variable that has a significant difference in the two groups. This means that the characteristics of the two groups considered being homogeneous.

Differences in BDNF Levels in Normal Babies and IUGR

In the initial process of testing normal data distribution, the BDNF level shows normal data distribution which can be seen in Table 2. Therefore the data displayed is average. Table 2 shows that there are differences in BDNF levels between normal infants with IUGR.

Table 2: BDNF levels for each group of research subjects

	N	BDNF Levels (ng/mL)		*P value*
		Mean	SD	
Normal	31	1.58	0.23	0.001*
IUGR	31	1.25	0.35	

* p < 0.05. Independent t-test.

Differences in MMP-9 Levels in Normal Babies and IUGR

In the initial process of testing the distribution of normal data, MMP-9 levels show normal data distribution which can be seen in Table 3. Therefore the data displayed is in the form of a mean.

Table 3: MMP-9 levels for each group of research subjects

	N	MMP-9 Levels (ng/mL)		*P value*
		Mean	SD	
Normal	31	1.09	0.20	0.03*
IUGR	31	1.25	0.35	

* p < 0.05. Independent t-test.

Table 3 above shows that there are differences in MMP-9 levels between normal infants with IUGR.

Discussion

Differences in BDNF Levels in Normal Babies and IUGR

There were significant differences between the median BDNF levels in normal infants, which were 1.58 ± 0.23 ng/ml and BDNF levels in IUGR infants

were 1.25 ± 0.35 ng/ml which can be seen in Table 2.

Previous studies have shown that a decrease in serum BDNF at the end of pregnancy is associated with a risk of low birth weight [16]. Also, other studies have also found the role of BDNF in energy angiogenesis and homeostasis in the uteroplacental system which allows in influencing the aetiology associated with impaired placental growth and the fetus including one of them IUGR [8].

One of the most studied neurotrophin members is BDNF. Several studies have highlighted the critical role of neurotrophin in particular NGF and BDNF during pregnancy [14]. BDNF has been reported to have a role in embryo implantation, placental development, fetal growth from mid-gestational to advanced gestation by increasing trophoblast cell growth and survival in mice suggesting a possible role in the development of fetoplacental units [17].

In the study stated that one of the forms of neurotrophin is BDNF, a molecule that regulates placental and brain development. Placental development is very important during pregnancy because it forms the interface between the maternal-fetal circulation and is essential for fetal nutrition and oxygenation. Also, BDNF also tends to influence the birth and development of neurons through two mechanisms, namely angiogenesis and cell growth, defence and maturation [18].

However, in other studies, there were no significant differences in BNDF levels when observed between term infants with IUGR and term infants with normal birth weight (AGA/Age of Proper Pregnancy) measured from the mother, fetus, and neonatal side. This result may be due to differences in the characteristics of the sample under study where more criteria are used in determining the sample to be taken. These criteria include the presence or absence of polyhydramnios, congenital infections, and chromosome assessment for mothers over 35 years to assess the risk of fetal anomalies. Also, the assessment of smoking behaviour and drinking habits is also assessed. This study also included mothers with severe anaemia and diabetes while both were exclusion criteria in this study [19].

Differences in MMP-9 Levels between Normal Babies and IUGR

There is a difference between MMP-9 levels in normal infants which is 1.09 ± 0.20 ng/ml and the mean MMP-9 level in IUGR infants is 1.25 ± 0.35 ng/ml which can be seen in Table 3.

In line with the research conducted who found that placentas from pregnancies with IUGR showed a decrease in the release of MMP-2, MMP-9 and TIMP-1 compared to normal pregnancies [20]. Other studies also found that a decrease in MMP-9 levels affected the pathological processes underlying the occurrence of preeclampsia and IUGR in both preeclampsias and normal pregnancies with IUGR [21]. Different results were found in other studies that MMP-9 levels had no significant relationship with gestational age and infant birth weight. These different results may be due to using premature infants as respondents while this study assessed fairly month's infants [22].

Matrix metalloproteinases (MMPs) are members of the protease family that can degrade extracellular matrix (ECM) and connective tissue proteins [23]. MPMP-9 is expressed by the uterus and plays a role in remodelling uterine tissue in the estrus cycle, menstrual cycle and pregnancy in both animals and humans. MMP-9 has also been found to increase in the myometrium and aorta of pregnant mice which shows its role in the uterus and vascular remodelling MMP-9 also plays a role in angiogenesis by allowing endothelial cells to escape and move into new tissues and also by releasing proangiogenic factor matrix [24].

MMPs are families of zinc-dependent endopeptidases that divide the ECM component and are involved in embryo implantation, placental development and also in the process of labour. Research shows that the development of the maternal-fetal circulation and the success of pregnancy in humans require the invasion of trophoblast cells into the endometrial stroma and the lining in all three myometria. This invasion ability is mediated by MMPs where the most important role is held by MMP-9 [5].

Deficiency of MMP-9 can disrupt trophoblast invasion and will lead to abnormal and superficially implanted placenta as in preeclampsia. Low MMP-9 levels are found in patients with preeclampsia and cases of IUGR [5]. Experiments in pregnant rats proved that placentas that did not have MMP-9 showed smaller implantation spaces and embryos with a significantly reduced body weight that matched the characteristics of IUGR [6].

In this study only assessing IUGR, not classifying whether symmetrical or asymmetrical. This research can use to determine the aetiology and pathophysiology of IUGR by looking at BDNF and MMP-9 levels and as a preventive measure in preventing the occurrence of IUGR in pregnant women by avoiding the risk factors for low BDNF levels, namely age (< 20 or > 33 years), iron deficiency anemia (Hb < 11gr /dl), and avoiding depression as assessed from the EPDS questionnaire (Edinburgh postnatal depression scale) (score < 13). In a cross-sectional study, IUGR can be diagnosed if the neonatal body weight is below the 10th percentile based on the Lubchenco growth curve. Pretty month neonates who were born at 37 to 42 weeks' gestation were weighed within < 24 hours.

In conclusion, BDNF of moderately mature neonates was significantly higher in normal birth weight compared to intrauterine growth restriction and

the moderately high MMP-9 neonates were significantly higher in intrauterine growth restriction compared with normal birth weight.

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Clinical Science

Safety Profile and Efficacy of Chemoembolization with Doxorubicin - Loaded Polyethylene Glycol Microspheres in Patients with Hepatocellular Carcinoma

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AIM: This study was designed as a preliminary investigation of safety and efficacy of LifePearl, polyethylene glycol microspheres loaded with doxorubicin for treatment of locally untreatable (i.e., unresectable and not suitable for local thermal ablation) hepatocellular carcinoma (HCC).

MATERIAL AND METHODS: Patients with locally untreatable HCC (mono- or bilobar disease, ECOG performance status 0-1, Chilg-Pugh score < 11) were analysed for this single arm Unicenter retrospective study. All the information were acquired through our local hospital information system. DEB-TACE was performed with 100-200 microns LifePearl loaded with 75-150 mg of doxorubicin depending on tumour size. One interventional radiologist with experience of more than 350 TACE procedures and one fellow in radiology performed all embolisations.

RESULTS: Twenty subjects with 29 tumours were treated (mean age 66.2 years). Child-Pugh status was A for 12 pts. (60%), B for 6 pts. (30%) and C for 2 pts. (10%). Three patients had insignificant ascites. Most patients (70%) underwent < 3 DEB-TACE procedures. Average doxorubicin dose was 71.1 mg per procedure. One patient had procedure-related SAE (acute pancreatitis) within the postembolization period which was induced due to non-target embolisation of the superior pancreaticoduodenal artery. Six-month freedom from procedure-related SAE or death was 95% (one necrotizing pancreatitis). Tumor response or stable disease was achieved in 95% (19/20) of subjects. Freedom from tumor progression or death at 6 months was 95%. One-year survival rate was 90% overall.

CONCLUSION: The results from this investigation suggest that LifePearl microspheres, Terumo loaded with doxorubicin can provide an excellent local tumour control with very few side effects in a relatively homogeneous group of patients with locally untreatable HCC.

Abstract

Introduction

Patients with locally untreatable HCC (i.e., unresectable and not suitable for local thermal ablation) have few treatment options [1]. Systemic chemotherapy with sorafenib has shown to modestly prolong survival in patients with advanced stage disease [1], [2] and transarterial chemoembolization (TACE) is recommended for patients in intermediate stage disease [1]. Meta-analyses have shown that TACE performed with doxorubicin-loaded beads has similar efficacy but fewer side effects than conventional lipiodol-based TACE [3], [4]. So far there are four types of drug-eluting microspheres on the market. The first three types are based on polyvinyl alcohol (PVA) and the last one developed are LifePearl made from polyethylene glycol. In our centre, we started using this polyethylene glycol platform since 2016. So far, there are only a couple of preliminary studies in the literature, regarding the safety profile of polyethylene glycol particles.

This investigation was designed in a retrospective fashion to evaluate safety and efficacy of LifePearl microspheres loaded with doxorubicin for DEB-TACE treatment of locally untreatable hepatocellular carcinoma, specifically the size of 100 and 200 microns and 75-150 mg of doxorubicin.

Material and Methods

Study design

This investigation represents a retrospective, Unicenter, single-arm feasibility study of chemoembolization with 100 and 200 microns doxorubicin-loaded LifePearl microparticles for treatment of locally unresectable HCC.

Study Patients

Patients eligible for the study were adults with a confirmed diagnosis of HCC according to the European Association of Study of the Liver (EASL) criteria [6] and were staged according to BCLC criteria [1]. Eastern Cooperative Oncology Group (ECOG) [7] performance status was 0 and 1, and we included patients with Child-Pugh score of < 11 points (i.e. A, B or C 10) [8]. Twenty patients with 29 tumours were enrolled. Tumor size ranged between 1.5-15 cm in largest diameter (mean diameter = 5.67 cm) both mono or bilobar disease. Lack of main portal vein trunk or common bile duct invasion was confirmed with preprocedural multidetector computed tomography (MDCT). Patients had laboratory values in the following ranges: white blood cell count > 3500/ml, absolute neutrophil count > 1500 cells/ml, INR < 1.8, partial thromboplastin time < 38 s, platelet number > 5 x 10^4 ml, blood bilirubin level < 20 mmol/L, aspartate aminotransferase (AST) level and alanine aminotransferase (ALT) within five times of normal range of each organ, serum creatinine level < 2.5 mg/dL, hemoglobin > 8.0 g/dL, and alkaline phosphatase < 630 IU/L.

Embolisation Procedure

LifePearl microspheres are polyethylene glycol particles that can be loaded with anthracyclines such as doxorubicin, epirubicin, idarubicin or other chemotherapeutic drugs, such as irinotecan [5], [9], [10], [11]. Patients in this study were treated with 100 and 200 microns diameter LifePearl microspheres, which are calibrated to ± 25 microns. One 2 ml syringe of microspheres can be loaded with up to 75 mg of doxorubicin, with little shrinkage observed after drug loading [9]. Doxorubicin loading was performed according to the manufacturer's instructions. A procedural doxorubicin dose of 75 mg/m^2 body surface area was targeted. A minimum of two treatments per lesion, separated by 4 weeks interval were performed. Thus, patients with bilobar disease were treated with at least 4 sessions (two per liver lobe).

Chemoembolization procedures were performed with antibiotic prophylaxis, analgesic and antiemetic medication at the physician's discretion. Angiography of the hepatic and mesenteric arteries was performed before chemoembolization to confirm anatomical eligibility and identify tumour feeder arteries. Hepatic segmental or subsegmental arteries supplying the lesion were selectively catheterised with microcatheter while ensuring sufficient flow to the tumour, and a mixture of 100 or 200 microns of doxorubicin-loaded microspheres and non-ionic contrast agent was slowly injected. Bland microspheres were also used in some cases if blood stasis was not achieved after the delivery of the desired drug dose.

Safety and efficacy endpoints

Safety was observed as freedom from severe adverse events (SAE) at 30 days and 6 months, and efficacy as freedom from tumour progression at 6 months after chemoembolization. Secondary points were the rate of local tumour control and 12-month survival.

Adverse event monitoring was observed and recorded during the treatment and follow-up phases. Tumour imaging (contrast-enhanced CT) was performed, and measurements were taken within 4 weeks before the first DEB-TACE procedure (baseline) and 4 weeks following every embolisation procedure. Repeat DEB-TACE procedures were performed when follow-up imaging studies showed residual enhancement until complete response according to the modified Response Evaluation Criteria in Solid Tumors (mRECIST) criteria was achieved. Imaging was repeated at 4 to 6 weeks intervals to determine the need for additional DEB-TACE procedures and was scheduled to be performed 3 and 6 months following the last DEB-TACE procedure and 12 months from the initial treatment. Clinical and laboratory assessments were also repeated at each of these visits. Tumour response was assessed based on mRECIST criteria [12]. "Best overall response" was defined as the smallest measurement of hypervascularized tumour tissue recorded from the start of treatment until disease progression/ recurrence. Images were evaluated by the interventional radiologist who performed the DEB-TACE procedures.

Results

From February 2016 to July 2018, 20 patients with 29 tumours were treated at our department for interventional radiology. Patient and lesion characteristics are summarized in Table 1. There was no statistically significant difference among patient groups regarding the aetiology of cirrhosis ($p > 0.05$). ECOG performance status of 0 is significantly more included than ECOG 1 ($p < 0.05$).

Table 1: Baseline patient characteristics

Characteristic	N (%) (*N = 20*)	P
Sex (male/female)	15/5	P = 0.0016
Age, years (median, range)	66.2 (55-80)	
Aetiology of cirrhosis		
Alcohol abuse	5 (25.0)	
Hepatitis B	7 (35.0)	P > 0.05
Hepatitis C	0 (0)	
Unknown/other	8 (40.0)	
Child-Pugh classification		
A	10 (50.0)	
B	8 (40.0)	P = 0.0058
C	2 (10.0)	P = 0.0258
ECOG performance status		
0	16 (80.0)	P = 0.0001
1	4 (20.0)	
BCLC classification		
A	12 (60.0)	P = 0.0009
B	6 (30.0	
C	2 (10.0)	
Prior liver surgery	1 (5.0)	
Prior RFA therapy	1 (5.0)	
Liver lobes involved		
1 (Monolobar HCC)	17 (85.0)	P = 0.0000
2 (Bilobar HCC)	3 (15.0)	
Tumor size (largest diameter; *N = 29*)		
< 3 cm	10 (34.5)	
3 ≤ 5 cm	5 (17.2)	P > 0.05
5 ≤ 10 cm	9 (31.0)	
> 10 cm	5 (17.2)	
Range	1-15 cm	

Most of the patients (85%) had monolobar disease (P value < 0.05) and 90% had early or intermediate stage HCC, BCLC A and B, significantly more than C (P < 0.05). Only two patients had prior therapy (surgery or radiofrequency ablation) before chemoembolization; none had systemic chemotherapy.

All patients that were included in the study underwent at least two successful DEB-TACE procedures, except one patient who had one chemoembolization only because we were confident that we achieved a complete response after the first TACE which was confirmed with several consecutive imaging controls.

Safety

By 30 days from their first embolisation procedure with drug-loaded microspheres, 95% (*n* = 19) of patients remained free from procedure-related severe adverse events. One patient (5%) experienced acute pancreatitis which was confirmed by clinical signs, laboratory tests and imaging studies. After carefully reviewing the DEB-TACE procedure in this patient we realised that it was due to reflux of drug-loaded microparticles in the superior panreatoduodenal artery because tumor was supplied from the gastroduodenal artery and partially from the right hepatic artery. The patient had a history of cirrhosis of the liver, previous liver surgery due to HCC, Hepatitis B infection, portal hypertension, chronic erosive gastritis and presented with BCLC B HCC. This complication was treated with conservative medical treatment and prolonged hospitalisation. The patient recovered and was discharged from hospital 10 days later. Prolonged postembolization syndrome occurred in 6 patients, followed by moderate abdominal pain, slightly elevated temperature, nausea/vomiting and loss of appetite. These patients remained in the hospital for 3 nights and then were discharged in good clinical condition. The occurrence of post-embolisation syndrome symptoms after embolisation procedures is summarised in Table 2. Post-embolization syndrome events were grade 1 or 2.

Table 2: Occurrence of post-embolisation syndrome following DEB-TACE procedures

	1st DEB-TACE (*N* = 20)	2nd DEB-TACE (*N* = 19)	3rd DEB-TACE (*N* = 10)	4th DEB-TACE (*N* = 3)	5th DEB-TACE (*N* = 2)
Postembolization syndrome, n (%)	11 (55.0)	8 (42.1)	7 (70.0)	2 (66.6)	1 (50.0)
Abdominal pain	7 (35.0)	4 (21.0)	6 (60.0)	1 (33.3)	0 (0)
Nausea/vomiting	1 (5.0)	2 (10.5)	1 (10.0)	1 (33.3)	0 (0)
Fever	1 (5.0)	2 (10.5)	3 (30.0)	0 (0)	1 (50.0)

According to the index of dynamics, a decrease in postembolization syndrome is registered from first to third DEB-TACE for 36.4%, and first to fifth DEB-TACE for 90.9%.

By 6 months from their first DEB-TACE procedure, 95% (N = 19) of patients were free from procedure-related serious adverse events or death. All patients survived at least 6 months after treatment.

Imaging to evaluate tumour response was available for all 20 patients. According to mRECIST criteria for best response, complete response was achieved in 10 patients (50%), partial response in 6 (30%), stable disease in 3 (15%) and progressive disease in 1 (5%). Significantly more patients had complete response versus stable and progressive disease (P = 0.0181 and P = 0.0014). At 6 months, 95% of patients were free from tumour progression or death. The one patient who had tumour progression had three embolisation procedures before progression was observed. At 6 months, 95% of patients were free from tumour progression or death. The one patient who had tumour progression had three embolisation procedures before progression was observed.

One-year survival rate was 90% (18/20). One death was due to other comorbidities, not tumour itself, the other one was due to progressive HCC disease. Both of these patients were staged as BCLC C disease before treatment.

Discussion

The results from this study demonstrate a high rate of local tumour control and only one severe adverse event among patients treated with doxorubicin-loaded PEG microspheres. In a preliminary single-center retrospective study by Veloso Gomez et al., [21] in 302 patients with HCC, treated with polyethene glycol microspheres, more than 80% of patients had objective tumour response

and the one-year survival rate was 93.5%. However, in this study, a large number of patients (142) had Barcelona Clinic Liver Cancer stage A disease and 134 had BCLC stage B disease which is a good prognostic factor for HCC patients. Also, mean index tumour size in this study was 3.7 cm, and in our study median tumour diameter was 5.7 cm. The incidence of serious adverse events or Grade 3-4 toxicities following doxorubicin-eluting embolic therapy for HCC has generally been low in previous studies [13], [14], [15] and was comparably low in our investigation. For example, Prajapati et al., [13] reported an overall adverse event rate of 30% within 30 days of embolisation, with the majority of complications Grade 1-2 and no Grade 4 toxicities in a retrospective study od 121 patients. In a randomised study, approximately 24% of 93 patients treated with drug-eluting embolic agent reported serious adverse events within 30 days, and two patients died [14]. In this study, no deaths or systemic toxicities occurred within 30 days, and only 1 patient had procedure-related serious adverse event. Although the heterogeneous nature of HCC patients characteristics, variable treatment regimes, and evaluation criteria across studies limit comparisons between studies, results from previous studies of doxorubicin-loaded microspheres treatment of HCC provide background for the findings observed in our investigation.

Post-embolization syndrome, or symptoms like abdominal pain, nausea, vomiting, and fever has been reported in 5-100% of patients in previous studies [5], [13], [14], [15], [16], [17]. Richter et al. reported tumour response rate with complete or partial tumour response observed in 67% of patients in MIRACLE I study [20]. In our study, we found that 80% of the patients had a complete or partial response to intraarterial therapy and only 1 patient (5%) displayed tumour progression during follow up. The one-year survival rate in MIRACLE I study was 56% with a greater rate among patients with Child-Pugh A liver cirrhosis (75%) [20]. In our study, one-year survival rate was 90%, but compared to the patients in MIRACLE I, in our study Child-Pugh A and B cirrhosis was found in 90% of patients. We had 2 patients with no significant ascites while in MIRACLE I, 10 patients had severe ascites. Previously reported one-year survival rates among different studies varied between 58 and 92% [5], [15], [18], with stratifying factors such as the presence of ascites and Child-Pugh classification of B or C associated with poorer survival [13], [18], [19]. The proportion of patients with ascites or liver function with Child-Pugh B and C are relatively low in our group of patients but not very different than other studies (i.e., no patients with ascites or Child-Pugh C were included in the studies from Reyes et al., [15] or Malagari et al., [5].

The limitations of this study are that it is a retrospective one and that the sample size is relatively small. Study sample is with relatively homogeneous clinical characteristics. The results from this investigation suggest that LifePearl polyethylene glycol microspheres, when loaded with doxorubicin, can provide excellent local tumour control with a very low rate of procedure-related complications in a well-selected group of patients with locally untreatable HCC.

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Comparison of Mean VEGF-A Expression Between Acute Ischemic Stroke Patients and Non-Ischemic Stroke Subjects

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Abstract

BACKGROUND: Glucose and oxygen supply to neurons are disrupted during acute ischemic stroke, resulting in hypoxia. This event, in turn, activates the transcription of hypoxia-inducible factor (HIF-1), which is responsible for activating genes responsible for angiogenesis, including vascular endothelial growth factor (VEGF). VEGF and their receptor systems exert complex mechanisms of angiogenesis, including the stimulator, inhibitors, angiogenic and modulator. VEGF-A is the primary regulator of angiogenesis, both during physiological and pathological conditions. Nevertheless, the role of VEGF on the prognosis of hypoxia remains controversial.

AIM: The purpose of this study was to address if there is any difference between the mean expression of VEGF-A between acute ischemic patients and non-ischemic stroke subjects.

METHODS: This was an observational study with a cross-sectional design, the population in this research is the acute ischemic stroke patients and non-ischemic stroke subjects, which were admitted on Emergency Room and later treated in the Stroke Unit, Dr Sardjito General Hospital, Yogyakarta, Indonesia. Subjects were recruited using the purposive method, yielding a total of 64 subjects on both groups. Diagnosis of acute ischemic stroke was established using a head CT scan. Patients who meet the inclusion criteria and willing to participate in the study were asked to provide informed consent. Laboratory analysis was conducted during the first 24 hours after being treated at Stroke Unit, Dr Sardjito General Hospital, Yogyakarta, Indonesia, with venous blood was withdrawn VEGF-A levels between acute ischemic stroke and non-ischemic stroke subjects were subsequently compared. Categorical variables (including gender) were tested using either chi-square or Fisher exact test. Interval data was examined using student t-test if data distribution was normal.

RESULTS: As many as 35 acute ischemic stroke and 35 non-ischemic stroke patients were included in the study, among whom were 18 men (51.43%) and 17 women (48.57%) among stroke patients and 21 (60%) men and 14 (40%) women among subjects without stroke. The average of the subject's age on stroke and non-ischemic stroke group was 58.51 and 48.57 years old. VEGF-A levels were significantly higher in the non-stroke group (561.77 ± 377.92) compared with stroke group (397.78 ± 181.53) with p = 0.02.

CONCLUSION: expression of VEGF-A in acute ischemic stroke group was lower when compared with the non-stroke group.

Introduction

VEGF-A is the primary regulator of angiogenesis, both during physiological and pathological conditions, including those related to malignancy or hypoxia of any cause including acute ischemic stroke [1]. Hypoxia triggers smooth muscle cell proliferation and endothelial cells to form new blood vessels so that it will improve the function of oxygenation. Hypoxia activates several important genes and signal pathways under the condition above [2].

As a result of hypoxia, the gene will be controlled by the transcription of Hypoxia-Inducible Factor 1 (HIF-1). HIF-1 will activate genes responsible for angiogenesis, for example, Vascular Endothelial Growth Factor (VEGF) [3]. Oxygen functions as a regulator of VEGF production, hypoxia causes an increase in VEGF levels and returns to the baseline level within 24 hours, and then the cell returns to normoxia condition [4]. VEGF levels will be reduced in

hyperoxia in vitro and in vivo [5]. Nevertheless, the role of VEGF in hypoxia remains controversial, considering several studies showing different results.

This study aims to prove that there are differences in the mean expression of the Vascular Endothelial Growth Factor-A (VEGF-A) between acute ischemic stroke patients with non-stroke patients.

Material and Methods

This was an analytical observational study. The inclusion criteria in this study were acute ischemic stroke patients both male and female, of 35 to 70 years old, with a maximum 5-day duration between the time of the attack and hospital admission, (2) stroke onset was defined as the last time the patient was known by others in a healthy condition and had not shown any signs of neurological deficit, (3) the presence of one of the neurologic deficits are decreased consciousness, hemiparesis, seventh and/or eighth cranial nerve palsy,, dysarthria, aphasia, or hemianopsia, (4) proven acute ischemic stroke with head CT scan.

The eligible criteria in this study were: (1) intracerebral haemorrhage patients or the space-occupying process for other reasons, (2) any history of previous strokes.

Non-ischemic stroke groups including non-infectious low back pain patients who were treated in the Neurological Ward or general check-up patients who are known to have no degenerative abnormalities that have vascular influences, such as hypertension, diabetes mellitus or dyslipidemia, taken in the same period, both male and female.

Subjects were recruited by a consecutive method, in which every new ischemic stroke patient who came through an Emergency Department and treated at the Stroke Unit Dr Sardjito General Hospital, who fulfilled the inclusion criteria and willing to participate in the study after being explained with the risks and benefits. All participants included in the study were asked for informed consent.

These steps must be carried out until the specified sample size reaches the number of samples. The participants above' identity and characteristics were then recorded in the case form.

History taking and physical examination of all included subjects were performed by a neurology resident and those who were suspected of having acute ischemic stroke underwent additional head CT scan.

Laboratory analysis was carried out once, at 24 hours after being treated at the Stroke Unit Dr Sardjito General Hospital, and blood was withdrawn from a vein, then VEGF-A levels were examined by ELISA method.

Comparative analysis was made on subjects with acute ischemic stroke with non-stroke subjects concerning VEGF-A levels. The level of significance used is 0.05 or 95% confidence interval ($p < 0.05$), with 80% power. All statistical analyses were performed using SPSS for Windows version 20.

Results

This study began in August to December 2011. A total of 11 subjects were not included in this study, among whom 8 of them refused to participate in the study and three subjects were excluded because of acute renal insufficiency. Seventy subjects, therefore, were included in the study, i.e., every 35 subjects with acute ischemic stroke and non-ischemic stroke cases.s. The basic characteristics of the research subject were obtained through descriptive analysis.

Of the total subjects in the stroke group based on sex, 18 (51.437%) subjects were of males, and 17 (48.57%) subjects were of females. Subject comparison based on sex were then considered homogenous. In contrast, in the non-ischemic stroke group, there were more female than male subjects (21 [60%] vs 14 [40%]).

Table 1: Basic Characteristics of Research Subjects

	Stroke Groups	Non-Stroke Groups	p
Male	18 (51.43%)	14 (40.00%)	-
Female	17 (48.57%)	21 (60.00%)	-
Age (years)	58.51 ± 9.89	48.57 ± 11.36	-
VEGF-A level	397.78 ± 181.53	561.77 ± 377.92	0.02

Furthermore, based on the subjects' age, the average stroke group was 58.51 years old, higher than the non-ischemic stroke group with an average of 48.57 years old. While VEGF-A levels were significantly higher in the non-ischemic stroke group, i.e.561.77 ± 377.92, compared to the stroke group (397.78 ± 181.53) with $p = 0.02$.

Discussion

VEGF-A levels in the stroke group were lower than that of non-ischemic stroke group. This result is different from other studies, in which VEGF-A levels were found at higher levels. For instance, in cases of malignancy, an increase in VEGF-A expression can be observed in lung adenocarcinoma, six colorectal carcinomas, pancreatic carcinomas, breast carcinoma, and ovarian carcinoma [7], [8], [9], [10].

There are several explanations as to why in the stroke group there was a lower VEGF-A level compared with the non-ischemic stroke group. Stroke is caused by several potential risk factors associated with a decrease in VEGF levels, for example, elderly, diabetes mellitus and also the possibility of early complications of kidney failure, although the results of laboratory tests have not shown signs of kidney failure. Low serum albumin levels are often found in elderly patients. Hypoalbuminemia was reported in 19% of stroke patients [11].

Furthermore, albumin significantly reduced VEGF expression during hypoxia [12].

Hypoalbuminemia is associated with endothelial dysfunction in all causes of death in patients with chronic kidney disease and cardiovascular mortality in terminal kidney disease patients. However, it is not yet clear whether endothelial dysfunction is a direct result of a decrease in albumin levels or due to other factors, such as chronic inflammation and dyslipidemia, and indeed it is often found in conditions associated with hypoalbuminemia [12].

In contrast to previous in vitro studies, in this study, we found that a decrease in VEGF-A levels is a response to hypoxia. An explanation might be related to the study of Lerman et al., in mice, by which the production of VEGF by fibroblasts in diabetic conditions-because of the presence of glucose intolerance-did not increase the regulation of VEGF under hypoxic conditions [14]. Hypoxia causes glucose intolerance in vivo, so in this study, VEGF production may be influenced by glucose intolerance, because stroke patients may have risk factors for diabetes mellitus [15].

There is a study on changes in VEGF levels in the circulation which are likely to be influenced by glucose transporters, on the blood-brain barrier [16] [17]. Besides, VEGF also mediates the induction of endothelial fenestration, thus increasing the transport of small molecules such as sucrose and fluorescein to penetrate the blood-brain barrier [18], [19]. Based on these mechanisms on the blood-brain barrier, a decrease in VEGF after acute hypoxia can reduce glucose transfer across the blood-brain barrier which ultimately is neuroprotective.

Furthermore, a decrease in VEGF-A levels in the stroke group compared with the non-ischemic stroke group might be partly explained by the fact that astroglial cells are the most abundant cells in the central nervous system and play an important role in the pathology of brain tissue. Although never proven directly, astrocytes are thought to have a neuroprotective role in protecting neurons from oxidative stress during a stroke. The hypothesis is based on the ability of astrocytes to act as a buffer, to transport and metabolise amino acids, glucose, and other important molecules, and also improve antioxidant regulation and free radical scavenger in the area of ischemia. Reactive gliosis is the response of the astrocytes at the time of central nervous system injury, including cerebral ischemia, and reactive astrocytes undergo changes in morphology and expression in various molecules [20].

Reported by Bareto et al., that although there is extensive cell proliferation, especially in microglia and neutrophils/monocytes at one week after the stroke, however, there are some adult astrocytes that re-enter into the cell cycle, and this is concentrated around the infarct area [21].

Astrocytes are known to be more resistant to oxidative stress than neurons and act as neuroprotectors through their ability to take potassium and glutamate and release mitogenic factors. But in response to injury, astrocytes will pull off its endfeet from the blood vessels, which will result in increased permeability, as well as due to proliferation, it will cause scars on the glial cells [22].

Hypoxia/ischemia causes activation of adaptive mechanisms and changes in gene expression in injured areas to counteract the pathological progression caused by inducing transcription of Hypoxia-Inducible Factor (HIF-1). The primary target of HIF-1 is a gene that plays a cytoprotective role, VEGF. Astrocytes can secrete VEGF under physiological conditions, and then hypoxia will induce both mRNA and protein [23].

Other factors also cause a decrease in VEGF expression due to hypoxia, namely Glia Cell-Derived Conditioned Medium (CM) [24]. Furthermore, it was explained that hypoxia which causes astrocytes could inhibit an increase in VEGF mRNA expression Conditioned Medium (CM) or C6 CM glioma cells. During normoxia, CM astrocytes and C6 CM glioma cells do not change the expression of VEGF mRNA. Thus hypoxia which causes an increase in VEGF protein will be inhibited by CM glia cells.

Studies are documenting the potential of VEGF therapy to repair peripheral neuropathy in diabetes [25]. Other studies reported that intramuscular gene transfer with VEGF-bound plasmids was able to improve motor and sensory function in a rabbit model who had peripheral ischemic neuropathy [26]. Besides, VEGF administration therapy in post-stroke patients can reduce the extent of cerebral infarction because VEGF will stimulate angiogenesis and neurogenesis in areas near the penumbra [27], [28]. The administration of specific antibodies to block the function of VEGF in injured animal models apparently can increase the size of the lesion and reduce angiogenic activity and astroglia in the striatum [29].

There are also other studies that support the role of VEGF in controlling damage from brain injury, for example, the administration of VEGF to spinal cord contusions can improve cellular levels, and the administration of VEGF significantly increases nerve

regeneration when the Matrigel implant is inserted into sciatic nerve injury [30], [31], [32].

Based on these findings, for example in models of stroke, diabetic neuropathy, and spinal cord injury, VEGF therapy significantly increases cellular and functional improvement, so it is reasonable that when a cellular injury occurs due to hypoxia, it appears that there is a decrease of VEGF expression. Another possibility of decreasing VEGF-A expression is the activity of Glia Cell-Derived Conditioned Medium (CM), both CM astrocytes and C6 CM glioma cells because in acute stroke there will be an increase in proliferation of glial cells, including astrocytes.

Another theory that VEGF will increase its expression in the acute phase of brain injury so that it can affect endothelial permeability which results in cerebral oedema, for example in patients with acute stroke, is still controversial, and further research is needed with a focus on VEGF receptor activity that might answer the controversy.

The limitations of this study were: (1) the activity of glial cells during hypoxic conditions is difficult to control, (2) the maximum time taken for taking blood in patients is 6 hours from the time of onset, and (3) the number of other variables that can affect VEGF-A expression in acute ischemic stroke group.

It was concluded that the expression of VEGF-A in the acute ischemic stroke group was lower than in the non-ischemic stroke group.

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Clinical Science

Comparing of Tp-Te Interval and Tp-Te/Qt Ratio in Patients with Preserved, Mid-Range and Reduced Ejection Fraction Heart Failure

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Keywords: Heart Failure; Transmural dispersion; Tp-Te interval; fQRS; Tp-Te/QT

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Abstract

BACKGROUND: Heart failure (HF) is classified in three class: HF with preserved EF (HFpEF); normal or LVEF ≥ 50%, HF with reduced EF (HFrEF); LEVF < 40% and newly HF mid-range EF (HFmrEF); LVEF 40-49%. On Electrocardiography (ECG) T wave, Tpeak-Tend (Tp-Te) interval reflects transmural dispersion of repolarisation (TDR) which of these indexes have been proposed as predictors of risk for ventricular arrhythmia (VA) in many cardiac diseases.

AIM: Aim of this study to asses these indices of TDR among three HF class.

METHODS: Total of 192 patients were included in this study.

RESULTS: Many of indices like Tp-Te, Tp-Te/QT wasn't different between groups (P > 0.05). But mean Q-Tpeak (QTp), S-Tend (S-Te) and S-Tpeak (S-Tp) were found significantly different between groups (P < 0.05). Again S-Te was found different according to having fragmented QRS (fQRS) on ECG (P = 0.031). Comparing to mitral inflow E/A parameters showed significant differences for Tp-Te, Tp-Tec, Tp-Te/QT, Tp-Te/QTc and Tp-Tec/QTc parameters. Finally, we found correlations between S-Te and white blood cell (WBC) (r = - 0.171; P = 0.037) and S-Tp and WBC (r = - 0.170; P = 0.038) and between S-Te and fQRS (r = 0.158; P = 0.031).

CONCLUSIONS: We didn't find differences for many of indices of TDR like Tp-Te interval between groups except QTp, S-Te, S-Tp intervals. Also, S-Te and fQRS showed significant correlation. For prediction of ventricular arrhythmia and cardiovascular death newer indexes on ECG are needed to be established in the future which will make us facilitate to distinguish high risk patients.

Introduction

Hear failure (HF) is a clinical syndrome which shows typical symptoms and signs due to reducing cardiac output and increasing of intracardiac pressures in many circumstances. The prevalence of HF is nearly 1-2% of the general population. The HF is classified into 3 groups according to the measurement of the left ventricular ejection fraction (LEVF). HF with preserved EF (HFpEF): normal or LVEF ≥ 50%; HF with reduced EF (HFrEF): LEVF < 40% and HF with mid-range EF (HFmrEF): LVEF is between 40-49%. HFmrEF depicts a new group of patients with different which is a deserving attraction with different characteristic a treatment features [1], [2]. Mortality rates of cardiac failure for HFrEF, HFmrEF and HFpEF were accounted approximately with 154-115 and 87 deaths per 1000 person-year, respectively [3]. Sudden cardiac arrest or death (SCD) is one of the important cause of mortality in these patients because of reentrant ventricular arrhythmia (VA). This re-entry is being occurred highly due to local dispersion of myocardial repolarisation and this total ventricular dispersion of repolarisation (DVR) facilitates VA and cardiac arrest [4]. Cardiac myocardial transmural dispersion of repolarisation (TDR) or DVR was described in previous reports with three different myocardial cell layers: endocardial, epicardial and mid-myocardial M cells. M cells have

the longest action potential duration with prone to action potential prolongation with external factors. On surface Electrocardiography (ECG), the repolarization of the epicardial layer ends at the peak of T-wave but M cells' repolarization continue until the end of T wave and by measuring the time between the peak and end of the T wave, which is called as Tp-Te interval and reflects TDR [5], [6], [7]. QTc (corrected), Q-Tpeak (QTp), Tpeak-Tend (Tp-Te), and Tp-Te/QT have been defined as predictors of risk for VA or SCD in various clinical scenarios like in HF patients, Brugada syndrome, hypertrophic cardiomyopathy, Long-QT syndrome and bradyarrhythmia or general population [8], [9], [10], [11], [12], [13]. The Tp-Tec interval and Tp-Tec/QT ratio were also found to be more accurate measurements of the TDR or DVR compared to the QT, QTd (QTdispersion), and Tp-Te interval [14]. Different cutoff values for Tp-Te interval have been proposed or found in previous studies [8], [15]. In groups of patients with increased risk of VA, the Tp-Te was often more than 100 millisecond (ms) in various clinical scenarios like acute myocardial infarction and HF [4], [16]. Although meaningful clinical usage of Tp-Te for prospective risk stratification for VA events and mortality in patients with cardiomyopathy has been demonstrated before, further risk stratification within three separate high-risk population with HF would be of clinical value [4]. Fragmented QRS (fQRS) is another risk predictor index on surface ECG for electro-mechanical dyssynchrony, VA, SCD and poor prognosis for patients with HF and hypertrophic cardiomyopathy [17], [18], [19], [20], [21], [22]. The purpose of this study was to assess If there is a distinction of various indices of TDR in three group of patients with HF (HFrEF- HfmrEF- HFpEF) and there is a relationship between fQRS and these indices in patients with HF.

Methods

Study Population

The study consisted of 192 patients who were admitted to our institute with HF between November 2016 and May 2017. After the diagnosis of HF was made according to the previous guideline [1]. Demographic data including age and sex and clinic data of history of diabetes mellitus (DM), hypertension (HT), hyperlipidemia (HPL), coronary artery disease (CAD), baseline rhythm of ECG (atrial fibrillation (AF) or sinus rhythm (SR), as well as laboratory data and used medication and being on a diet were obtained at baseline. Patients were classified to their baseline LVEF measurements as HFrEF (LVEF < 40%), HFmrEF (LVEF: 40-49%) and HFpEF (LVEF > 50%). Patients with prior pacemaker implantation, cancer, or other major illnesses were excluded. Patients with abnormal thyroid function test, abnormal electrolyte values and on antiarrhythmic drug treatment were also excluded.

Approval of Ethics Committee

The study protocol was approved by the Ethics Committee at Afyon Kocatepe University, and informed consent was obtained from each patient.

ECG

All 12-lead ECGs were recorded using a General Electric MAC 5000 (GE Healthcare, Milwaukee, WI, USA) at 25 mm/s with standard lead positions. All records were magnified by 200%, and QT intervals were measured. Automated ECG analysis of the baseline ECG was performed at a central laboratory (GE Healthcare, Wauwatosa, WI, USA) using the commercially available GE Healthcare Marquette 12SL ECG analysis program, which uses validated algorithms for measurement [23]. To eliminate both interobserver variability and bias, all measurements were measured in each of the 12 leads by a single observer who was blinded to all clinical findings. QT intervals were taken to be from the onset of the QRS complex to the end of the T wave. The end of the T wave was defined as the intersection of the tangent to the down slope of the T wave and the isoelectric line when not followed by a U wave or if distinct from the following U wave. If a U wave followed the T wave, the T-wave offset was measured as the nadir between the T and U Waves. The Tp-Te interval was defined as the interval from the peak of the T wave to the end of T wave [24]. Q-Tpeak (QTp) was measured from onset of QRS to peak of T wave, and in the case of negative or biphasic T waves, Q-Tpeak (QTp) was measured to the nadir of the T-wave. The Tp-Te value reported was the average value of obtained in all precordial leads. The Tp-Te/QT ratio was calculated as the ratio of Tp-Te in that lead to the corresponding QT interval.

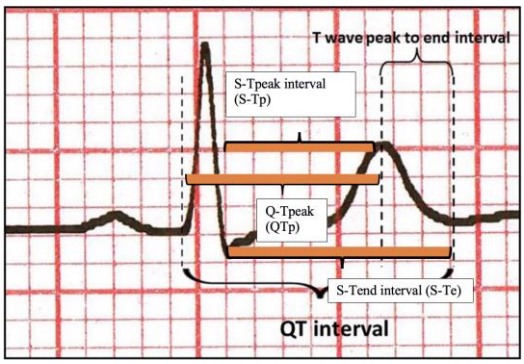

Figure 1: Demonstration of the T wave peak to end and QT intervals [24]

Other novel indexes were described as S-Tend (S-Te) interval and S-Tpeak interval (S-Tp). S-Te and S-Tp were measured from nadir of S wave to

peak of T and end of T wave in precordial limbs (Figure 1). Bazett's formula (n/ RR) was applied to the all the indices to find heart rate corrected form which was shown as 'c' in this text (for example QTc) [25]. The corrected intervals are expressed in the same units as the original parameters, as recommended by Molnar et al., [26].

fQRS included various RSR patterns and was defined by the presence of an additional R wave (R prime), notching in nadir of the S wave, notching of R wave, or the presence of more than one R prime (fragmentation) in two contiguous leads corresponding to a major myocardial segment [23] (Figure 2).

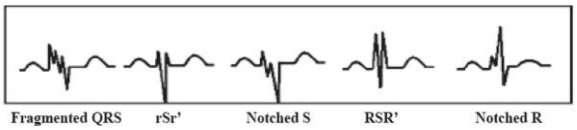

Figure 2: Different types of fragmented QRS (fQRS) [23]

Echocardiography

A Vivid 5 pro echocardiographic unit (GE, USA) with 3,5 MHz probe was used. The echocardiographic study was performed in standard accepted positions which all of the echocardiographic measurements (M-mode, two-dimensional and Doppler echocardiography), were performed and/or reviewed by experienced staff cardiologists, compliant with the recommendation of the American Society of Echocardiography. Mitral inflow was determined by continuous and pulse wave Doppler echocardiography at the tips of the mitral leaflets. Early diastolic mitral peak flow velocity (E), late diastolic mitral peak flow velocity (A), E/A ratio were measured. Left ventricular diastolic dysfunction (LVD-Dys) was defined as a mitral continuous-wave (CW) Doppler E<A as compliant with the recommendation of previous guideline [27], [28].

Statistical analysis

Continuous variables were expressed as mean ± SD (Standard deviation), and categorical variables were presented as frequencies (%, per cent). Continuous and categorical measures were compared with *t*-tests or 2 statistics, as appropriated. For correlations, appropriate calculations were done. *A p* value < 0,05 was accepted as a statistically significant. All analyses were performed using SPSS Version 16.0 (SPSS Inc. Chicago, IL, USA).

Results

Baseline descriptive analysis

The 192 patients were included in our study with 68 women (35.4%) and 124 men (64.6%). Many of baseline features which were borne in Table 1 were similar between groups except pulse rate, left ventricular end-diastolic diameter (LVDD) and left ventricular end-systolic diameter (LVSD) which were higher in first group (for pulse rate *P* = 0.001; for LVDD *P* = 0.002; for LVSD *P* = 0.001, respectively). History of HPL, CAD, AF and SR ratios were similar between groups (for all *P* value > 0.05) however DM was found higher in group 2 (*P* = 0.006), and HT was found higher in group 1 and 2 (*P* = 0.017).

Table 1: Baseline frequency and descriptives analysis of some features of groups

Features	Count	LVEF% < 40 Group 1 n:66 (34.4%)	LVEF% 40-49 Group 2 n:69 (35.9%)	LVEF% >50 Group 3 n:57 (29.7%)	Total n:192 (100%)	$P^{¥}$
Gender						
Women	Count & percent in total	20 (10%)	27(14%)	21 (10%)	68 (35%)	
Male	Count & percent in total	46 (24%)	42 (22%)	36 (19%)	124 (65%)	0.543 *
Age	Mean ± SD	71.5 ± 13.2	68.8 ± 13.3	67.6 ± 13.3	69.4 ± 13.3	0.086 #
	Min: 22 Max: 97				Min: 22 Max: 97	
S-BP (mmHg)	Mean ± SD	127 ± 26.5	132 ± 25.4	135 ± 27.2		0.568 #
	Median (25%-75%)	130 (110-148)	128 (110-150)	130 (120-160)		
D-BP (mmHg)	Mean ± SD	69 ± 14.5	67 ± 14.5	71 ± 12.7		0.574 #
	Median (25%-75%)	70 (60-81)	70(60-80)	70(62-80)		
Pulse rate per minute	Mean ± SD	99 ± 22.7	98 ± 26.4	82 ± 19.2		0.001 #
	Median (25%-75%)	98 (84-114)	94 (79-114)	79 (69-98)		
LVEF %	Mean ± SD	31 ± 5.5	44 ± 2.9	53 ± 2.8		0.001 #
	Min: 18 Max: 58					
	Median (25%-75%)	32 (26-36)	45 (41-47)	53 (51-55)		
NT-ProBNP	Mean±SD	310 ± 116.9	305 ± 120.5	330 ± 135.7		ns #
NYHA 1-2	Count & percent in total	46 (23.9 %)	44 (22.9%)	40 (24.4%)		ns #
NYHA 3-4	Count & percent in total	20 (10.4%)	25 (13.0%)	17 (8.8%)		ns #
LVDD (mm)	Mean ± SD	54 ± 6.8	51 ± 7.9	49 ± 6.1		0.002 #
	Median (25%-75%)	53 (49-60)	51 (46-54.5)	51 (46-54.7)		
LVSD (mm)	Mean ± SD	40 ± 8.1	35 ± 7.7	32 ± 6.5		0.001 #
	Median (25%-75%)	39 (33-47)	35 (29-40)	32 (27-35)		
IVS (mm)	Mean ± SD	10 ± 1.6	10 ± 1.4	10 ± 1.3		
	Median (25%-75%)	10 (10-11)	11 (10-12)	11 (10-11)		0.782 #
DM (missing n = 6)	Count & percent in total	27 (15%)	37 (20%)	16 (8%)	80 (43.2%)	0.006*
HT (missing n = 8)	Count & percent in total	45 (25%)	46 (25%)	29 (16%)	120 (65.6%)	0.017*
HPL (missing n = 16)	Count & percent in total	16 (9%)	21 (12%)	23 (13%)	60 (34.3%)	0.173*
CAD (missing n = 13)	Count & percent in total	39 (22%)	40 (22%)	28 (16%)	107 (60.1%)	0.071*
AF	Count & percent in total	14 (7%)	21 (11%)	11 (6%)	46 (24.1%)	
SR	Count & percent in total	51 (27%)	48 (25%)	46 (24%)	145 (75.9%)	0.291*

*: Chi-Square test. #: Independent samples non-parametric Kruskal-Wallis test. ¥ : P < 0,05 is accepted statistically significant. SD: Standard deviation, mm: millimetre, S-BP: Systolic blood pressure, D-BP: Diastolic Blood pressure, LVEF: Left ventricular ejection fraction, NYHA: New York Heart Association functional classification, LVDD: Left ventricular diastolic diameter, LVSD: Left ventricular systolic diameter, IVS: interventricular septum, DM: Diabetes mellitus, HT: Hypertension, HPL: Hyperlipidemia, CAD: Coronary artery disease, AF: Atrial fibrillation, SR: Sinus rhythm.

As shown in Table 2 there wasn't a significant difference between groups for interventricular septum

(IVS), posterior wall (PW), right atrium (RA), right ventricular (RV) dimensions, P-time, QRS-time and fQRS (all $P > 0.05$). But there were significant differences for left atrium diameter (LA), T wave time and QT time *(*$P = 0.047$*,* $P = 0.003$*,* $P = 0.007$*,* respectively). However, after cross-tabulation between groups the adjusted significance was found ($P > 0.05$) for LA. After then we compared the groups each other for T and QT times we found significant difference only in between group 1 and 3 for T time ($P = 0.002$) and between group 1 versus (vs) 3 and 2 vs 3 ($P = 0.009$ and $P = 0.042$, respectively) for QT time.

Table 2: Baseline frequency and descriptives analysis of groups

Features	Count	LVEF% < 40 Group 1 n:66 (34.4%)	LVEF% 40-49 Group 2 n:69 (35.9%)	LVEF% > 50 Group 3 n:57 (29.7%)	Total n:192 (100%)	$P^{¥}$
PW (mm)	Mean ± SD	9.8 ± 1.4	10.4± 1.2	10.3 ± 1.3	10 ± 1.3	0.332#
LA (mm)	Mean ± SD	45 ± 8.2	41± 8.0	41 ± 9.6	42 ± 8.6	0.047#
RV (mm)	Mean ± SD	31 ± 5.9	29 ± 5.9	27 ± 3.6	29 ± 5.5	0.075#
RA (mm)	Mean ± SD	34 ± 6.7	31 ± 7.4	31 ± 10.1	32 ± 8.0	0.067#
P time (ms)	Mean ± SD	70 ± 41.2	62± 46.1	79 ± 43.5	70 ± 44.0	0.067#
QRS time (ms)	Mean ± SD	91 ± 16.5	90 ± 12.7	88 ± 13.7	90 ± 14.4	0.542#
T time (ms)	Mean ± SD	127 ± 29.0	141 ± 41.7	148 ± 35.0	138 ± 36.6	0.003#
QT time (ms)	Mean ± SD	345 ± 49.9	348 ± 53.8	375 ± 54.6	355 ± 54.0	0.007#
fQRS	Present count & percent in total	37 (19.3%)	40 (20.8%)	28 (14.5%)	105 (55%)	0.566*
	None count & percent in total	28 (14.6%)	29 (15.1%)	29 (15.1%)	86 (45%)	

*: Chi-Square test. #: Independent samples non-parametric Kruskal-Wallis test. ¥ : P < 0,05 is accepted statistically significant. SD: Standard deviation, mm: millimetre, ms: millisecond, PW: Left posterior ventricular wall, LA: Left atrium four-chamber diameter, RV: Right ventricular four-chamber diameter, RA: Right atrium four-chamber diameter, fQRS: Fragmented QRS.

As shown in Table 3 we found the significant difference between groups for only QTp and RR interval measurements (QTp: 271.5 ± 51.0 ms vs 272.4 ± 45.1 ms vs 293.1 ± 53.1 ms; $P = 0.028$ and RR interval; $P = 0.0001$).

Table 4: Independent samples Kruskal-Willis and Pearson Chi-Square test results

Features	Count	LVEF%<40 Group 1 n:66 (34.4%)	LVEF%40-49 Group 2 n:69 (35.9%)	LVEF%>50 Group 3 n:57 (29.7%)	Total n:192 (100%)	$P^{¥}$
S-Tp (ms)	Mean±SD	222.0 ± 43.0	219.7± 42.3	241.3 ± 53.3	227.0 ± 46.8 Min:120 msn Max: 502 msn	0.032
	Median (25%-75%)	220 (193-240)	220 (200-243)	240 (207-260)		
S-Tpc (ms)	Mean ± SD	279.6 ± 42.7	277.6 ± 35.8	276.2 ± 45.5	277.9 ± 41.0 Min:170 msn Max:435 msn	0.631
	Median (25%-75%)	278 (257-308)	275 (251-304)	268 (240-307)		
S-Te (ms)	Mean ± SD	296.0 ± 47.5	296.7 ± 47.8	318.9 ± 56.7	303.1 ± 51.3 Min: 199 msn Max: 582 msn	0.028
	Median (25%-75%)	288 (266-320)	292 (271-320)	320 (280-358)		
S-Tec (ms)	Mean ± SD Min: 18 Max: 58	370.8 ± 48.8	376.4 ± 44.2	365.7 ± 45.4	371.3 ± 46.1 Min: 235 msn Max: 544 msn	0.432
	Median (25%-75%)	372 (342-403)	374 (342-404)	360 (336-399)		

*: $P < 0,05$ is accepted statistically significant. S-Tp: Measurement from nadir S wave to T peak. S-Te: Measurement from nadir S wave to T end. SD: Standard deviation, ms: millisecond, c: Heart rate-corrected form with Bazett's formula (n/ RR).

We found the mean Tp-Te in total population as 76.0 ± 17.5 ms with minimum: 40 ms and maximum: 120 ms and didn't show any difference between groups (Tp-Te: 73.4 ± 15.4 ms vs. 77.5 ± 19.0 ms vs. 77.2 ± 17.9 ms; $P = 0.291$) with all kind of new indexes of TDR as QTpc, Tp-Tec, Tp-Te/QT, Tp-Te/ QTc, Tp-Tec/QTc and PR interval (QTpc: $P = 0.644$*;* Tp-Te > 100 ms: $P > 0.05$; Tp-Tec: $P = 0.213$; Tp-Te/QT: $P = 0.463$; Tp-Te/QTc: $P = 0.253$; Tp-Tec/QTc: $P = 0.367$, PR: $P = 0.547$).

After then again comparing to groups for QTp and RR interval between each other showed there was a significant difference only in between group 1 and 3 for QTp time ($P = 0.049$) and however in group 1 vs 3 and 2 vs 3 ($P = 0.0001$ for both) for RR interval time.

As shown in Table 4 and Table 5 when comparing to groups for new indices we found the significant differences for S-Tp and S-Te measurements (S-Tp: 222.0 ± 43.0 ms vs. 219.7 ± 42.3 ms vs. 241.3 ± 53.3 ms; $P = 0.032$; S-Te: 296.0 ± 47.5 ms vs. 296.7 ± 47.8 ms vs. 318.9 ± 56.7 ms; $P = 0.028$). But S-Tpc ($P = 0.631$) and S-Tec ($P = 0.432$) weren't found to be different between groups.

Again we compared the groups each other for S-Te and S-Tp and found there was a significant difference only in between group 1 and 3 for S-Te time ($P = 0.043$). Other many of the biochemistry features and list of used drugs were not different between groups (for all P value > 0.05), however ratio of used of mineralocorticoid receptor antagonist (MRA) drugs, blood urea nitrogen (BUN), creatinine, glucose and WBC levels were found significantly different between groups (for all P-value < 0.05).

When comparing of incidents of TDR according to having of E/A < 1.0 (LVD-Dys) that we found significant differences between groups for Tp-Te, Tp-Tec, Tp-Te/QT, Tp-Te/QTc and Tp-Tec/QTc ($P = 0.005$, $P = 0.003$, $P = 0.002$, $P = 0.016$, $P = 0.002$, respectively). LVD-Dys was found a significant predictor of many of arrhythmic indices.

Table 5: Comparing to groups with each other for S-Te and S-Tp

Groups	Adj.Sig. $P^{¥}$ for S-Te	Adj.Sig. $P^{¥}$ for S-Tp
Group 1 vs. Group 2	> 0.05	> 0.05
Group 1 vs. Group 3	0.043	0.077
Group 2 vs. Group 3	0.081	0.054

*: $P < 0,05$ is accepted statistically significant.

Table 6 shows the comparing of incidents of TDR according to having fQRS or not on surface ECG. Only S-Te was found significantly different between groups (309.2 ± 47.4 ms versus 295.5 ± 55.0 ms; $P = 0.031$).

This Table showed us fQRS was found relevant only with S-Te however not with Tp-Te and other important indices. Finally, we made correlation analysis and we found significant correlation between S-Te and WBC (r = - 0,171; $P = 0,037$) and S-Tp and WBC (r = - 0,170; $P = 0,038$) and between S-Te and fQRS (r = 0,158; $P = 0,031$).

Table 6: Differences of incidents of TDR according to having fQRS or not on surface ECG showed only S-Te was significantly different between groups

Variable of indices	Count	fQRS Present n: 105 (55%)	fQRS None n: 86 (45%)	P**
QTc (ms)	Mean ± SD	439.5 ± 37.5	442.0 ± 41.8	0.544
QTp (ms)	Mean ± SD	282.7 ± 43.8	272.8 ± 57.1	0.197
QTpc (ms)	Mean ± SD	343.6 ± 44.9	342.2 ± 47.3	0.780
Tp-Te (ms)	Mean ± SD	78.0 ± 17.6	73.6 ± 17.2	0.098
Tp-Tec (ms)	Mean ± SD	95.1 ± 24.0	92.3 ± 24.1	0.339
Tp-Te/QT	Mean ± SD	0.216 ± 0.05	0.214 ± 0.05	0.481
Tp-Te/QTc	Mean±SD	0.182 ± 0.07	0.170 ± 0.04	0.256
Tp-Tec/ QTc	Mean±SD	0.214 ± 0.05	0.215 ± 0.05	0.720
S-Tp (ms)	Mean±SD	230.7 ± 43.5	222.3 ± 50.4	0.157
S-Tpc (ms)	Mean±SD	279.6 ± 39.8	275.7 ± 42.6	0.415
S-Te (ms)	Mean±SD	309.2 ± 47.4	295.5 ± 55.0	0.031
S-Tec (ms)	Mean±SD	374.0 ± 44.2	368.0 ± 48.5	0.250

*: Non-parametric Mann-Whitney U test. **: Chi-Square Test. *: $P < 0,05$ is accepted statistically significant. SD: Standard deviation, ms: millisecond, c: Heart rate-corrected form with Bazett's formula (n/ RR). fQRS: Fragmented QRS.

Discussion

Repolarisation parameters on ECG

In an earlier report by Sicouri and Antzelevitch identified distinct functional four type ventricular cells in a canine model, endocardial, M cells (in deep subepicardium layer), epicardial and Purkinje fibres. They found that action-potential duration-rate relation in which of cells in the M region relative to cells in neighbouring tissues is such that a prominent dispersion of repolarisation and refractoriness develops that area when stimulation rate is slowed. Intramural renentry during ischemia and bradycardia-induced could be facilitated by that midmyocardial reentry which occurs by delays of activation [7]. Yan et al., found that repolarization of the M cell was at the nearly same time with the end of the T wave, whereas repolarization of the epicardial cells was at the same time with the peak of the T wave in canine ventricle model so that the interval between the peak and the end of the T wave (Tp-Te) depicts the TDR (difference in repolarization times between epicardium and the M region). The Action-potential duration (APD) of endocardial cells was usually intermediate. Ascending part of T wave is drawn by voltage gradient difference between M cell-epicardial cell and descending part is drawn by difference between endocardial cell-M cell. When the T wave is upright, the epicardial response is the earliest to repolarise and the M cell action potential is the last. It concluded that the duration of the M cell action potential determines the QT interval, whereas the duration of the epicardial action potential determines the Q-Tpeak (QTp) interval. QT dispersion is used a parameter to determine ventricular arrhythmia risk. Also measuring Tp-Te interval depicts the TDR. Transmembrane action potentials (APs) recorded from the right ventricle are usually longer than those from the left, and APs from the apical regions are generally longer than the base region. These apico-basal repolarisation gradients have been proposed to determine the electrocardiographic T wave [5], [6], [12], [29]. According to these studies, the Tp-Te interval in precordial ECG leads was suggested to depicts the index of TDR. More recent studies have also provided to help estimation of TDR in more complex T waves, including negative, biphasic and triphasic T waves [30].

Clinical Implications of Repolarization Indices

Patients with non-heart failure

Conlon et al., found mean Tp-Te and Tp-Te/QT ratio significantly were prolonged in patients with coronary artery ectasia comparing to control group (Tp-Te: 95.5 ± 9.01 ms vs. 84 ± 5.62 ms and Tp-Te/QT: 0.22 ± 0.02 vs. 0.20 ± 0.01, $P < 0.05$ for all) [31]. Tenekecioglu et al., found mean Tp-Te, Tp-Te/QT and Tp-Te/QTc ratio were significantly higher in patients with coronary slow flow phenomenon (Tp-Te: 85 ± 13.7 ms vs. 74 ± 9.9 ms and Tp-Te/QT: 0.24 ± 0.03 vs. 0.20 ± 0.02 and Tp-Te/QTc: 0.20 ± 0.03 vs 0.17 ± 0.02 all of *P*-value < 0.001) [32]. Can Yontar et al., demonstrated mean Tp-Te, Tp-Te/QT ratio, Tp-Te/QTc ratio were higher in patients with mitral valve prolapse comparing to normal healthy patients (Tp-Te: 100.2 ± 22.1 ms vs. 74.6 ± 10.2 ms; Tp-Te/QT: 0.24 ± 0.0 vs. 0.20 ± 0.0; all *P*-value < 0.001) [24]. Castro Hevia et al. found Tp-Te interval is a suitable risk predictor for VA in patients with Brugada syndrome (BS). Most of these arrhythmia recurrences were in patients maximum QTc > 460 ms, and an average value of Tp-Te > 100 ms. The Tp-Te and Tp-Te dispersion were significantly longer in patients experiencing a recurrence compared with those who did not (104.4 and 35.6 ms vs 87.4 and 23.2 ms; $P = 0.006$ and $P = 0.03$; respectively) [10]. These results were congruent with another trial with BS. Tp-Te duration in lead V1 (87 ± 30 ms vs. 71 ± 21 ms; $P = 0.017$) was significantly longer and TpTe/QT ratio (0.24 vs. 0.19; $P = 0.019$) was significantly larger in patients with VA. They found a cutoff value of Tp-Te ≥ 77 ms and Tp-Te/QT ratio of ≥ 0.205 for predicting cardiac events with a good sensitivity and specificity level [33]. In hypertrophic cardiomyopathy patients with VA events, mean Tp-Te interval and Tp-Te/QTc ratio were longer than without events and control group (Tp-Te: 82.6 ± 9.8 ms vs. 74.6 ± 9.3 ms; Tp-Te/QTc: 0.202 ± 0 vs.0.181 ± 0; $P < 0.001$ for all) [34]. Another trial which included acute ST-elevation myocardial patients with coronary interventional therapy showed pre-coronary intervention (pre-CI) Tp-Te was prolonged in patients that died during follow-up. The optimal cutoff point was determined to be 100 ms for the pre-CI Tp-Te [16]. In acquired bradycardic patients The QT interval, QTc interval, and Tp-Te interval were closely related to the risk of Torsade de Pointes (TdP). The best single discriminator for TdP

was the Tp-Te. Also, having a Tp-Te ≥ 85 ms with Long-QT2-like morphology almost were proposed to predict the occurrence of TdP [13].

Patients with heart failure

An investigation by Morin *et al*. found increased Tp-Te was associated with 14% increase in risk for VA (P = 0.04), and Tp-Tec was found to be more powerful predictor (P < 0.01) in 327 HFrEF patients. Increasing of Tp-Tec was associated with a 19% increase in the risk of death (P < 0.01). The cutoff point of Tp-Te was 103.5 ms for VA and 126.7 ms for all-cause mortality in 2 years [4]. Lellouche et al. found baseline QTc dispersion and Tp–Te dispersion was significantly higher in patients with ICD (intracardiac defibrillator) therapy after a 1-year follow-up (P = 0.08). After multivariate analysis postimplantation Tp–Te was the only independent predictor of ICD therapy (P = 0.02). A cutoff point of Tp–Te: 110 ms level had specificity 74% and sensitivity 77% in predicting ICD therapy [35]. Evaluation of 101 consecutive patients with HF by Xue et al., after CRT-D (cardiac resynchronisation therapy-intracardiac defibrillator) therapy Tp-Te was shortened (107 ± 23 ms at baseline to 94 ± 24 ms at the 1-year follow-up). Shortened Tp-Te group experienced lower VA episodes, compared to non-shortened Tp-Te (12% vs. 39%, P = 0.002) [36].

In general population risk stratification

Panikkath et al., showed Tp-Te, QTc, QRS dispersion and Tp-Te/QT ratio were significantly prolonged in SCD cases compared to control (Tp-Te: 89.4 ms vs 76.1 ms; Tp-Te/ QT: 0.22 vs 0.19; P < 0.05 for all) [8]. Tp-Te was proved to be a good risk predictor for VA in various cardiac diseases including HF patients. None of these trials didn't compare heart failure patients according to their LVEF which was our prime aim.

A clinical and echocardiographic feature of our study population

Newly classification of HF patients in three groups attracted attention on new HF class which named HFmrEF [1]. HFmrEF has different features comparing to HFpEF and HFrEF [2]. Our main aim was to find interesting results from this new group for indexes of TDR and fQRS. We found our HFmrEF patients have many features somewhat different from HFpEF but nearly the same characteristics with HFrEF. Previous reports stated HFmrEF tends to have a higher rate of HT, DM and CAD and increased LV diastolic stiffness compared to HFpEF [3]. DM and HT were seen more in HFmrEF and HFrEF than HFpEF (P < 0.05). But CAD was seen the same in three groups (P > 0.05). LV-Dys was found same in groups (P > 0.005). Patients with HFrEF had higher creatinine level than others (P < 0.0001).

Although we determined the cutoff level of Tp-Te > 100 ms as a higher, but our examination found only 11.5% of patients had Tp-Te value higher than cutoff level. Mean Tp-Te was found 76 ms in our patients which was lower than the accepted cutoff levels in other reports including HF patients even reference predictive level for SCD for the general population [4], [8], [35], [36]. But Tp-Tec levels were found higher than some previous cutoff levels [13], [33]. Other important indices like QT, QTc, Tp-Te/QT, Tp-Te/QTc, Tp-Tec/QTc didn't show any significant difference between groups. When looking into results more precisely in tables, our mean Tp-Te/ QT and Tp-Te/ QTc values in all groups were found higher than important cutoff levels in some trials [8], [24], [31], [32], [34]. But after careful examination, some indices were found meaningful higher than accepted cutoff points according to some trials, mean Tp-Te/QT level in our HFmrEF patients was higher than patients with BS. [33] However after an investigation of newer indices in this study which was including QTp, S-Tp, S-Te as well as their heart rate corrected forms QTpc, S-Tpc, S-Tec showed only QTp, S-Tp and S-Te were significantly different between groups (All for P-value < 0.05, in Table 3-4). For QTp HFrEF and HFpEF showed the significant difference but HFmrEF was same with HFrEF and HFpEF. For S-Te again HFrEF and HFpEF showed the significant difference, but HFmrEF was same with HFrEF and HFpEF. However for S-Tp although HFmrEF wasn't different from HFrEF but different from HFpEF and again HFpEF was different from HFrEF. Which is the useful distinct index between these three groups, S-Tp or S-Te or QTp? Exactly what is the value of these new indexes in prediction for VA or serious events in HF patients, we don't know now. Patients with HFpEF had more prolonged QTp, S-Te, and S-Tp than others which may imply more protected LVEF shows some different depolarisation-repolarisation features than HFmr and HFrEF. We need to investigate these indexes in a prospective study with more patients to find true answers. Another finding of our study was about the relationship between fQRS and indexes of TDR showed there was a significant relationship between fQRS and S-Te with important correlation (P = 0.031). Again important indexes were found relevant, but we need more studies on how we can use them in real life for prognosis of patients. And finally, patients with LVD-days had a significant relationship with prolonged Tp-Te and some other indices (in table 7) which may suggest that this kind of indices can use to show stages progressive ventricular disease with other subtle traces like diastolic dysfunction.

Limitations

Some important limitations of this study should be mentioned. This study was a cross-sectional study to find differences of TDR on ECG. In

conjunction with different level of LVEF, some of the previously reported indexes of TDR were supposed to be different, but we could not find. But we could find different newer indices of TDR. Maybe low number of patients with rather missing data for some measurement which could affect to find real differences of these indexes of TDR we showed some interesting data for TDR indices in three different HF groups and finally we can say there are a needed more trials with more patients to establish and evaluate difference or relationship among these indices deeply.

In conclusion, although in our study mean Tp-Te interval levels were lower than other reports and didn't show any differences between three different HF groups. QTp, S-Te, S-Tp intervals were found to be different between the HF groups. S-Te and fQRS showed a correlation. For prediction of VA and cardiovascular death newer indexes on ECG are needed to be established in the future which will make us facilitate to distinguish high risk patients. Maybe Pathological and electrophysiological feature of TDR must be evaluated in the future.

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Hospital-Based Study of Maternal, Perinatal and Neonatal Outcomes in Adolescent Pregnancy Compared to Adult Women Pregnancy

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Abstract

BACKGROUND: Adolescent pregnancy, defined as a pregnancy in girls aged 10 to 19 years. Adolescent mothers are at high risk for maternal and neonatal complications.

AIM: To compare maternal, perinatal and neonatal outcomes in adolescents and adult women aged 20-24 years.

MATERIAL AND METHODS: This retrospective cohort study included all singleton pregnancies during a three-year period (January 2016-December 2018) who gave birth in a Clinical Hospital in Tetovo, Republic of Macedonia. After exclusion criteria, a total of 932 cases were reviewed and divided into two groups: one of the teenage mothers (< 19 years old) (115 women) and the other of adult mothers (20-24 years old) (817 women).

RESULTS: Of the total number of 5643 births, 128 (2.27%) were from adolescent pregnancies. Of them, nulliparous adolescent women were 115 (2.04%). Adolescents compare to adult mothers had a higher rate of urinary tract infections (33% vs. 22%), increased rate of maternal anemia (26% vs. 15%), preterm birth, small for gestational age newborns (25.2% vs. 17.1%), lower high school attendance (0 vs. 21.9%) and inadequate prenatal care. Spontaneous labour was more common in adolescents (73% vs 63.5%), while Caesarean sections were less common than in women aged 20-24 years (25.2% vs 33.5%). The rate of other perinatal outcomes was not significantly different between the 2 groups.

CONCLUSIONS: The results of the study showed that the frequencies of some maternal, perinatal and neonatal complications were considerably higher in adolescent mothers.

Introduction

Adolescent pregnancy has been defined as a pregnancy in women aged between 13-19 years [1] and as a social problem distributed worldwide, has serious implications on maternal and child health, especially in the developing countries [2]. More than 16 million babies (11% of all births globally) are born to adolescent girls [3]. Globally, adolescent birth rates for every 1000 births in the year 2016 were 0.045 in the World, 0.047 in Arab World 0.038 in the Middle East & North Africa, 0.022 in OECD members, 0.020 in North America, 0.019 in Asia, 0.010 in European Union. Among all countries of the world, the highest rate (206/1000) belongs to Niger. The countries with the lowest birth rates among adolescents in the range of age 15-19 are Slovenia and Denmark (0.004), Hong Kong and Switzerland (0.003), South Korea (0.002). In our country, the adolescent birth rate was 0.017 in the year 2016 [4].

Teenage mothers are at high risk of maternal and neonatal complications include maternal anemia, hypertensive disease in pregnancy, preterm birth, urinary tract infection [5], postpartum hemorrhage, eclampsia and cephalopelvic disproportion, as well as adverse infant outcomes including preterm birth, poor fetal growth, low birth weight, neonatal mortality [6], respiratory diseases and birth trauma, besides a higher frequency of neonatal complications and infant mortality [7]. Although adolescent pregnancies, especially unintended pregnancies, might carry a greater risk of adverse consequences in developing countries with limited health resources and restrictive abortion laws, pregnancy and childbirth among young women in developed countries can also pose challenges to their social, economic and physical well-being. Studies on complications in teenage pregnancy

have yielded conflicting results, and opinions of different authors vary in this regard [8], [9]. Given the characteristics of adolescence, pregnancy during the period is different from other age groups and creates different feelings in women. Pregnancy during adolescence is considered a social issue associated with medical, emotional and social outcomes for the mother, child and family [11]. Early marriage, in some traditional rural communities, low educational level, low level of sexual education and contraceptive use, high rate of poverty are important factors in the rate of adolescent pregnancy. Adolescent mothers are more likely to have poor prenatal health behaviours and poorer health status [12]. In these group of women, pregnancy and delivery are not only associated with adverse pregnancy outcomes, but also associated with low school achievement, increased health care costs, and living in poverty [13].

This study aimed to determine maternal, perinatal and neonatal outcomes in nulliparous singleton adolescent pregnancies compare to nulliparous singleton adult pregnancies aged 20-24 years.

Material and Methods

This is a retrospective comparative hospital-based cohort study of all singleton pregnancies and deliveries that occurred in a teenage group (< 19 years old) compared with an adult group (aged 20-24 years) at Clinical Hospital in Tetovo, Republic of Macedonia, which is the regional secondary referral center for the region of north-western region of the country. Data were collected from the hospital's electronic database in the period between January 2016 and December 2018. Inclusion criteria included maternal age of 15 to 24 years, primigravida, gestational age more than 22 weeks. Because most of the adolescents (85%) were nulliparous women [14], we limited the analysis to nulliparous women. Age between 20 and 24 years as a control group, was considered since this age-group is generally regarded as safe for childbirth. Exclusion criteria were second or more pregnancy, multiple pregnancies, chronic diseases, diabetes mellitus, any known systemic disorders and gestational age less than 21 weeks. The total number of subjects was 932 women, and we divided these mothers into a teenage mother group (115) and a non-teenage mother group (817).

Study parameters

The choice of study parameters was based on previous literature and clinical relevance. Maternal parameters reviewed were maternal demographic characteristics, antenatal complications and mode of delivery. Maternal age was defined as the completed age of the mother at the time of delivery and was further categorised into 2 groups: adolescence (< 19.9 years) and adult (between 20-24.9 years of age). The adult classification was considered to be the control group. We used the World Health Organization's definition of adolescent pregnancy that is a pregnancy in a woman aged 10-19 years [15]. The area of residence at the time of delivery was divided into an urban and rural area. The maternal educational level was classified as primary (class 1-9), secondary school (class 9-13) and high-level school. A number of prenatal visits was categorised as less than four or four and more.

Mode of delivery was vaginal delivery, instrumental vaginal delivery (vacuum extraction or forceps) and caesarean section. Cervical laceration, perineal tear, postpartum haemorrhage, and uterine curettage were delivery outcomes which also were studied.

Antenatal complications included urinary tract infection (asymptomatic bacteriuria, acute cystitis or pyelonephritis) [16], anemia (hemoglobin concentracion < 11 g/dL) [17], preterm rupture of membrane (PROM) [18], gestational hypertension (GH, blood pressure > 140/90 mmHg in women with proteinuria < 0.3 g/24h urine collection), pre-eclampsia (PE, blood pressure > 140/90 mmHg and proteinuria > 0.30 g/24h urine collections in women) [19] third trimester bleeding including placenta previa and placental abruption [20].

Perinatal outcomes included preterm delivery (< 37 completed weeks) [21], intrauterine fetal death (delivery of a dead infant after 22 week' gestation) [22], low Apgar score (A/S) at 1st minute < 7 and admission to neonatal intensive care unit (NICU), birth weight adjusted for gestational age according to the previously published curves standardized for gestational age [23] (divided into small-for-gestational-age (SGA, defined as < -2 SD), average-for-gestational-age (AGA) and large-for-gestational-age (LGA, defined as > +2 SD). Gestational age at birth was calculated as a number of weeks from the first day of the last menstrual cycle until the delivery date. Descriptive analyses were carried out by calculating the numbers and percentages for categorical variables and calculating mean and standard deviation for continuous variables.

Bivariate analyses for the association between maternal age and development of maternal, fetal and neonatal complications were carried out. P-values were calculated using Pearson's chi-squared test or the Student's t-test as appropriate. Odds ratios and 95% confidence intervals were calculated for categorical variables and categorized continuous variables. P-value < 0.05 was considered to be statistically significant.

Results

During the study period, in our hospital were born 5643 infants. Overall 128 (2.27%) infants were born to adolescents aged 19 years and younger. Of the total deliveries, only singleton pregnancies were included in this study. In the beginning, 5491 adult pregnancies were recruited for the control group. After the restriction of analyses to singleton primiparous women, 817 adult pregnancies aged 20-24 were analysed as a control group. The singleton pregnancies were divided into two groups: pregnant nulliparous teenage women (n = 115, 2.04%) and as the control group, nulliparous pregnant women aged 20-24 (n = 817, 14.54%).

The age of the study group patients ranged from 15 to 19 years, with a mean age of 18.02 ± 0.98 years. Women aged 17-19 years represented 92% of the total number, while only 8% of women were aged between 15-16 years. The age of the control group ranged from 20 to 24 years, with a mean age of 22.42 ± 1.54. Maternal demographic characteristics are shown in Table 1. All the demographic characteristics of the teenagers versus the adult women differed significantly, except for the level of secondary school. Pregnant teenagers were more live in a rural area (77.4% vs. 61.9%, p = 0.0001, OR = 2.1, 95% CI = 1.33-3.33), were more likely to be Roma ethnicity (39.1% vs. 88.8%, p = 0.0001, OR = 6.85, 95% CI = 4.37-10.71) and have low educational level (63.5% vs.31.9%, p = 0.0001, OR = 3.70, 95% CI = 2.46-5.56).

Table 1: The demographic characteristics of women in the two age groups

	Maternal age (years)		p-value	Odd ratio	95% CI
Characteristics	< 19 (n. 115)	20-24 (n. 817)			
Maternal age	18.02 ± 0.98	22.42 ± 1.54			
Educational level n (%)					
Primary school	73 (63.5)	261 (31.95)	0.00001	3.70	2.46-5.56
Secondary school	42 (36.5)	377 (46.15)	0.0572	0.67	0.44-1.00
High school	0	179 (21.90)	0.00001	0.03	0.00-0.22
Ethnicity n (%)					
Macedonian	2 (1.73)	123 (15.05)	0.00001	0.09	0.02-0.40
Albanian	66 (57.39)	597 (73.07)	0.0009	0.5	0.33-0.74
Roma	45 (39.13)	72 (88.81)	0.00001	6.85	4.37-10.71
Others	2 (1.73)	25 (3.05)	0.5643	0.52	0.12-2.21
Area of residence n (%)					
Rural	89 (77.40)	506 (61.93)	0.0001	2.1	1.33-3.33
Urban	26 (22.60)	311 (38.06)	0.0012	0.47	0.30-0.75

Complications during pregnancy. Registered obstetrical characteristic and comorbidities are presented in Table 2. The association between the age-group of mothers and number of antenatal visits and folic acid intake was significant (p < 0.01). When compared with adult mothers, the proportion of anemia (26.0% vs.15.1%) and urinary tract infection (33% vs. 22%) were significantly higher in teenage mothers (p = 0.0042, OR = 1.97, 95% CI = 1.24-3.11and p = 0.013, OR = 1.74, 95% CI = 1.14-2.66 respectively).

There were no significant differences between the teenage and adult group in GH (3.47% vs. 7.71%), pre-eclampsio (0.86% vs. 1.10%), placenta previa (1.73% vs. 1.71%), placental abruption (1.73% vs.1.22%), PROM (22.6% vs. 31.9%), IUFD (0.86% vs.1.46%), preterm birth (10.4% vs. 16.9%), (p = 0.12, p = 1, p = 1, p = 0.65, p = 0.49, p = 1, p = 0.07, respectively).

Table 2: The characteristics of pregnancy in the two age groups

	Maternal age (years)		p value	Odd ratio	95% CI
Characteristics n (%)	<19 (n.115)	20-24 (n.817)			
Antenatal visits					
< 4 times	67 (58.26)	148 (18.11)	0.0001	6.30	4.18-9.51
> 4 times	48 (41.73)	669 (81.88)	0.0001	0.15	0.10-2.23
Folic acid intake	82 (71.30)	767 (93.88)	0.0018	0.16	0.09-0.26
Anemia	30 (26.08)	124 (15.17)	0.0042	1.97	1.24-3.11
GH	4 (3.47)	63 (7.71)	0.12	0.43	0.15-1.20
Pre-clampsia	1(0.86)	9 (1.10)	1	0.78	0.09-6.27
Urinary tract infection	38 (33.04)	180 (28.03)	0.0132	1.74	1.14-2.66
Placenta previa	2 (1.73)	14 (1.71)	1	1.01	0.22-4.52
Placental abruption	2 (1.73)	10 (1.22)	0.6531	1.42	0.30-6.60
PROM	26 (22.60)	261 (31.94)	0.49	0.62	0.39-0.98
IUFD	1 (0.86)	12 (1.46)	1	0.58	0.07-4.56

PROM-Preterm rupture of membranes, IUFD-Intrauterine fetal death, GH-gestational hypertension.

Delivery characteristics. The outcomes of deliveries are presented in Table 3. The teenage mothers had significantly higher proportion (73% vs. 63.5%), of normal vaginal delivery compared to the adult mothers, (p = 0.048, OR = 0.64, 95% CI = 0.41-0.99). The association between the age of mothers and operative and instrumental mode of delivery was non-significant (p = 0.08 and p = 0.76 respectively).

Perineal ruptures of 1st and 2nd degrees and cervical lacerations were in significantly higher proportion in adult mothers compared to teenage mothers (p = 0.049, OR = 0.06, 95% CI = 0.01-0.49). Postpartum blood transfusion and instrumental revision of uterus were non-significant different between adolescent and adult mothers.

Table 3: Association between outcomes of deliveries and maternal age

	Maternal age (years)		p value	Odd ratio	95% CI
Variables n. (%)	<19	20-24			
Vaginal delivery	84 (73.04)	519 (63.52)	0.048	0.64	0.41-0.99
Caesarean Sectio	29 (25.21)	274 (33.53)	0.085	0.66	0.42-1.04
Vacuum extraction	2 (1.73)	24 (2.93)	0.760	0.58	0.13-2.50
Blood transfusion	11 (9.56)	92 (11.26)	0.750	0.83	0.43-1.60
Perineal tear	0	42 (7.73)	0.049	0.06	0.01-0.49
Complications of third stage of labor	4 (4.65)	50 (9.20)	0.39	0.56	0.19-1.58

The fetal outcome. The teenage mothers had a significant higher proportion (25.1%) of SGA deliveries compared to the adult mothers (17.1%), (p = 0.039, OR = 1.63, 95% CI = 1.03-2.57). It was less common for newborns of adolescents to have low Apgar score < 7 at 1st minute (3.47% vs. 13.21 %) compared to adult mothers, (p = 0.001, OR = 0.23, 95% CI = 0.08-0.65).

There was no statistically significant difference in AGA and LGA newborns, preterm birth and admission to NICU between two age groups (Table 4).

Table 4: The newborn status between two age groups

	Maternal age (years)		p value	Odd ratio	95% CI
Status of the newborn, n. (%)	< 19	20-24			
AGA	78 (67.82)	601 (73.56)	0.2178	0.75	0.49-1.54
LGA	8 (6.95)	76 (9.30)	0.4894	0.72	0.34-1.55
SGA	29 (25.21)	140 (17.13)	0.039	1.63	1.03-2.57
Preterm birth (<37 weeks)	12 (10.43)	138 (16.89)	0.0794	2.01	1.25-3.23
A/S <7	4 (3.47)	108 (13.21)	0.0012	0.23	0.08-0.65
NICU	8 (6.95)	96 (11.75)	0.15	0.56	0.26-1.18

AGA-Average for gestational age, LGA-Large for gestational age, SGA-Small for gestational age, A/S-Apgar score, NICU-Neonatal intensive care unit.

Discussion

In this study, we investigated the correlation between maternal age and the risk of adverse maternal, perinatal and neonatal outcomes in our hospital. 2.40% of births occurred to women younger than 19.9 years old. 2.04% of teenage mothers were with the first pregnancy. This proportion is higher compared to the national level of 1.7% of all pregnancies [4]. The results demonstrated that our population had a high teenage birth rate, similar to most of the studies from developed and developing countries. UNFPA reported the similar results of the adolescent birth rate per 1000 women aged 15 to19 in other countries, like a Serbia 15, Albania 20, Hungary 23, Slovakia 24 birth rate per 1000 women [25]. Low levels of literacy adversely affect reproductive, sexual health and quality of life. An early start of childbearing greatly reduces the educational and employment opportunities of women and is associated with higher levels of fertility [2]. In our study, the educational level is significantly higher among the adult mothers compared to teenage mothers. Younger maternal age is associated with being unmarried, primiparous and under-educated, heavy smoking and inadequate prenatal care, which may cause adverse pregnancy outcomes as reported in Taiwanese national survey and a study from Slovenia [27], [28]. Our results showed that pregnant teenagers were more live in rural area and the adolescent birth rate is significantly higher among Roma minority compared to the other ethnicities. The high adolescent birth rates among Roma populations are linked to the practice of child marriage which remains prevalent in Roma communities. Roma girls often have no choice but to follow tradition, leave school and get married in young age, thus perpetuating a cycle of lack of education, poverty and early childbirth [29], [30].

The results of the study showed that adolescent pregnancy is related to poorer prenatal care compared to adult women. This finding is supported by other studies in Tertiary Centers in Slovenia, Greece and Turkey [28], [31], [32]. In addition to the fact that teenage pregnancy is more likely to occur in a socially deprived society, social factors themselves can also affect the adequacy of prenatal care among teenagers. Because of economic and social barriers, teenage mothers are less likely to attend prenatal care clinics, which can affect maternal and neonatal outcomes. This conclusion is supported by the study for evaluating the social determinants of teenage pregnancy in the United Kingdom and Korea [6], [33].

Consumption of prophylactic folic acid tablets was significantly lower among teenage mothers. These results are consistent with those reported by other research studies in Turkey and the United States. Kirbas et al., (2016) reported a lower prevalence of both preconception and prenatal folic acid supplementation in adolescents compared with healthy pregnant women aged 20-34 years [34]. Branum et al., (2013) demonstrated a 2.5 times lower prevalence of supplement use among pregnant women aged < 25 years compared with older women [35]. However, two other studies researched in North Mexico and Brasil did not reveal a significant difference in folic acid intake between pregnant adolescents and adults [9], [36]. Consistent with other studies, the results showed significantly higher risks of maternal anaemia and urinary tract infections in adolescent mothers. This high proportion of anaemia may be attributed to the fact that teenage pregnant women are usually uneducated and are likely to come from relatively low-income families, so they do not appreciate the importance of regular antenatal care, blood tests for anaemia and taking iron supplements during pregnancy to prevent and treat anaemia. Studied from Slovenia, Oman and India reported for high rate of anemia in adolescent women ($p = 0.012$, 0.005, 0.001, respectively) [28], [37], [38]. In the present study a high prevalence of urinary tract infections in pregnant adolescents (33% vs 22% in adults), also is reported in other studies from Finland, Turkey, Romania and North-West Russia (OR 2.5, 0.72, 1.10, 1.17 respectively) [23], [32], [39], [40].

In contrast no excess risk was found in a Latin American study in which teenagers were analysed in sub-groups by age [41]. Researchers speculate that teenagers might be sexually more active during pregnancy and have reduced resistance to infections compared with older women. This reason placed them at a higher risk of urinary tract infections [23].

Previous studies carried out in industrialized countries, like a Korea, Canada, Slovenia and Iran have revealed no excess risks of preeclampsia among adolescents [6], [10], [28], [42], whereas higher risks have been reported in developing countries, like a Turkey and Zambia [43], [44]. Our results do not support earlier findings of a higher risk of gestational hypertension and preeclampsia in teenage mother's compared to adults [45]. The relatively small number of pregnant mothers aged 15-16 years in our study places some uncertainty on this finding. In our study pregnancy comorbidities placenta praevia, placental abruption, PROM, IUFD, occurred similarly across age groups. These results for placenta praevia and placental abruption support the findings of most authors [10], [32], [46] in the previous studies. More

frequent preterm birth in adolescents was also found in other studies. Mayo et al., (2017) reported for the highest prevalence of preterm birth among the youngest (13-year-olds, 14.5%) and lowest among the oldest (20-year-olds, 6.7%) mothers. Keskinoglu et al., (2007) reported for the rates of preterm birth and low birthweight of teen mothers were 18.2% and 12.1%, respectively [47], [48]. In the Slovenian study from Korencan et al., (2017) have reported that young mothers aged < 19 years had increased rates of preterm birth compared to 20-24-year-olds (7.9% vs.5.4%) [28]. The mechanisms responsible for preterm birth are still unclear: the immaturity of cervical blood supply in young mothers stimulates prostaglandin production that could lead to preterm birth [28]. The other explanation of preterm delivery for adolescents is that short cervix (< 25mm) and small uterine volume may also be more common among younger mothers [32]. Furthermore, competition between the fetus and adolescent mother for nutrition, and relative nutritional deprivation of both the fetus and adolescent mother may explain the risk of maternal anaemia, low birth weight, and preterm delivery [49]. We did not find significant difference in the prevalence of PROM between adolescents and adult women. The findings of our study are not consistent with those of previous studies from Fleming et al., (2013) and Pergialiotis et al., (2015) who reported for significantly higher incidence rates of PROM ($p < 0.001$, RR 1.16 respectively) in teenage mothers compared to adult mothers [10], [31].

The rate of cesarean section was higher in adult women compared with teenagers. These findings are supported by others [28,48]. Fleming N et al., (2013) and Ganchimeg et al., (2013) also reported for lower rate of cesarean delivery in adolescents compared to adult women (OR 0.57 and OR 0.75, respectively) [10], [50] who attributed this to the presence of more functional myometrium, greater connective tissue elasticity, and lower cervical compliance that allowed for more spontaneous vaginal deliveries in teenage women [34], [38]. In our study, adolescent mothers were significantly more likely to have a vaginal delivery, as reported in any studies from Brasil (65%) [51] and from Germany where younger maternal age was associated with a higher chance of spontaneous delivery (OR 2.07 95% CI 1.45-2.93) [52]. Concerning vaginal operative delivery, our findings suggest a lower risk in women < 19 years of age compared with those between 20 and 24 years of age. A lower risk for instrumental delivery in adolescents has been cited by Torvie et al., (2015) (RR, 0.87; 95% CI, 0.78-0.97) compared to adults and Usta et al. (2008) reported that vacuum was used more frequently in multiparous controls (0.2 vs 2.7%, $p = 0.011$) [53], [54], but other authors found a contradictory result. Shah et al., (2011) presented that the teenagers had instrumental deliveries more often than non-teenagers (7.1% vs. 2.2%, $p < 0.01$) [55]. The teenagers were also less prone to perineal damage, and cervical laceration compare to adult mothers. There are conflicting data regarding the risk of major perineal lacerations in adolescents [13], [28]. A previous study of 325 women aged 16-19.9 found an increased risk of any perineal laceration compared to women aged 20-24.9 (4.53% vs 2.80%), OR 0.82 (95% CI, 0.71-0.95) [13]. The incidence of uterine curettage after childbirth was lower among teenagers, but differences were non-significant. Some authors reported for the higher incidence in the teenage group [32], but others did not find a significant difference between both groups [6], [55].

Regarding neonatal outcomes, this study confirmed the higher risk of SGA among infants of adolescent mothers, as found in the most previous studies [23], [39] and more commonly birth of LGA and AGA among infants of adult mothers. A previous study of 3891 women aged < 19,9 found higher rate of SGA infants compared to 9479 women aged 20-24 (13.77% vs. 10.40%, $p < 0.001$) [39]. The results are corresponding with previous results of other authors [32], [56]. Tyrberg (2013) et al., found that the rate of SGA infants is higher in adolescents compared to adult women (3.5% vs.2.3%) while the rate of LGA infants is higher in adult mothers (3.2% vs 1.8%) [56]. Other authors did not find a significant difference between both groups [57].

Previous studies have not found an increased risk of admission to a NICU [23], [32] in infants born to adolescent women compared to adult women's babies. In our study, we have found a decreased risk of the need for neonatal transfer in adolescents compared to adults. Other authors reported contradictory results [43]. Usynina A et al., (2018) reported for a higher rate of neonatal transfer to higher level hospital of adolescent women compared to adults (11.2% vs 9.8%, $p = 0.042$) [40]. Lower Apgar score in a 1st minute was more common in newborns of adult mothers, compared to adolescent mothers. Some studies have found lower Apgar scores similarly in adolescents' newborns [31] while others have not [39].

In this study, adolescent mothers gave birth to 2.27% of all infants born in our hospital from 2016 to 2018. The results of the study show that adolescent mothers have a lower educational level and were from rural areas, more are Roma ethnicity, have a lower number of antenatal visits and a lower rate of intake of folic acid. The adolescent pregnancy is related to higher risks for anaemia, urinary tract infection, preterm birth and SGA newborns. Adolescent mothers were more likely to have a vaginal delivery, lower risk of cesarean delivery and a lower rate of instrumental vaginal delivery, so the labour in adolescents must be treated similarly as with adult women, to reduce the operative intervention based on age alone. With optimal antenatal care, high standard labour and delivery management, postnatal psychological counselling and support of adolescents, we will reduce adverse maternal and neonatal outcomes.

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Neurological Alterations in Type 1 Diabetes Mellitus Among Adolescents

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Abstract

BACKGROUND: Diabetes mellitus (DM) is a group of chronic disorders of metabolism characterised by high blood glucose levels. There is an increased prevalence of Type 1 DM in children and adolescents with its adverse complications especially microvascular ones (retinopathy, nephropathy and neuropathy) that might cause multiple organ damage.

AIM: To study the relation between DM and neurological affection.

METHODS: Fifty-nine children with type I DM, divided randomly into 2 groups, aged 8-18 years old of both sexes were enrolled in this cross-sectional study. All children were subjected to full history taking, physical, neurological and systemic examination.

RESULTS: There was an affection of motor power in both upper limbs as well as lower limbs. Also, we found that there was an affection of the superficial peripheral sensation affecting both upper and lower limbs.

CONCLUSION: Neurological assessment of children with diabetes mellitus type I should be a routine to early discover these manifestations which can have a deteriorating effect on the child's health.

Introduction

Diabetes mellitus (DM), is known to be a disorder of metabolism characterized by elevated blood sugar concentrations for a long time, which leads to a great number of nutritional, neurological, audiological and cognitive impairment symptoms that can be a direct consequence of metabolic disease or its management, or they could be secondary symptoms [1]. Diabetes is caused by either insufficient pancreatic production of insulin or the improper responsiveness of body cells to its insulin. Few studies have examined the effect of DM on hearing and cognitive functions in children [2]. There are some debates concerning the affected function in spite of known neuro-cognitive problems seen in DM, their rising about disease occurrence and their underlying progression. Learning the whole effect of T1DM on the brain especially glycemic control, is crucial [3], particularly in childhood and adolescence, which are critical periods of brain matter and cognitive skills development [4], [5]. This is the hardest time for T1DM adjustment because of its complicated therapeutic self-care rules, particularly during that age [6]. Researches have shown that dominance over one's surroundings, an essential constituent of resiliency, is correlated with life satisfaction, life quality and better daily performance particularly in school [7]. Better glycemic control is associated with resiliency and better life quality, that are both related to improved school performance in children with T1DM [8].

Material and Methods

Fifty-nine already diagnosed and under insulin treatment cases, their age ranged from 8-18 years were recruited from the Child Health Clinic, neurology clinic and paediatric neurology clinic medical research and Paediatric Neurology Clinic in Centre of Medical Excellence at National Research centre “MRCE”. Our inclusion criteria include children known to have type 1 diabetes mellitus, from both sexes. Those with chronic medical conditions known to affect a child`s health, blood sugar, patients with a history of known chronic neurological disorders and those with a history of any other chronic endocrinal disorders were excluded. We started collecting data and filling the sheets prepared for the study from parents and caregivers, by detailed history taking, disease duration, frequency and dose of insulin injection “Medication History”, detailed nutritional history [9].

Thorough clinical examination, anthropometric measurements were obtained using the standardised equipment, following the recommendations of the International Biological Program. Systemic examination including cardiac examination, chest, and abdominal examination were performed for detection of any clinical and nutritional problems — complete neurological assessment including examination of cranial nerves, motor power, sensation and mental status. Written informed consent was taken from parents of all participants before enrollment in the study and after full explanation of their role in the study. The consent was approved by The Ethical Committee of The National Research Center under the registration number 16358.

Results

The male to female ratio (frequency table) is shown in Table 1.

Table 1: Shows male to female ratio (Frequency table)

	GENDER		Total
	Male	Female	
Count	21	38	59
% within cases	35.59%	64.41%	100%

Gender distribution according to controlled and uncontrolled case is shown in Table 2.

Table 2: Shows gender distribution according to controlled and uncontrolled cases (Frequency table)

	GENDER		Total
	Male	Female	
Count	21	38	59
Controlled cases	16	17	33
Non-controlled cases	5	21	26

In our study we enrolled 59 cases with T1DM of them 21 were males (35.59% of all patients), 16 cases were controlled, and 5 cases were uncontrolled and 38 female patients (64.41%) of the 17 cases were controlled and 21 cases were uncontrolled, as shown in Table 1, 2, with a male/female ratio is 1:1.8.

Table 3: Mean value ± SD of height, weight, BMI of 59 cases

Vari-ables	Male cases (21 cases)			Female Cases (38 cases)		
	Height (cm)	Weight (Kg)	BMI (Kgm^{-2})	Height (cm)	Weight (Kg)	BMI (Kgm^{-2})
Range	145.3-159.7	44.1-57.9	17.35-27.37	135.1149.5	38.150.9	16.95-22.37
Mean ± SD	151.6 ± 4.02	46.7 ± 3.07	21.08 ± 3.67	148.6 ± 3.92	43.2 ± 4.03	20.3 ± 3.9

In our study regarding the anthropometric measurements, we found that the mean height, weight, and BMI among male cases are (151.6 cm, 46.7 Kg, 21.08 Kg/m^2) respectively, while in females all these measurements are (148.6 cm 43.2 Kg, 20.3 $Kg/m^{2)}$ respectively, Table 3, which means that anthropometric measurements were affected among diabetic cases.

Table 4: Distribution of motor power affection in Upper limbs in group I (Males) and group II (Females)

Crosstab						
			Motor UL affection			Total
			Normal	Grade I	Grade II	Grade III	
Group	I	Male	13	4	2	2	21
		% within Male Group	62%	19 %	9.5%	9.5%	100.0%
	II	Female	22	7	4	5	38
		% within Female Group	57.9%	18.4%	10.5%	13.2%	100.0%

In our results, regarding the distribution of affection of motor power in both upper limbs, we found that 62% of male patients had no affection of motor power, while 38% had variable stages of motor affection (19% had grade II affection, 9.5% had grade III, and 9.5% had grade IV motor power affection), compared to females we found that 57.9% of female patients had no affection of motor power, while 42.1% showed variable stages of motor affection (18.4% had grade II affection, 10.5% had grade III, and 13.2% had grade IV motor power affection) (Table 4).

Table 5: Distribution of motor power affection in lower limbs in group I (Males) and group II (Females)

Crosstab							
			Motor LL after therapy				Total
			Normal	Grade I	Grade II	Grade III	
Group	I	Male	10	6	4	1	21
		% within Male Group	47.62%	28.58%	19%	4.8%	100.0%
	II	Female	19	11	3	5	38
		% within Female Group	50%	28.9%	7.9%	13.2%	100.0%

Regarding the distribution of the affection of motor power in both lower limbs, we found that 47.62% of male patients had no affection for their motor power in lower limbs, while 52.38% showed different stages of motor affection (28.58% had grade II affection, 19% had grade III, and 4.8% had grade IV motor power affection), while in comparison with females we found that 50% of female cases had no affection for their motor power, while 50% showed different stages of motor affection (28.9% had grade II

affection, 7.9% had grade III, and 13.2% had grade IV motor power affection), Table 5.

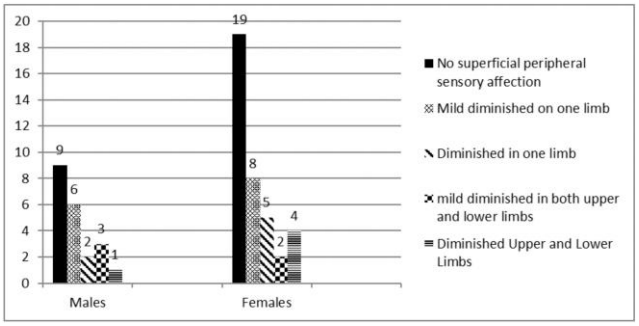

Figure 1: Distribution of superficial sensation at baseline in group I (Males) and group II (Females)

Regarding the distribution of the superficial peripheral sensory affection, we found that 47.4% of all cases had normal superficial peripheral sensation. While 52.6% showed different staged of Superficial peripheral sensory affection (23.7% had mild diminished superficial sensation in one limb, 11.9% had Superficial peripheral sensory affection on one limb, 8.5% shows mild diminished affection of both upper and lower limbs, and also 8.5% shows diminished superficial peripheral sensation in both upper and lower limbs) for both sexes (Figure 1).

Table 7: Distribution of deep sensation affection at baseline in group I (Males) and group II (Females)

	Deep sensation		Total
	Not affected	Affected	
Male	17	4	21
Female	32	6	38
Percentage	83.05%	16.95%	100%

Regarding the deep sensory affection, we found that 83.08% of all cases had normal deep sensation, while 16.95% showed some deep sensory affection for both sexes Table 7.

Discussion

It is almost presumed that there is trivial or no sex prejudice among either Type I (insulin-dependent) or Type II (non-insulin-dependent) DM. Type I diabetes is the unique major organ-specific autoimmune disease to demonstrate sex equality. The whole sex ratio is nearly similar in patients diagnosed < 15 years old. Communities with the maximal occurrence usually show male prejudice; while communities with minimal risk, mostly of non-European origin, particularly show female dominance. On the contrary, male dominance is a constantly seen in European origin people aged 15-40 years, with approximately 3:2 male: female ratio [10].

Bulletin of the WHO, 2013, [11] stated that the average incidence of diabetes mellitus did not markedly influenced by sex and was shown to be low or high in females than in males when analysed by African subregion. Growth measures are essential signs of child health and affected by elements like glycemic control in diabetic youth. A study done in India showed that growth was negatively affected by children with DM when compared with healthy ones. Children diagnosed earlier, need maximum concern to improve growth [12].

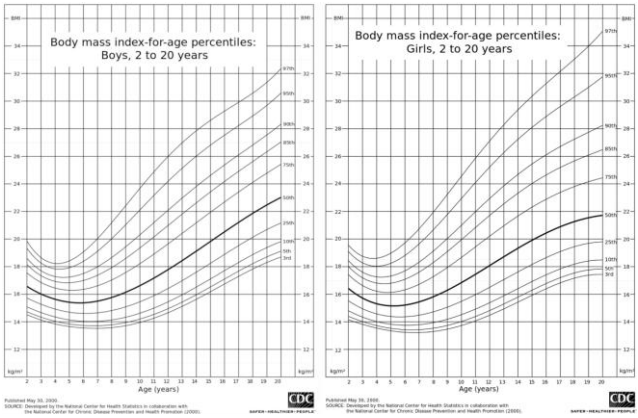

Figure 7: BMI for age percentiles for boys 2 to 20 years of age (Left). BMI for age percentiles for girls 2 to 20 years of age (Right)

Using the growth charts, BMI was used differently in youth; instead of its comparison against constant thresholds for underweight and overweight, the BMI is compared against percentile for children similar in sex and age Figure 7 [13]. A BMI that is < 5th percentile is referred to underweight & > 95th percentile is referred obese. Children with a BMI between the 85th and 95th percentile is referred to be overweight [14]. New researches in UK have shown that girls between 12 and 16 of age have bigger BMI than boys similar in age by nearly 1.0 kg/m^2 [15], while Vaman et al., 2013, [12] stated that children having T1DM were shorter for their age-matched controls and children that were on intensive insulin therapy were less influenced than those in the traditional regimen in spite of non-significant difference. They were less in weight than normal peers. Toddlers who were diagnosed before 3 years old were the shortest, whereas the tallest were those diagnosed after 14 years old [12].

Both motor power and cardiorespiratory performance can be affected by adolescents having T1DM. There is a unique relationship between metabolic control and cardiorespiratory performance confirming how essential lifestyle changes in the management and caring of diabetes in childhood are. Routine follow up of the motor, and cardiorespiratory fitness by the Euro fit battery tests could be of great help to determine the child's need of specific exercises which help in better physical fitness and glycemic control of youth with type 1 diabetes. So, more researches are needed to demonstrate the

processes by which diabetes causes decreased fitness and to study the influence of lifestyle modification on improving cardiovascular performance [16].

Peripheral neuropathy is common morbidity of DM that affects patients in adulthood, but early manifestations can occur in childhood and adolescence. Therefore, it is claimed that yearly investigations for the prompt discovery of nervous system dysfunction, has to be ordered for all youths with T1DM and children with diabetes of more than 3 years duration [17]. Screening should include simple non-invasive tests and patients diagnosed with subclinical neuropathy must be motivated to reach near-normoglycemia by scheduled insulin regimen, as perfect glycemic control is the successful way to avoid or postpone diabetic neuropathy besides other diabetes sequelae. Nowadays medications are capable of decreasing symptoms but unable to prevent the development of diabetic neuropathy [17].

In conclusion, T1DM children are at risk of having neurological morbidities which affect their lifestyle; so, adjustment of insulin therapy is considered of maximum importance in adjusting blood glucose level and preventing neurological complications.

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Clinical Science

Clinical Presentation and Laboratory Characteristics in Acute and Recurrent Erysipelas

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Abstract

AIM: Typical feature of erysipelas, especially on the lower limbs, is the tendency to reoccur. The study aimed to identify clinical and laboratory characteristics of acute and recurrent erysipelas.

MATERIAL AND METHODS: We prospectively included patients diagnosed with erysipelas on the lower limbs in the period from January 2016 to December 2017. Patients were divided into two groups: patients with the first episode and recurrent erysipelas. The groups were compared by their demographics, clinical and laboratory characteristics.

RESULTS: The study included 187 patients with the first episode of erysipelas and 126 patients with recurrent erysipelas. Both groups were homogeneous in terms of demographic characteristics, gender and age. Mean age of patients with the first episode of erysipelas was 64.18 ± 12.5 years; patients with recurrent erysipelas were inconsiderably mean younger (62.98 ± 12.5 years). Patients in both groups had a significantly different anatomical localisation of skin infection ($p = 0.008$). Tibial localisation was more frequent in patients with the first episode of erysipelas 77% vs 62.7%, while recurrent erysipelas was more frequent on the foot 36.5% vs 23%. No significant difference was found, about the affected side of the limb ($p = 0.95$). Patients with recurrent erysipelas had a pronounced inflammatory response, seen through significantly higher values of C reactive protein ($p = 0.02$), granulocytes ($p = 0.03$), fibrinogen ($p < 0.0001$), and higher body temperature, (37.22 ± 0.97 $p = 0.006$). Length of hospital stay was increased in the recurrent group.

CONCLUSION: Erysipelas is more frequent in older people; it has seasonal character and tendency to reoccur. Identifying clinical and laboratories characteristics of those at risk may prevent recurrence and long term comorbidities.

Introduction

Erysipelas is an infectious disease caused most often by β – hemolytic streptococci group A (*Streptococcus pyogenes*), rarely by streptococci groups B, C, G and occasionally Staphylococci [1]. It's a common condition, characterised with a warm, painful well-demarkated area of erythema – oedema, with prominent lymphatic involvement [2]. Systemic manifestation such as fever chills regional lymphadenopathy and leukocytosis are sometimes present and may occur hours before the skin abnormalities appear [3]. The diagnosis of soft tissue infections relies basically on the clinical picture [3]. Studies have shown that erysipelas is a potentially serious infection often resulting in recurrence, long-term morbidity and prolonged hospital stay. The most common site of erysipelas is lower legs [4], [5], although any area of the body can be affected. Its presentation on the lower legs, usually is unilaterally [6]. Predisposing factors include chronic oedema/lymphoedema, chronic venous insufficiency, obesity, any disruption of cutaneous barrier as possible sites of bacterial colonisation, previous episode of erysipelas, diabetes and immune suppression [5], [6], 7]. Complications occur in nearly 31% of cases [8] and generally present as recurrent

erysipelas, abscesses, necrotising fasciitis, phlegmon, deep venous thrombosis, skin ulcers and bacteremia [9]. Recurrence is a common complication, the percentage of recurrence is around 12-29% [4], [10]. The mainstay of the treatment is systemic antibiotics. In recurrent cases, long term antibiotic prophylaxis is recommended [3], [11] but also a vigorous control of the risk factors can reduce the rate of reoccurrence. The aim of the study was a prospective analysis and comparison of patients with acute and recurrent erysipelas, with particular consideration of demographic, clinical and laboratory characteristics in both studied groups.

Material and Methods

All patients ≥ 18 years of age both hospitalised and outpatients, diagnosed with erysipelas on lower legs were recruited in the study, between January 2016 and December 2017 in the dermatology department in General Hospital in Skopje. The diagnosis was clinical, made by the investigator.

The patients were divided into two cohorts. First cohort – patients with a first and single episode of erysipelas (no recurrence group – NR). Second cohort – patients with recurrent erysipelas (RE). NR – in this group erysipelas was defined as diffuse, superficial skin infection, which causes acute erythema, swelling and pain [3]. RE included patients with second/multiple episodes of erysipelas that meets the criteria of the first episode, at the same anatomical localisation, at least 1 month to one year from the initial diagnosis. In this group patients with anamnestic or medical records for recurrence in or outside the study were also included. The required data for all recruited patients were obtained through clinical examination and patient interview, as well as medical documentation.

The analysed data included demographic characteristic (age, sex), the season of erysipelas occurrence, clinical characteristic of erysipelas, length of hospitalisation, initial values of laboratory parameters. These two groups will be compared based on their demographic characteristics, clinical manifestations, laboratory findings. For the statistical analysis, the necessary percentages were computed and linear regression analyses performed. For continuous variables that were normally distributed the mean and standard deviations were presented. For non-continuous data, the median was presented. Categorical variables were tested with chi-square and logistic regression. The statistical significance was defined at $p < 0.05$.

Results

Comparison of demographic, clinical and laboratory characteristics between the two cohort

The study included 187 with a first/single episode of erysipelas (NR group) and 126 with recurrent erysipelas (RE). Both groups were homogeneous in terms of demographic characteristics sex and age ($p = 0.64$, $p = 0.4$ consecutive) (Table 1). Male patients were insignificantly more frequently presented in the RE group-52.4% (66), 46.5% (87), while female patients were insignificantly more common in the NR-53.5% (100) and 47.6% (60).

Table 1: Comparison of demographic and clinical features in both cohorts

Variable	NR group Patients with the first episode of erysipelas	RE group Patients with recurrent erysipelas	P-value
Age n (%)			
Mean ± SD	64.18 ± 12.5	62.98 ± 12.5	[B]p = 0.4
Min - max	26 – 86	33 – 88	
Gender n (%)			
Male	87 (46.52)	66 (52.38)	[A]p = 0.64
Female	100 (53.48)	60 (47.62)	
Patient included in the study n (%)			
Hospitalized	152 (81.28)	105 (83.33)	[A]p = 0.64
Outpatient	35 (18.72)	21 (16.67)	
Length of hospital stays (LOS) n (%)			
Mean ± SD	7.43 ± 4.3	8.35 ± 5.0	[B]p = 0.12
Min - max	1 – 28	2 – 30	
Febrility n (%)			
Mean ± SD	36.94 ± 0.78	37.22 ± 0.97	[B]p = 0.006
Min - max	36 – 40	36 – 40.2	
Anatomical localisation of erysipelas (%)			
Tibial	144 (77.01)	79 (62.7)	[A]p = 0.008
Foot	43 (22.99)	46 (36.51)	
Femoral region	0	1 (0.79)	
The side of affected extremity (%)			
Right	77 (41.18)	54 (42.86)	[A]p = 0.95
Left	91 (48.66)	59 (46.83)	
Billateral	19 (10.16)	13 (10.32)	

The mean age of patients with the first episode of erysipelas was 64.18 ± 12.5 years, and patients with recurrent erysipelas were insignificantly younger (62.98 ± 12.5 years).

Most patients in both groups were hospitalized-81.3% (152) patients in NR group and 83.3% (105) patients with RE ($p = 0.64$) (Table 1). The length of hospitalisation was significantly lower in the NR group (7.43 ± 4.3 vs 8.35 ± 5.0, $p = 0.12$). Recurrent erysipelas located in the lower extremities, the high temperature on admission, increased markers of inflammation, significantly prolonged the hospital stay.

Recruited patients from both groups (Table 1) had a significantly different anatomical localisation of erysipelas ($p = 0.008$). In NR group 77% vs 62.7% of patients had tibial localisation, while in patients with RE the infection was more often localised on foot - 36.5% vs 23%. One patient had erysipelas in the femoral region, and it was recurrent. About 10% of patients in both groups had bilateral erysipelas. More frequent was the unilateral left-sided localisation of the disease in both groups-48.7% (91) all 46.8% (59). No statistically significant difference was found between

both groups, regarding the affected side of the limb (p = 0.95). Patients with RE had a significantly higher value for the febrility, compared with NR group (37.22 ± 60.97 vs 36.94 ± 0.78, p = 0.006).

Table 2: Comparison of laboratories parameters / inflammatory markers in both cohorts

Variable	NR group Patients with the first episode of erysipelas	RE group Patients with recurrent erysipelas	P-value
C reactive protein			
Mean ± SD	41.94 ± 51.9	56.83 ± 61.9	$^{B}p = 0.02$
Min - max	3.02 – 203	3.02 – 203	
Leucocytes			
Mean ± SD	9.61 ± 3.7	10.44 ± 4.4	$^{B}p = 0.08$
Min - max	2.1 – 28.5	2.2 – 27.86	
Granulocytes			
Mean ± SD	7.05 ± 3.5	7.98 ± 4.1	$^{B}p = 0.03$
Min - max	0.9 – 26.8	1.1 – 25.18	
Sedimentation rate			
Mean ± SD	123.48 ± 21.3	126.24 ± 20.5	$^{B}p = 0.25$
Min - max	20 – 175	101 – 173	
Antistreptolysin O (ASO) titer			
Mean ± SD	195.82 ± 289.3	224.31 ± 316.5	$^{C}p = 0.064$
Min - max	68.3 (50.9-209)	103 (52.5-227)	
Fibrinogen			
Mean ± SD	4.48 ± 2.1	5.66 ± 3.7	$^{C}p < 0.0001$
Min - max	4 (3.5-5.1)	5.3 (3.9-6.7)	
D dimers			
Mean ± SD	19.35 ± 192.6	12.45 ± 125.5	$^{C}p = 0.42$
Min - max	0.62 (0.33-1.33)	0.72 (0.35-1.39)	

Most of the patient – 72.2% had erythematous erysipelas (226 patients), vesiculobullous and hemorrhagic erysipelas in the form of ecchymoses or purpuras was observed in 27.8% (87) of patients.

Comparative analysis of the two groups of patients in relation to certain laboratory parameters (Table 2) showed that patients with RE had significantly higher values of C reactive protein (CRP)(p = 0.02), granulocytes (p = 0.03), and fibrinogen (p < 0.0001), and had a insignificantly higher serum leukocyte values (p = 0.08), Sedimentation rate (p = 0.25), Antistreptolysin O (ASO) titer (p = 0.064), and D dimers (p = 0.42). CRP in serum had an average value of 41.94 ± 51.9 in the NR group and 56.83 ± 61.9 in the RE group. The granulocyte values were on average 7.05 ± 3.5 in the NE group, and 7.98 ± 4.1 in the RE group. The mean and median values of fibrinogen in the NR group were 4.48 ± 2.1, 4, and in the RE 5.66 ± 3.7, 5.

Figure 1 illustrates the percentage of erysipelas cases of admissions per season. The greatest prevalence of cases admissions (in and outpatients) was observed in summer (29.7%) and spring (26.8%); for autumn, the prevalence was 24.6% and lowest in the winter 18.8% (p = 0.9). The peak in the summer has been previously reported [7].

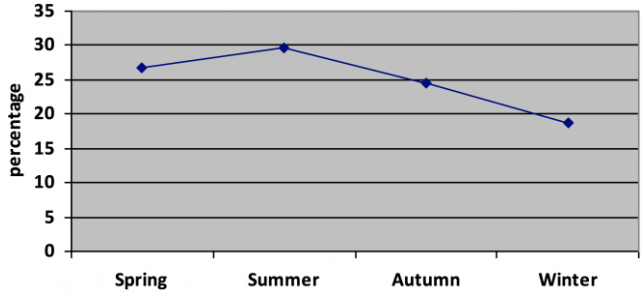

Figure 1: Percentage of erysipelas cases admissions per season

Discussion

The study confirmed that recurrent erysipelas is more common in the elderly population. The mean age in both cohorts patients has no significant difference, and no significant gender predomination has been established, which is consistent with other studies [6]. The greatest prevalence of erysipelas was found in > 60-year-old patients (more than 60% of the cases), and the mean age was 64.18 ± 12.5 year in the NR group and 62.98 ± 12.5 years in RE. Recent studies have reported a similar mean age [5], [6].

Regarding the anatomical localisation, tibial localisation was more common with patients with the first episode of erysipelas, while patients with recurrent erysipelas had more frequent foot location. We noted that the latter is in line with other results obtained regarding RE-obesity, insulin-dependent diabetes and neuropathy (not included in this paper). The feet are prone to chronic swelling, especially in obese patients, with diabetes where diabetic neuropathy and angiopathy may be present. Injuries to the feet, especially in neuropathy and dermatomycosis, are the point of entry of the infection.

Patients with RE had a stronger inflammatory response, which is evident from the higher initial values of the C reactive protein, the granulocytes, fibrinogen. They also have a longer hospital stay compared to the NE group, which is consistent with other studies [12]. In conclusion, erysipelas is more frequent in older people; it has a seasonal character with the highest peak in summer**.** Patients with recurrent erysipelas, located in the lower extremities, especially foot, with high temperature on admission, increased markers of inflammation, seem to be risk factors of prolonged hospital stay.

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The Influence of Oral Multivitamins Supplementation on Selected Oxidative Stress Parameters and Lipid Profiles among Sudanese Patients with Type-2 Diabetes

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Abstract

AIM: The objective of the current study was to assess the influence of oral multivitamins supplementation on some oxidative stress parameters (serum Vitamin A, C, E, Zinc, Malondialdehyde (MDA)) and lipid profile among Sudanese patients with type- 2 diabetes mellitus (T2DM).

MATERIAL AND METHOD: Three hundred Sudanese patients with T2DM and Hundred healthy subjects (control group) were enrolled in this cross-sectional study. Blood was collected after overnight fasting for 10-12 hrs. Fasting plasma glucose (FBG), lipid profiles, Glycosylated haemoglobin (HbA1c%), Serum zinc, Malondialdehyde (MDA), Vitamins A, E, and C levels were measured using standardised laboratory techniques. Data was collected with the help of a structured questionnaire and direct interview.

RESULTS: Biochemical parameters of the study population were shown a highly significant difference (P value < 0.05), between the means of serum vitamin A, C, E, Zinc, MDA, HbA1c, triglycerides, HDL, FBG, total cholesterol and LDL. Significant differences in serum vitamin A, C, E, Zinc, MDA, triglycerides, HDL and FBG between people with diabetes who used multivitamins and diabetics who did not use it (P-value < 0.05).

CONCLUSION: The study observed a significant increase in serum levels of vitamin A, C & E and other biomarkers parameters in patients with T2DM who take oral multivitamins supplements; such improvement may lead to minimising the diabetic complications. Further studies are needed to explore the possible therapeutic role of multivitamins supplements for T2DM patients.

Introduction

According to international diabetes Federation (IDF), there were over 2.247.000 cases of diabetes in Sudan in 2017 with the prevalence of 10.9% (confidence interval: 5.6-17.7%), which expected to reach 14.2% by 2045 [1]. High prevalence of type- 2 diabetes mellitus (T2DM) and prediabetes was observed among Sudanese [2], [3]. T2DM is a progressive condition in which the body becomes resistant to the normal effects of insulin and gradually loses the capacity to produce enough insulin in the pancreas. Diabetes has been known as an oxidative stress disorder caused by an imbalance between free radical formation and the ability of the body's natural antioxidants [4]. Oxidative stress is hypothesised to play an important part in the development of late diabetes complications. In the absence of an appropriate compensatory response by the endogenous antioxidants, such as vitamins C and E, catalase, glutathione, and superoxide dismutase, oxidative stress dominates resulting in the activation of stress-sensitive intracellular signalling pathways [5]. Most patients with diabetes have lipid metabolism disorders, most common forms are decreased high density lipoprotein (HDL) and increased triglyceride, cholesterol and LDL in patients' serum.

High doses of vitamins C and E have been shown to decrease blood glucose, plasma cholesterol and triglyceride in T2DM patients [6]. Vitamins and minerals play an important role in glucose metabolism, so understanding the impact of vitamin and mineral deficiencies and the potential utility of

supplementation is relevant to the prevention and management of T2DM. The majority of diabetic individuals should receive daily vitamins and minerals within the ranges of recommended values from consumption of natural food sources and/or fortified foods [7]. Several epidemiological studies have investigated the association between dietary intake and plasma levels of some micronutrients and plasma lipid concentrations in a variety of populations. Serum levels of Vitamin C, magnesium and zinc have been reported to be correlated inversely with serum levels of cholesterol [8], [9].

The purpose of the present study was to assess the influence of oral multivitamins supplementation on some oxidative stress parameters and lipid profiles among Sudanese patients with T2DM.

Material and Method

This cross-sectional hospital-based study enrolled three hundred patients with known T2DM admitted to Bahri diabetes centre, Sudan. Hundred healthy subjects (non-diabetic) were enrolled as a control group. Patients were received either 500 mg or 1000 mg randomly daily of oral multivitamins which contains the recommended daily allowance of vitamins and minerals supplement for not less than 6 weeks. About 7.0 ml of venous blood were obtained from the candidates after overnight fasting (10-12) hrs. The collected blood was drawn in three containers (heparin, EDTA and fluoride oxalate). The whole blood was used immediately after collection for testing HbA1c%. The blood in all containers was gently mixed and then centrifuged to obtain plasma. FBG, lipid profiles, zinc, MDA, Vitamins A, E, and C antioxidant were measured by using standardised laboratory techniques. Data such as age, weight and height were collected with the help of a structured questionnaire and direct interview. Body mass index (BMI) was calculated by = Weight (kg)/Height (m^2). Ethical clearance of research was obtained from the Ethical Committee of Bahri diabetic center-Bahri and the Ethical Committee of Faculty of Medical Laboratory Sciences, University of Science and Technology, Omdurman, Sudan. Verbal and written consent was obtained from each participant before enrollment.

Data were analysed using statistical package for social science (SPSS) software [version 21, Chicago, IL, USA). The variables analysed were age, weight, BMI, and biochemical parameters about T2 diabetes status and intake of a multivitamin. Chi-squared test for association and an independent T-test for continuous variables were used to test for significance at *P* value < 0.05.

Results

In the current study, 300 patients with T2DM and 100 healthy subjects were included. The mean ages of the study and the control groups were 50.1 ± 14.0 and 51.2 ± 11.1 years respectively (Table 1).

Table 1: Characteristics of the study groups

Variables	Control group (non-diabetics) (n = 100)	Study group (T2DM) (n = 300)	*P* value
Age (years)	50.1 ± 14.0	51.2 ± 11.1	0.06
Weight (kg)	74.5 ± 12.2	79.7 ± 22.8	0.032*
BMI (w/h^2)	25.2 ± 3.2	29.5 ± 8.1	0.0004*

*Significant differences (P value < 0.05).

The means of serum vitamin A, C, E, Zinc, MDA, HbA1c, triglycerides, HDL, FBG, total cholesterol and LDL have shown a highly significant difference (*P* value < 0.05) between the study group and healthy subjects (Table 2).

Table 2: Comparison of the means of some blood parameters (Vitamins (A, E& C), MDA, zinc, HbA1C, FBG and lipid profile) between T2DM and non-diabetics

Variables	Control group (non-diabetics) (n = 100)	Study Group (T2DM) (n = 300)	*P* value
Vitamin A (µg/dL)	81.2 ± 21.8	50.3 ± 20.0	0.001*
Vitamin E (µ/mL)	15.6 ± 4.8	5.2 ± 1.8	0.001*
Vitamin C (µg/mL)	10.0 ± 2.2	3.9 ± 1.3	0.0003*
MDA (µM)	2.4 ± 1.1	6.7 ± 6.2	0.001*
Zinc (µmol/L)	100.5 ± 12.9	77.2 ± 9.8	0.001*
HbA_1c%	4.9 ± 0.3	7.5 ± 1.4	0.001*
FBG (mg/dL)	101.5 ± 11.9	160.4 ± 65.5	0.0002*
Triglycerides (mg/dL)	107.1 ± 20.1	124.6 ± 79.1	0.033*
Total Cholesterol (mg/dl)	117.3 ± 20.9	164.8 ± 45.6	0.0006*
LDL (mg/dL)	86.6 ± 20.6	109.0 ± 34.7	0.0003*
HDL (mg/dL)	51.9 ± 6.2	41.8 ± 11.9	0.0008*

* Significant differences in all blood parameters between control and test group (*P* value < 0.05).

Concerning intake of multivitamin (n = 194, 64.7%) of patients with T2DM on multivitamin, while (n = 106, 35.6%) of them were not. Significant differences in serum vitamin A, and E, Zinc, MDA, triglycerides, HDL and FBG between people with diabetes who used oral multivitamins and diabetics who did not use it (*P*-value < 0.05). No significant differences in total cholesterol and LDL between T2DM patients who used multivitamins and for those who did not use it (*P* value > 0.05) (Table 3).

Table 3: Comparison of blood parameters (Vitamins (A, E& C), MDA, zinc, HbA1C, FBG and lipid profile) among test group according to Intake of multivitamins

Variables	Intake (n = 194)	No Intake (n=106)	*P* value
Vitamin A (µg/dL)	61.2 ± 14.6	30.3 ± 11.0	0.001*
Vitamin E (µ/mL)	6.1 ± 1.3	3.4 ± 1.4 (1.0 - 9.0)	0.002*
Vitamin C (µg/mL)	4.3 ± 1.2	3.2 ± 1.3	0.08**
MDA (µM)	4.7 ± 4.3	10.4 ± 7.3	0.01*
Zinc (µmol/L)	80.3 ± 8.6	71.7 ± 9.3	0.003*
HbA_1c%	6.9 ± 0.7	8.7 ± 1.6	0.03*
Triglycerides (mg/dL)	116.5 ± 64.2	139.2 ± 99.6	0.02*
Total Cholesterol (mg/dl)	162.6 ± 39.4	168.9 ± 55.2	0.259**
LDL (mg/dL)	108.3 ± 32.0	110.5 ± 39.3	0.598**
HDL (mg/dL)	43.9 ± 11.2	37.8 ± 12.2	0.0002*
FBG (mg/dL)	133.0 ± 43.8	210.6 ± 69.1	0.001*

* Significant differences in Serum Vitamin A, and E, Zinc, MDA, Triglycerides, HDL, HbA_1c and FBG between diabetics who used multivitamins and diabetics who did not used it (P-value < 0.05); ** No significant differences in vitamin C, total cholesterol and LDL between diabetics who used multivitamins and diabetics who did not used it (P value > 0.05).

There was a significant negative correlation between the duration of diabetes and vitamin A, C &E in addition to MDA and zinc levels (Figure 1).

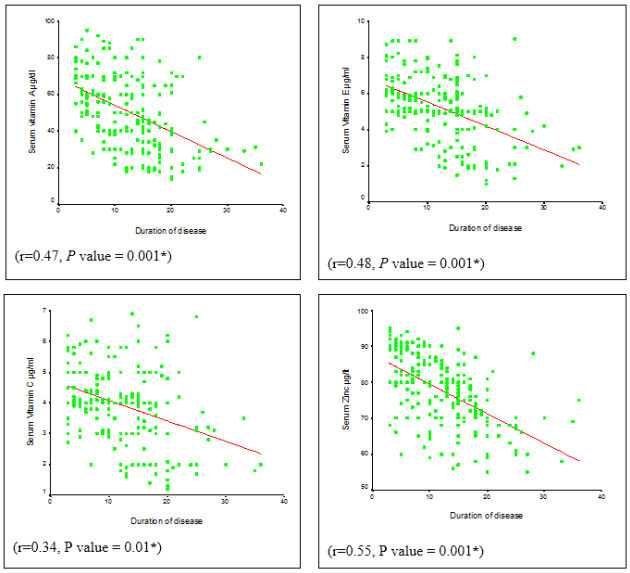

Figure 1: Scatter plot shows the relationship between the duration of the disease and the numbers of biomarker parameters among T2DM patients (N = 300)

Discussion

High prevalence of Type 2 diabetes mellitus (T2DM) and prediabetes was observed among Sudanese [2], [3]. The objective of this study was to assess the influence of oral multivitamins supplementation on some oxidative stress parameters (serum Vitamin A, C, E, Zinc, MDA) and lipid profile among Sudanese patients with T2DM. The results showed a significant increase in the means of the plasma levels of fasting glucose, cholesterol, LDL, triglycerides, MDA and HbA1c of the test group when compared with healthy control group subjects. While, the means of the plasma levels of antioxidant vitamins (A, E, C), HDL and zinc showed a significant reduction when compared with that of the control group. This finding which indicates the excessive glycosylation of haemoglobin and poor control of diabetes is by the results reported by some studies among patients with T2DM [10], [11], [12].

Increased serum cholesterol, LDL, triglycerides and reduce in HDL indicate the presence of dyslipidemia. Some factors may contribute to the changes in lipid metabolism in patients with T2DM, including insulin resistance and/or relative insulin deficiency, and hyperglycaemia [13]. The marked significant reduction in vitamins A, E, C and zinc, has been reported by various studies indicating metabolic abnormalities, which is related to increased cardiometabolic risks. Such reduction may be attributed to the increase in the need to control the excessive oxidative stress produced by abnormalities in glucose metabolism [14], [15]. In both types of diabetes, there is a massive oxidative and nitrosative stresses which have been associated with intensive changes on both antioxidant enzyme systems and total antioxidant capacity causing peroxidative damage to lipids, proteins, nucleic acids and carbohydrates which can be used as DM biomarkers [16].

In the present study, we subdivided the study group according to the multivitamin intake. The results showed a significant difference in serum vitamin A, and E, Zinc, MDA, triglycerides, HDL and FBG between T2DM patients who intake oral multivitamins and T2DM patients who did not (*P* value < 0.05), Our finding is inconsistent with a number of studies, Gazis *et a*l., observed that supplementation in diabetic patients considerably reduced HbA1c levels [17]. Prajapat *et al.* concluded that supplementation and normal diet might improve plasma glucose and lipid profile in patients with T2DM [18]. Sayed *et al.* observed that dietary regimen together with supplementation with zinc might be useful to suppress plasma glucose and to regulate insulin secretion of diabetics [19]. Another study conducted among Nigerians patients with T2DM concluded that vitamins supplementation was significantly increased GSH levels and lowered MDA levels [20]. Polidori *et a*l. found those very old age patients with T2DM have lower plasma concentrations of vitamin A &E [21]. The benefits of using multivitamins not only limited to the glucose and lipid metabolisms; Barringer *et al.*, noticed the reduction the incidence of infections in patients with T2DM who use multivitamins supplementation and minerals for One-year [22].

In conclusion, a significant difference of Serum vitamin A, E, C, Zinc and MDA between T2DM patients and healthy subjects was found in this study. Furthermore, a significant difference of some endogenous antioxidant levels was observed in patients with T2DM who administered oral multivitamins; such improvement may minimise the diabetic complications. Further studies are needed to explore the possible therapeutic role of multivitamins supplements for T2DM patients.

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Case Report

Arthroscopic Reduction and Percutaneous Osteosynthesis of Tibial Plateau Fractures

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Keywords: Tibial plateau fractures; Arthroscopic reduction; Percutaneous osteosynthesis

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Abstract

BACKGROUND: Tibial plateau fractures are complex lesions capable of causing severe consequences if not appropriately treated. They are often the result of high-energy trauma and, not rarely, are associated with significant soft-tissue and intra-articular injuries. Different therapeutic options can be managed in the treatment of these lesions. Minimally invasive surgery percutaneous technique-offers several advantages compared to other surgical techniques and allows, with less additional soft tissue damages, good reduction and stable fixation of the fracture.

CASE PRESENTATION: In this study, we assessed the results of the combined arthroscopic and radioscopic assisted reduction and internal fixation of tibial plateau fractures in 7 patients with Schatzker type II and III.

CONCLUSION: According to Hohl's and Rasmussen's grading system, all of the patients scored excellent and good results at 1 year follow up. We experienced no complications due to arthroscopy.

Introduction

Tibial plateau fractures are complex lesions capable of causing severe consequences if not appropriately treated. They are often the result of high-energy trauma and, not rarely, are associated with significant soft-tissue and intra-articular injuries. Different therapeutic options can be managed in the treatment of these lesions. Minimally invasive surgery percutaneous technique-offers several advantages compared to other surgical techniques and allows, with less additional soft tissue damages, good reduction and stable fixation of the fracture [1].

Methods

Between April 2013 and December 2016, seven lateral tibial fractures were treated with arthroscopic assisted osteosynthesis. The patients had a mean age of 42 years (22 – 56 years), male three and four female. The cause of injury was pedestrian hit by a car – three patients, fall from height two of them and two-sport injuries.

According to the Shatzker classification (image 1), four fractures were type II, and three were type III. Surgery was done when the swelling subsided on the average 4-the day (3-6 d). Mean follow up

period = 18 months (12 – 24 months).

Inclusion criteria were a proximal tibial fracture with > 3 mm articular step off. We excluded complex fracture type IV-VI. We obtained informed consent from all patients preoperatively.

We used the t-test for statistical analysis, and we considered a result of $p < 0.05$ statistically significant.

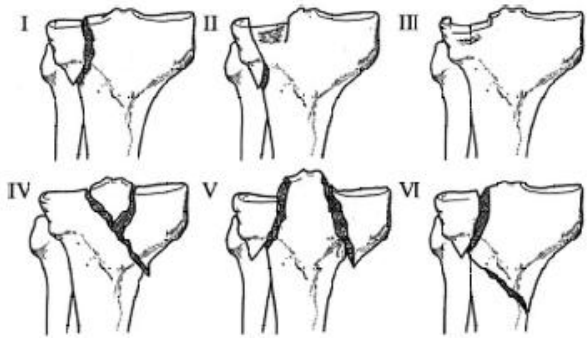

Figure 1: Shatzker classification

Surgical technique

The operating room setup requires positioning of the patient as a standard knee arthroscopy procedure and place for fluoroscopy C-arm monitor. The leg holder is placed more proximal than usual, exsanguinated with esmarch bandage, the contralateral limb is abducted ensuring that is it out of imaging. The standard arthroscopic portals are used. Concomitant pathology as meniscal and cartilage injury are diagnosed and treated.

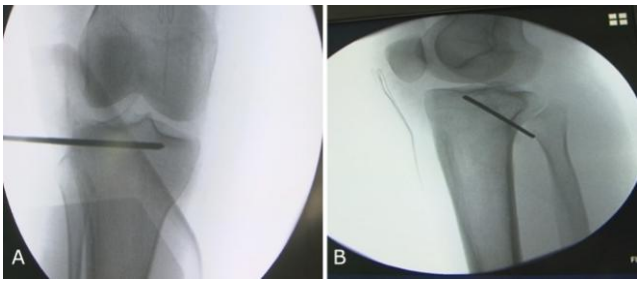

Figure 2: A) and B) k-wire starting position

According to the type of the fracture we placed the k-wire (Figure 2), and with joystick technique, we reduce the fracture, or we put the ACL guide pin percutaneously from the anteromedial tibia and with a cannulated bone tamp elevated the depressed fragment. Once satisfactory reduction under arthroscopic control is achieved (Figure 3) three to four 3, 5 or 4.5 partially or fully threaded screws are drilled and tapped. The bone tunnel was grafted to increase subchondral support.

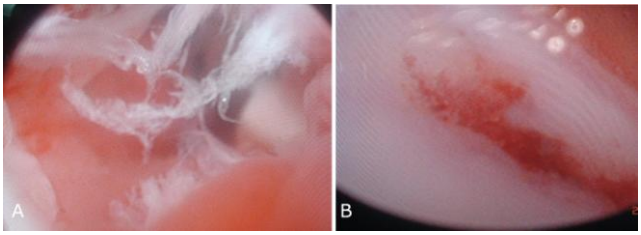

Figure 3: A) and B) Pre- and post-fracture reduction- arthroscopic view

Results

All treated fractures were successfully united, Average length of union 12 weeks (10 – 13 week). Preoperative fracture depression average 7 mm (3 – 10 mm).

At the clinical assessment subjectively all for the patient were satisfied with this technique. According to the Rassmussen criteria. 6 (86 %) were excellent, and one (14%) was good.

Concomitant lesions were noted, four of them had a lateral meniscal lesion, one medial meniscal and partial ACL tear. We partially resected the meniscal lesions, and we treated conservatively ACL partial tear.

We didn't find any statistical difference between the range of motion of injured and noninjured knee -135-140 degree ($p > 0.05$) Figure 4.

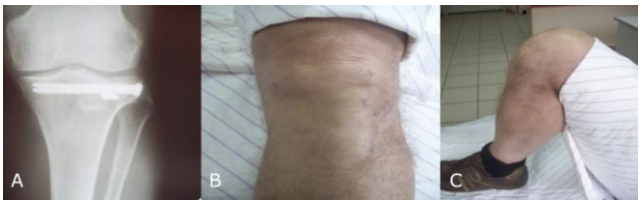

Figure 4: A), B), and C) Three months postoperative results

Discussion

Arthroscopically assisted percutaneous fixation which was first recommended by Caspari [2], and Jennings [3] has gradually become popular during the years. The advantages of this technique are an exact visualisation of the intraarticular fracture, accurate reduction, lower morbidity and immediate treatment of associated soft tissue lesions. Ohdera [4] reported no difference in the duration of surgery, clinical results between two open and arthroscopic technique.

It was reported that not all plateau tibial

fractures are amenable for arthroscopic assisted osteosynthesis. Hign energy fractures according to Shatzker IV-VI are associated with potential risk of fluid extravasation and compartment syndrome [5], [6], [7]. Torneta et al., [7] insisted in using arthroscopic assisted osteosynthesis only in Shatzker type I, II and III.

According to that all the fractures healed without angular deformity, no complications, infections or compartment syndrome, in conclusion, we can accept arthroscopic assisted osteosynthesis as an effective, minimally invasive, surgical method for the treatment of low energy plateau tibial fractures [8] Shatzker II and III types.

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Case Report

Scleromyxedema (Arndt - Gottron Syndrome) Developing Under Tenofovir Treatment for Hepatitis B: Unique Presentation in a Bulgarian Patient!

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Keywords: Scleromyxedema; Arndt - Gottron syndrome; Tenofovir; Hepatitis B; Diabetes mellitus; Survival benefit; Pathogenetic relationship; Treatment

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Abstract

BACKGROUND: Scleromyxedema, also referred to as the Arndt-Gottron (S-AG) syndrome or the systemic form of Lichen myxedematosus (LM), is a cutaneous mucinosis with a chronic course and high lethality from systemic involvement of other organs and systems. Interesting in several aspects is the association between scleromyxedema and viral hepatitis about: 1) hepatitis virus infection as a possible etiological factor for the development of scleromyxedema, 2) antiretroviral therapy for the treatment of hepatitis as a method of reversing scleromyxedema and 3) antiviral drugs as inducers of scleromyxedema.

CASE REPORT: We present a 53-year old patient who for nine months had been on tenofovir disoproxil 245 mg (0-0-1) therapy for chronic hepatitis B. Three months after the start of antiviral therapy (i.e. for a period of 6 months), the patient observed swelling, itching and hardening of the skin on the face, ears and hands, which subsequently spread throughout the trunk. Subsequent histological study of a skin biopsy revealed changes of scleromyxedema at an advanced stage, though immunoelectrophoresis of serum and urine excluded the presence of paraproteinaemia or para proteinuria. Systemic antihistamine and topical corticosteroid therapy were instituted. Bone involvement with possible plasmacytoma was excluded, and a myelogram showed evidence of an erythroblastic reaction of bone marrow.

CONCLUSION: We believe that drug-induced scleromyxedema is a rare but possible phenomenon. We describe the first case of tenofovir-induced scleromyxedema within the framework of chronic hepatitis B treatment.

Introduction

Scleromyxedema, a systemic form of lichen myxedematosus (LM) [1], is associated with significant mortality [2], [3], [4]. Interesting in this regard is the association of scleromyxedema with hepatitis virus [5]. Scleromyxedema may occur secondarily in patients with viral hepatitis C [5], [6]. According to some authors, antiviral therapy for the treatment of hepatitis leads to the reversal of scleromyxedema and, according to others, treatment with interferon alpha 2 leads to worsening of LM [7]. We describe a patient in whom we believe there is a possible association between the development of scleromyxedema and the use of tenofovir disoproxil for hepatitis B.

Case report

We present a 53-year-old man with type 2 diabetes mellitus, chronic hepatitis B, hepatic cirrhosis, duodenal ulcer, mild splenomegaly, chronic cholecystitis and hepatitis B associated nephropathy.

The patient was receiving treatment with insulin degludec 30 IU-0 -0 and insulin aspart 10 IU-14 IU-14 IU, and for the past nine months, he received tenofovir disoproxil 245 mg (0-0-1) for treatment of chronic hepatitis B. The patient was hospitalized for swelling, pruritus and hardening of the skin on the face, ears and hands, which subsequently spread to involve the trunk. Skin complaints began 3 months after the start of therapy with tenofovir. Dermatological examination revealed significant thickening and hardening in the areas of the face, neck, body and extremities, and generalised lichenoid papules were also found (Figure 1a, 1b, 1c, and 1d).

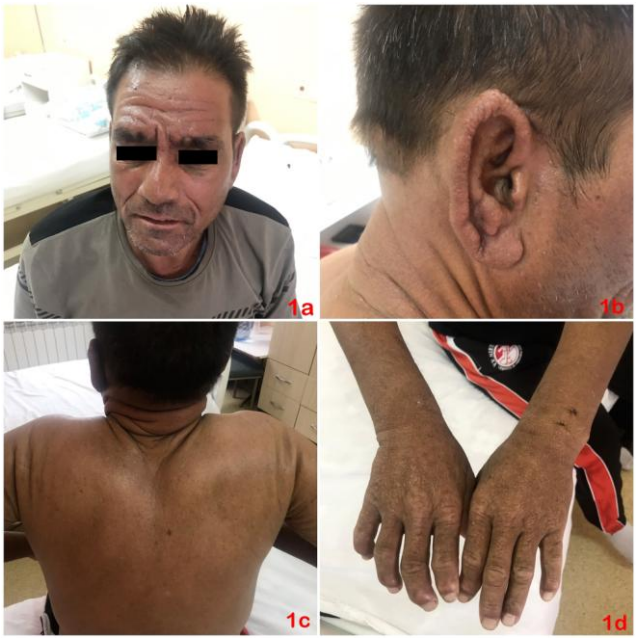

Figure 1: a) Hardening of the face skin; b) Skin-colored small papules on the ear skin; c) Hardening of the skin on the back and neck; d) Multiple disseminated papules on the skin of the hands and arthropathy

Based on clinical data, scleromyxedema, scleredema of Buschke and lichen amyloidosis were considered as possible diagnoses. A skin biopsy showed numerous fibroblasts and irregularly arranged collagen bundles with prominent mucin deposition (Figure 2), consistent with an advanced stage of scleromyxedema.

Double antihistamine therapy was initiated due to the presence of severe itching, and flumetasone pivalate/clioquinol was administered topically. The consultation was obtained from a gastroenterologist, who concluded that, given the patient's ongoing chronic hepatitis B and posthepatic cirrhosis, it would not be appropriate to start systemic corticosteroid therapy because of its immunosuppressive effect. Immunoelectrophoresis of serum and urine excluded paraproteinaemia or para proteinuria. During the hospitalisation, additional tests were performed. Skull and pelvic radiography excluded possible bone involvement with plasmacytoma, and ultrasound of the abdominal organs showed no paraneoplastic process.

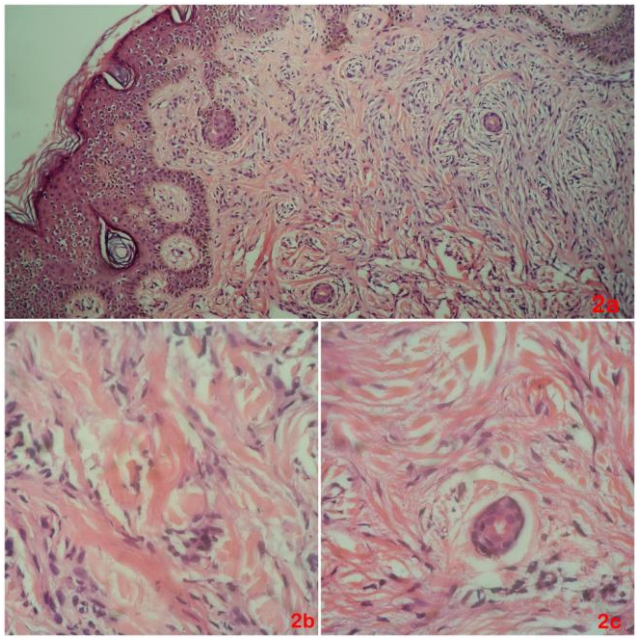

Figure 2: a) This skin biopsy shows a combination of numerous fibroblasts, mucin, and irregularly arranged collagen bundles; b) At higher magnification, there are irregularly arranged collagen and scattered spindled cells, representing fibroblasts, within a mucinous background; c) This image shows details of fibroblasts around the cross-sectional profile of an eccrine sweat duct

Laboratory data included CEA - 2.87 μg/ml (0-5), PSA-0.178 μg/ml (0-3,100), and AST-31 IU (0-200). A myelogram showed evidence of an erythroblastic reaction of bone marrow, a mild leukemoid reaction of granulocyte-neutrophil type, and a mild eosinophilic bone marrow response-consistent with reactive changes. The patient was referred to the haematology and oncology clinic for bone marrow puncture, bone scintigraphy, and further therapeutic recommendations. Ambulatory systemic therapy with Azathioprine 2 x 50 mg/day and topical-flumethasone pivalate/clioquinol was initiated. After a few months of treatment with Azathioprine 2 x 50 mg/day, a slight improvement in major symptoms was observed. It is planned to discontinue tenofovir therapy after consultation with a gastroenterologist. The viral load following azathioprine therapy was 0, but the therapy was stopped and after a 1-week treatment with intravenous immunoglobulin will be initiated. Treatment with intravenous IgG will be performed as follows: 2 g per kg/every month, divided into five days with 430 mg/day for 6 months.

Discussion

Scleromyxedema or Arndt-Gottron (S-AG) syndrome is a cutaneous mucinosis that mainly affects adults between 30 and 70 years of age and

whose aetiology is not fully understood [2]. The disease is defined as a systemic form of Lichen myxedematosus [5]. It is believed that in 80% of patients there is monoclonal gammopathy and there is probably an immune response of B-cells to antigenic mucin deposits in the dermis [3]. However, it is possible to observe scleromyxedema without underlying paraproteinemia, as in our patient [3]. A major sign of this disease is the histopathological triad of dermal mucin deposition, fibroblast proliferation, and fibrosis [2]. The clinical condition is characterised by widespread lichenoid papules which coalesce to form generalised plaques that cause extensive thickening and hardening of the skin [2], [3]. There may also be an atypical clinical presentation with cutaneous and subcutaneous nodules [8]. According to prevailing literature, the absence of thyroid disease should also be considered as one of the main criteria for the diagnosis of scleromyxedema [1].

Scleromyxedema is a chronic disease that may be accompanied by severe systemic manifestations such as gastrointestinal, neurological, cardiac, muscular, renal, pulmonary, or ophthalmological disorders, and these associations may cause significant disability and may be fatal [3], [4].

A rare variant that can be associated with a high risk of mortality is the so-called 'dermato-neuro syndrome' [9]. It is characterised by a prodromal flu-like period, followed by fever, convulsions and coma [9].

Of interest are the rare cases, such as this one, in which scleromyxedema is present within the framework of a hepatitis virus infection [5]. Much of the literature on this association describes scleromyxedema secondary to hepatitis C virus and successful treatment with antiviral therapy [5], [6]. According to these sources, hepatitis C infection should be considered as a possible etiological factor for the development of scleromyxedema [5], [6]. However, it is also possible that lichen myxedematosus may worsen in the setting of chronic hepatitis C therapy treated with interferon alfa-2a [7]. Also, there is a reported case of scleromyxedema development as a result of interferon beta-1a (IFN beta-1a) therapy following a diagnosis of multiple sclerosis (MS) [10]. At present, the mechanism by which IFN beta-1a may lead to the development of scleromyxedema has not been established [10]. In any event, we believe that the possibility of drug-induced scleromyxedema is worthy of serious consideration.

Also described in the literature is a case of papular mucinosis (PM) in association with HIV infection in a patient with highly active antiretroviral therapy (HAART) with tenofovir disoproxil/emtricitabine and lopinavir/ritonavir [11]. According to studies: 1) antiretroviral therapy (including tenofovir) in patients with HIV infection and lichen myxedematosus leads to a complete resolution of papular mucinosis and 2) tenofovir prevents hepatic fibrosis development and protects against bleomycin-induced dermal fibrosis by downregulating adenosine levels in the liver and skin [12], [13]. These data indicate that tenofovir, through a variety of mechanisms, may affect the pathogenetic chain of events leading to cutaneous mucinosis and fibrosis. Based on the previous data, we believe that we describe the first case of scleromyxedema induced by treatment with tenofovir for hepatitis B.

At the moment there is no specific definitive treatment of scleromyxedema [4]. There is a wide range of treatment options, such as high-dose corticosteroids, a variety of immunosuppressive and chemotherapeutic drugs, PUVA and UVA1 phototherapy, interferon-α, retinoids, thalidomide, bortezomib, and autologous stem cell transplantation, but intravenous immunoglobulin (IVIg) treatment is considered to be relatively effective and safe treatment, and appears to be the treatment of choice for scleromyxedema [3], [4], [8]. Also, some authors suggest that treatment with intravenous immunoglobulins is considered and recommended as First Line therapy [14]. They recommend a dose of 2 g per kg, divided into four/five partial doses on four/five days, with the interval between cycles from 4 weeks to maximally 6 weeks (as well as the one assigned to our patient) [14].

In conclusion, the exact mechanisms under which antiviral drugs affect skin deposits of mucin and cutaneous fibrosis have not yet been established. There are contradictory data according to which, on the one hand, drug therapy for hepatitis C worsens LM, and on the other, treatment with tenofovir in HIV-infected patients leads to a reversal of cutaneous mucinosis. However, they indicate that the pathogenesis of scleromyxedema may be associated with different drug groups. We believe that we are presenting the first case of tenofovir-induced scleromyxedema.

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Case Report

Challenging Diagnostics of Biofilm Associated Periprosthetic Infection in Immunocompromised Patient: A Clinical Case

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Keywords: Biofilms; Periprosthetic infection; Scanning electron microscopy

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Abstract

BACKGROUND: Periprosthetic joint infection (PJI) is a devastating complication of joint arthroplasty. The identification of microorganisms in biofilm-related PJI is challenging yet significant stage of the treatment process. Medical microbiology methods, such as pure culture isolation, remain the gold standard. However, the error rate of classical methods may vary from 10% to as high as 42% due to the inability to detect bacteria growing within biofilms. Other methods of detection are being explored to improve the management of PJI.

AIM: Accurate identification of PJI contributing microorganisms in a patient with acute postoperative PJI after total hip joint arthroplasty and systemic lupus erythematosus in anamnesis.

METHODS: We used microbial culture methods followed by scanning electron microscopy (SEM).

RESULTS: Perioperative an intraoperative cultural analysis of 8 different culture samples of tissue and prosthetic origin was insufficient for bacterial or fungal detection. Scanning electron microscopy revealed detailed biofilm visualisation on the surface of the prosthetic component. The biofilm exterior was composed of microbial clusters made of 10 or more cells with either pear- or bottle-shaped morphology, 3-6 mcm in length and 1.5-3 mcm in diameter. Rod-shaped microorganisms of 0.7-1 mcm length and up to 0.5 mcm in diameter were found adjacent to these clusters.

CONCLUSION: Additional methods for PJI agents' detection are time-and cost-effective in the case of the challenging diagnostics of biofilm-related PJI, particularly in immunocompromised patients. Using combined diagnostic approaches increases the accuracy of detection, justifies treatment strategies and improves clinical outcomes.

Introduction

Periprosthetic joint infection (PJI) is one of the most serious complications following total joint arthroplasty. The accurate identification of microbes contributing to PJI is critical for PJI detection and treatment. However, the identification may pose a significant challenge since the diagnostic tests report both false positive and false negative results. The reference standard for diagnosing of implant-related infections is the isolation of the pathogen in pure cultures and subsequent identification using the combination of tinctorial, physiological, biochemical methods [1].

Gram-positive bacteria contribute to the development of periprosthetic infection in 88% of cases. Infection by gram-negative bacteria is relatively rare (approximately 7%). The common reasons for gram-negative bacterial infection are empirical antibiotic therapy and prolonged surgical drain stay after endoprosthesis surgery. The widespread gram-negative agents are *Pseudomonas aeruginosa*, *Acinetobacter baumanii*, *Klebsiella pneumoniae*, *Proteus* spp., *Enterobacter* spp., *Escherichia coli* [2], [3], [4]. Despite recent technological advances in cultural methods, an overwhelming 10% to 42% microbial causes of all periprosthetic infections remain unidentified [5], [6].

The standard microbiology approach for microbial identification can be problematic due to the inability of biofilm-forming microorganisms to grow independently in pure culture [7] along with variability in time and specifics of cultivation methods for

different species [8], [9], [10].

Therefore, further studies are needed to improve the management and prevention strategies of PJI.

Scanning electron microscopy (SEM) is one of the effective methods for studying microbial morphology of microorganisms from clinical samples [11], [12]. However, comparative *in situ* studies of biofilm attached to the orthopaedic surfaces and microbial cultures are rare.

We hypothesised that etiologic agents of PJI which remain undetected using standard microbial culture methods could be identified using scanning electron microscopy approach.

Case Presentation

Clinical case: patient D., 44 years old woman, presented with acute postsurgical periprosthetic joint infection (April 2018).

Medical history: In January 2018, the patient underwent cementless total joint arthroplasty of the right hip. The postoperative period has been complicated by a fever of 38°C and purulent drainage of the surgical site. A month later, modular prosthetic components were revised and debrided with subsequent replacement. Following the replacement surgery, the patient has developed a fistula.

Past medical history revealed that the patient had chronic systemic lupus erythematosus, chronic bronchitis, and chronic gastritis in a remission period, genetic thrombophilia and stage 2 hypertonic disease.

The patient presented with the right hip fistula, right hip pain and activity limitations. In the perioperative period we suggested periprosthetic infection based on x-ray findings (Figure 1A, B and C) and cytological and microbiological analysis of the synovial fluid. During the surgery, total removal of prosthetic components followed by the debridement, articulating spacer installation and replacement of the right hip prosthesis with the x-ray control (Figure 1D).

Intraoperative samples of periarticular tissues, bone fragments of the prosthetic base and prosthetic components were obtained for histological and microbiological analyses.

Tissue samples were homogenised and synovial fluid samples were pelleted before inoculation. Blood agar containing 5% defibrinated blood, Baird-Parker egg yolk tellurite medium, Levine eosin methylene blue agar, Sabouraud agar and Columbia agar were used for microbiological cultures. Inoculated agar plates were incubated at 37º C for 24-48 h. Fungal cultures were incubated at 30º C for 120 h. “Anaerogas” gas generating pack sets and 72-120 h incubation at 3º C were used for anaerobic culture growth. The morphological observation was performed using ordinary staining (Gram) method.

Fragments of the periprosthetic membrane (n=3) and trabecular bone samples (n=3) of periprosthetic host bone were preserved at least 48 h in 4% formalin. Bone samples were decalcified in Richman-Gelfand-Hill solution after fixation. Then samples were dehydrated and embedded in paraffin for microtome sectioning. Slides with tissue sections of 5-7 mcm were stained with hematoxylin and eosin. The visualisation was performed using AxioScope.A1 stereomicroscope. For imaging, we used AxioCam ICc 5 and Zen blue («Carl Zeiss MicroImaging GmbH», Germany) software. For each sample, at least 10 images were obtained.

Metallic endoprosthetic femoral component and periprosthetic tissue samples for scanning electron microscopy was performed using standard patented procedures with authors’ modifications [11], [13] After platinum deposition in an IB-6 vacuum ion sprayer (Eiko, Japan), the surface of the implant component was examined with Zeiss EVO MA 18 scanning electron microscope (Carl Zeiss Group, Germany). The limitation was the height of the area in the study region of no more than 145 mm, which was due to the design limitations.

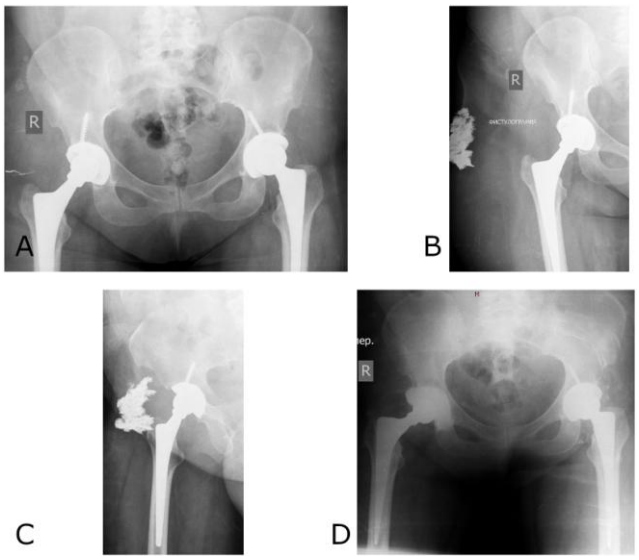

Figure 1: Radiographs of the pelvis: A) AP view before the revision operation; B, C) Fistulograph of the right hip joint before surgery; D) AP view after the intervention

Perioperative culture tests of the punctured material did not reveal bacterial growth. Leucocytosis in the joint fluid was 12.9 cells per microliter, and the rate of neutrophils was 67%.

Microbiological study of intraoperative samples revealed a small number of *Staphylococcus saprophyticus* cells (< 10^3 CFU/ml) in one sample (synovial joint fluid). No bacterial growth has been found after the cultivation of remaining samples. Tests

for both fungi and anaerobic bacteria were negative.

Histological analysis of the fragments of periprosthetic membranes was conducted according to the consensus classification of Krenn and Morawitz [14] and revealed some features of conformity to type I, caused by wear particles. In a reactively modified loose fibrous connective tissue with a high density of fibroblast-like cellular elements (Figure 2A), foreign microparticles of an optically transparent material were detected surrounded by giant cells of foreign bodies. In such areas, cell density was reduced; erythrocyte diapedesis and infiltration with monocytes and lymphocytes were noted (Figure 2B). Polymorphonuclear leukocytes were not detected in any of the visual fields.

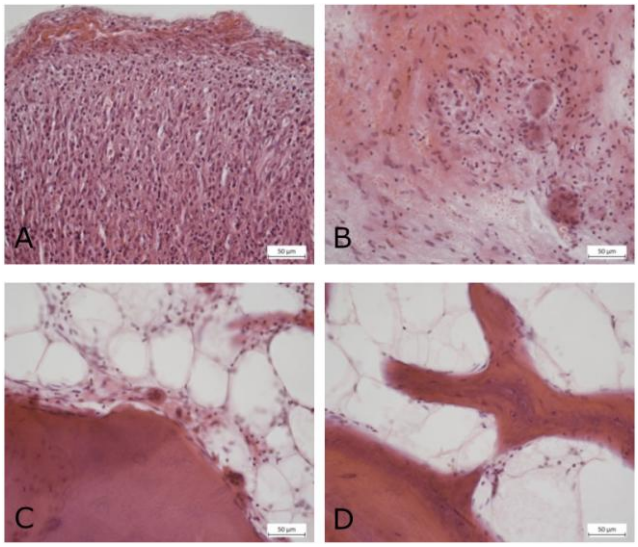

Figure 2: Reactive changes in the periprosthetic membrane: A) high cell density; B) diapedesis, clusters of giant multinuclear cells; C) osteoclastic resorption; D) osteoblastic remodelling of trabeculae in the spongy bone of the periprosthetic bed. Paraffin sections. Stained with hematoxylin and eosin. Magnification: 10 (lens), 40 (eyepiece). Scale bar = 50 microns

Histological study of spongy bone substance fragments revealed signs of remodelling, consisting of moderate osteoclastic (Figure 2C) and osteoblastic (Figure 2D) activity on the surface of bone trabeculae. The intertrabecular spaces contained fatty bone marrow with scattered foci of hematopoiesis; hyperemia and oedema were observed only on the border with the implant.

Scanning microscopy detected clusters of nanoscale globular bodies in the periprosthetic connective tissue (possibly immune complexes) (Figure 3A, and B) and perivascular accumulations of lymphoid cells (Figure 3D), characteristic of systemic lupus erythematosus.

The flat metal surface of the femoral component, free from adherent spongy bone substance fragments (Figure 4A), was covered with a biofilm shaped as amorphous layers (Figure 4B).

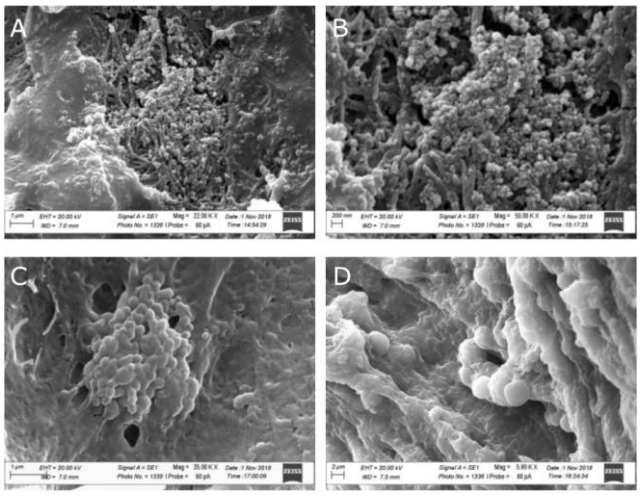

Figure 3: Morphological manifestations of systemic lupus erythematosus in the periprosthetic connective tissue: A, B and C) Deposits of nano-sized globular bodies; D) Perivascular accumulations of lymphoid cells; Electronic scans. Magnification × 22,000 (A), × 50,000 (B), × 35,000 (C), × 5,900 (D)

Microbial cells with different morphological characteristics were detected on its surface. Microorganisms with a length of 3 to 6 microns and a thickness of 1.5 to 3 microns had pear-like or bottle-like elongated shapes (Figure 4C, and D). The wide base of the cells was rounded; at the narrow end of each of them, there was a ring-shaped structure that resembled a “bottleneck”. The relief of the cell surface was uneven, poorly folded. Cells that were oriented in various directions were attached to a membranous substance on the component surface.

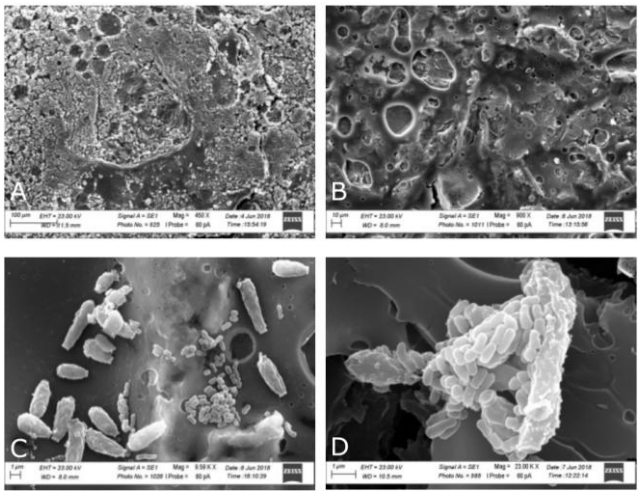

Figure 4: Areas of the metal implant surface with adhered fragments of spongy bone substance of the periprosthetic bed (a) and amorphous filmy substance (b). Biofilm on the surface of the metal implant: large bottle-shaped microorganisms with a characteristic "neck" and small rod-shaped microorganisms adhered on a membranous substrate (c), and a fragment of a structured biofilm (d). Electronic scans. Magnification × 450 (a) × 900 (b) × 9590 (c) × 23,000 (d)

Microorganisms were located in groups of up to 10 or more cells. Rod-shaped and transversely dividing bacteria with a diameter of 0.5 μm and a

length of 0.7 to 1 μm had a smooth surface. They formed clusters containing two or more cells that adhered nearby or on the surface of large cells (Figure 4 C, and D).

Discussion

At present, there has been an increase in the incidence of biofilm-related infections caused by saprotrophic or pathogenic fungi along with either Gram-positive or Gram-negative bacteria. The role of biofilm infections in orthopaedic surgery is tremendous due to the high virulence of biofilm-forming microorganisms and the high possibility of biofilm formation on the surfaces of commonly used orthopaedic components [15].

Microbial biofilms can be described as structured, high-functional aggregations of microbial cells belonging to one or most commonly several species, encased into biofilm matrix and adhered to the surface. The eradication of multi-species biofilms associated with implant surfaces can be problematic as a result of poor antibiotic activity against biofilms. Metabolic, structural and functional ties between microorganisms in biofilm do not make allowances for the cultivation of clear cultures in laboratory conditions, leading to false negative test results [16]. To improve the analytical outcome, ordinary microbiology approaches can be enhanced with sophisticated methods for cultivation of each separate microorganism for each case. However, routine use of some techniques is not cost-effective. Thus, ordinary medical microbiology methods are not always effective in PJI diagnosis and should be supported by other methods.

In this clinical case, repeated laboratory tests of diagnostic punctures and intraoperative samples did not reveal etiologic PJI agents despite clinically symptomatic acute inflammatory process. The single-time finding of *S. saprophyticus* in 1 out of 8 perioperative samples could not interpret *S. saprophyticus* as an etiological factor due to the low virulence of this bacteria. Taking into consideration the low number of *S. saprophyticus* cells in a synovial fluid sample, we believe that the contamination of one sample could occasionally occur.

Scanning electron microscopy has proven its effectiveness in the visualization and identification of biofilm-forming microbes [15], [16], [17]. In this clinical case, scanning electron microscopy revealed microbial association adhered to the endoprosthesis surface. Based on morphology, the larger microbial component of the biofilm was identified as yeast and smaller – as rod-shaped bacteria [18], [19].

Uncommonly, patient anamnesis included systemic lupus erythematosus (SLE). Immunodeficiencies in patients with SLE compromise the immunity so that conditional pathogens (fungal, bacterial, viral) can contribute to the infection development [20], [21]. In this case, additional methods for microbial detection are needed.

Therefore, in immunocompromised patients with periprosthetic infection, the utilization of scanning electron microscopy for accurate identification is advisable. Timely diagnostics is cost-effective and allows for justified, unbiased therapy decisions. The patient voluntarily signed informed consent to a surgical procedure and participation in the study.

Level of evidence: III (limitations due to the lack of patients, single case report)

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Case Report

Advanced Ulcerated Squamous Cell Carcinoma of the Hand with Locoregional Axillary Lymph Node Metastasis – Case Report and Literature Review

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Keywords: Cutaneous squamous cell carcinoma; Hand tumours; Surgery; Gout

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Abstract

BACKGROUND: Cutaneous squamous cell carcinoma (SCC) of the hand is the most common soft-tissue malignancy in this particular region. A literature survey suggested a higher rate of metastases in advanced SCC of the hand compared to head-and-neck cutaneous SCC.

CASE REPORT: An 84-year-old man presented with an ulcerated firm tumour on the dorsum of his right hand. A diagnostic biopsy confirmed the diagnosis SCC. Imaging suggested an involvement of the tendons of digits 3 and 4. A diagnostic ultrasound suggested a loco-regional axillary lymph node metastasis. After discussion in the interdisciplinary tumour board, amputation of the affected digits followed by lymph node excision was recommended.

CONCLUSIONS: Advanced SCC of the hand requires interdisciplinary management. Amputation is part of the surgical spectrum in advanced cases.

Introduction

Malignant skin tumours of the hand – although uncommon-may have a significant impact on professional and private life. About 15 % of all soft tissue malignancies occur in hand [1]. In a series of 407 patients cutaneous squamous cell carcinoma (SCC) comprised 70.8 % of all malignancies followed by basal cell carcinoma and melanoma.

The predominant localisation was on the dorsum of the hand [2], [3]. SCC of the palm is even more rarely [4]. Risk factors for SCC on the hand are exposed to ultraviolet light, radiation, arsenic or immunosuppression, and chronic ulcerations. SCC tend to invade subcutaneous adipose tissue, vessels, nerve sheaths, tendons, cartilage, and bone. The 5-year-rate of metastatic disease is about 5% [5].

Surgery provides a lower relapse rate than non-surgical treatment, i.e. 3% versus 33% [3]. A multidisciplinary approach is essential to manage the advanced disease. Amputation of fingers is rarely required but may become unavoidable in advanced disease [6].

Case Report

An 84-year-old man presented with an ulcerated firm tumour on the dorsum of his right hand. He reported that the lesion had been slowly growing

for more than two years but started to increase more rapidly for three to four months. Since then it became ulcerated and showed a tendency of bleeding. His medical history was remarkable for diabetes mellitus type II and arterial hypertension.

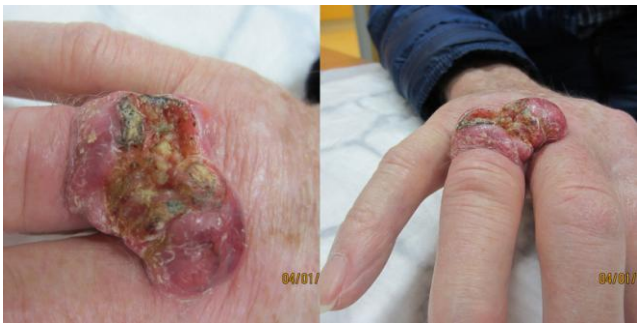

Figure 1: Clinical presentation of an advanced, ulcerated cutaneous squamous cell carcinoma of the dorsum of the right hand

On examination, we observed a firm skin coloured tumour with central ulceration above the metacarpophalangeal joints of the third and fourth right digits (Figure 1). The tumour was adherent to underlying structures. The size was 4.2 x 3.8 cm. The lesion was painful under pressure.

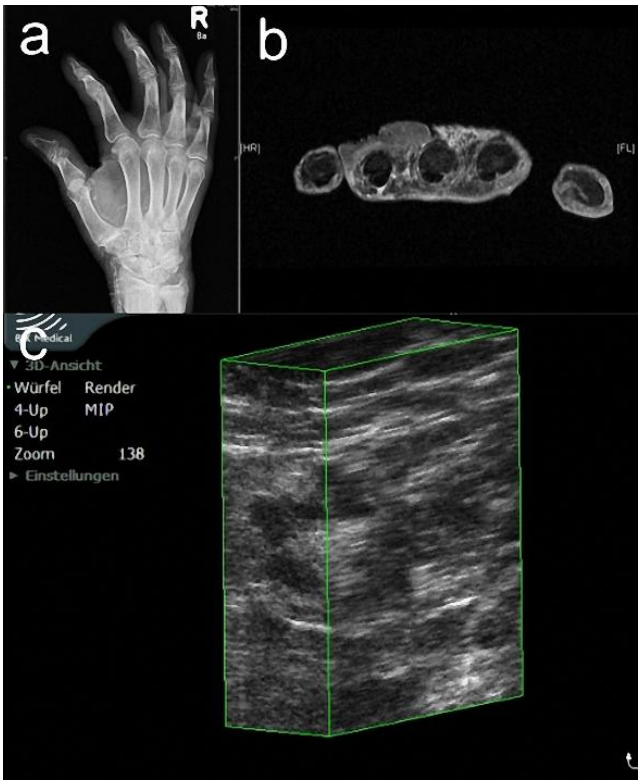

Figure 2: Imaging of the patients; a) X-ray of the hand excluded bony tumour involvement; b) MRI of the hand suggested the involvement of the tendons digitus 3 and IV; c) Ultrasound image of the axillary lymph node metastasis

A diagnostic biopsy was performed. Histopathologic examination revealed a cutaneous SCC. The staging was initiated. MRI of the hand suggested the involvement of tendons and bony structures. Diagnostic ultrasound detected a metastatic lymph node in the ipsilateral axilla. No distant metastases were detected (Figure 2). Laboratory investigations were unremarkable. In the interdisciplinary tumour board, a radical surgery with amputation of digits III and IV of the right hand was suggested followed by regional lymph node dissection.

An interdisciplinary team of hand surgeon and dermato-surgeon performed surgery of the primary tumour. After preparation of cutaneous palmar flaps, the tumour was excised with a safety margin of at least 5 mm. The vascular, nervous bundles of the 3rd and 4th digits were extensively electro-coagulated and cut. During metacarpophalangeal amputation, gout tophi were found in these joints and surgically removed. After preparation of flexor tendons, they were shortened and cut. The resulting defect was closed by palmar skin flaps (Figure 3).

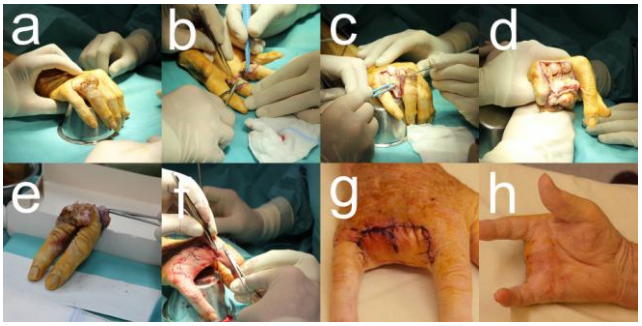

Figure 3: Surgery of the advanced cancer; a) Marking of the safety margins; b) Preparation of the palmar skin flaps; c) Electro-coagulation of vascular and nervous bundles; d) Exarticulation of digit 3 and 4; e) Surgical specimen; f) Suturing of the flaps; g) and (h). Palmar and dorsal view 6 days after surgery

Histopathologic examination of the amputation specimen demonstrated a desmoplastic SCC with a tumour thickness of 11 mm. The investigation confirmed an R0 resection with 7 mm safety margins. Tumour stage and grading was pT2cN1cM0, G2, stage III. Gout tophi were also confirmed (Figure 4). In the postoperative care, physiotherapy was performed including compression therapy and joint mobilisation. After successful and uneventful wound healing on the hand, the patient underwent axillary lymph node removal in the department of surgery.

Discussion

Advanced SCC of the hand has a poorer prognosis when it occurs in the skin overlying the dorsum of the proximal phalanges and web spaces), is ulcerated, has a macroscopic diameter greater than 20 mm, a tumour thickness greater than 4 mm, and an invasion to the subcutaneous fat or beyond [2].

Assessment of malignant tumours of the hand includes patient's history, clinical examination, imaging with computerised tomography (CT) or magnetic resonance imaging (MRI), and diagnostic biopsy for histopathology [1]. Since in the present patient, histopathology confirmed SCC. A diagnostic ultrasound suggested an isolated loco-regional axillary lymph node metastasis. The diagnosis of gout trophy was a surprise since no clinical or laboratory findings indicated a second metabolic disease – diabetes was already known and treated.

Amputation of a digit becomes the treatment of choice in case of tumour invasion to the periosteum, tumour size > 20 mm, or presence of satellite lesions. In a series of 74 patients with SCC on the hand, in 5.4 % finger amputation deemed necessary [7]. Regional node dissection should be performed if there are palpable nodes or imaging techniques demonstrating lymph nodes suspicious for metastasis [6]. The 5-year metastasis rate of cutaneous SCC, in general, has been estimated as 5%. Standardised effective medical drug therapy is yet not available. In a recent study on advanced and metastatic cutaneous SCC in general, the overall response rate to systemic therapies (mostly epidermal growth factor receptor-inhibitors) was 26% with a mean duration of response of only 5 months [8]. SCC on the hand has a higher propensity for metastases, ranging from 6% to 28% [9].

In the present case, metacarpophalangeal amputation followed by primary defect closure with skin flaps, and axillary lymph node dissection were performed [10]. The highest cure rates are achieved by surgical R0-resection [1]. The relapse rate after Mohs surgery is 1.2% [11]. The 5-year-survival rates are 88% to 92% for localized disease and 64% for advanced disease [12], [13].

In conclusion, the diagnosis of SCC of the hand should not be delayed ensuring the best possible treatment outcome. Tumour diagnosis can be hampered by other pathologies such as gout trophy in the present case. Treatment requires an interdisciplinary approach.

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Case Report

Angiolymphoid Hyperplasia with Eosinophilia Successfully Treated with Cryotherapy

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Keywords: Angiolymphoid hyperplasia with eosinophilia; Histopathology; Therapeutic approach

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Abstract

BACKGROUND: Angiolymphoid hyperplasia with eosinophilia is an uncommon, benign, vasoproliferative cutaneous neoplasm with uncertain origin. It preferably affects middle-aged adults, manifesting as plum-colored pruritic papules, nodules and plaques, which can persist indefinitely, relapsing over time. Different response/resistance to various therapeutic modalities and frequent recurrences impose a great therapeutic dilemma.

CASE PRESENTATION: Herein, we present a 77-year-old male patient with a 7-month-history of unrecognized cutaneous manifestations on his left shoulder and flank. Based on the investigations, the diagnosis of angiolymphoid hyperplasia with eosinophilia was established. We applied cryotherapy as a treatment of choice. The complete regression of the skin lesions and three years disease-free period was achieved.

CONCLUSION: Although surgery is the standard therapeutic approach, the disease recurs despite multiple surgical attempts. Therefore, we recommend cryotherapy as effective and safe treatment modalities for angiolymphoid hyperplasia with eosinophilia. Knowing the recurring nature of this disease, the patients with angiolymphoid hyperplasia should stay on short-term follow up in order to monitor if new lesions occur.

Introduction

Angiolymphoid hyperplasia with eosinophilia (ALHE) is a rare, benign vascular disease described for the first time by Wells and Whimster in 1969 [1]. It is characterized with prominent vascular proliferation with lymphocytic and eosinophilic infiltration of the skin [2]. This vascular tumor was also named epithelioid hemangioma (EH) by Weiss and Enzinger in 1982, in order to differentiate the lesion from the malignant vascular tumor, epithelioid hemangioendothelioma [3]. ALHE usually occurs in young to middle aged adults, and it usually affects women [4]. The most frequent involved sites are head and neck (face, ear and periauricular), rarely present in other areas and organs like shoulders, trunk, extremities, hands, breast, liver, spleen, orbit and bone [5]. Oral mucosa, conjunctiva and genital area can also be affected [6].

Clinically, ALHE shows solitary or multiple, dome-shaped, smooth, erythematous/violaceous papules/nodules [7]. There are no systemic symptoms, but sometimes, the lesions may be painful, pruritic or pulsatile due to its vascular nature [8]. Usually, there is no regional lymphadenopathy or peripheral eosinophilia, but approximately 20% of patients with ALHE may show eosinophilia [9]. ALHE grows slowly and can persist indefinitely [10]. Some cases clear up spontaneously, without any treatment, but more often, persistent and recurrent lesions require medical treatment [11].

We report a case of a male patient with a 7-months history of cutaneous lesions and histological characteristics compatible with ALHE. After cryotherapy, the complete healing of skin lesions was achieved. He is three years disease-free, without any recurrence.

Case Presentation

A 77-year-old man was referred to our clinic due to 7-months history of multiple, pruritic, occasionaly painful, erythematous/violaceus papulo/nodular and plaques-like lesions on left shoulder and trunk/flunk (Figure 1). During that period, lesions increased in number and size, without any signs of spontaneous remission. The patient noticed crusting and bleeding from his certain lesions. He has unremarkable medical and family history. No systemic symptoms or palpable regional lymphadenopathy were detected. A laboratory workup was conducted in addition to an excisional biopsy of one skin lesion. All laboratory investigations, including serum IgE level, were within normal range.

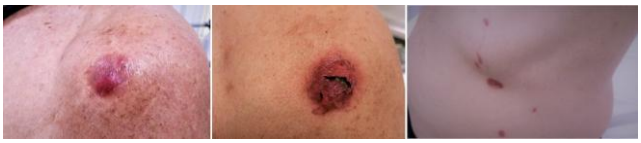

Figure 1: Angiolymphoid hyperplasia with eosinophilia: erythematous/violaceus colored papules, nodules involving the left shoulder and trunk. Superficial crusting and bleeding are observed

Skin biopsy from the lesion showed prominent vascular proliferation, epithelioid-like endothelial cells, with typical presentation of “hobnail” in the lumen of the vessels (Figure 2a and 2b). Polymorphous inflammatory infiltrate composed primarily of histiocytes and eosinophils with secondary lymphoid follicles formation were also noted (Figure 3). Immunohistochemical studies showed positive CD20, reflecting the B-cell nature of the infiltrate (Figure 4). According to these findings, the confident diagnosis of angiolymphoid hyperplasia with eosinophilia was established.

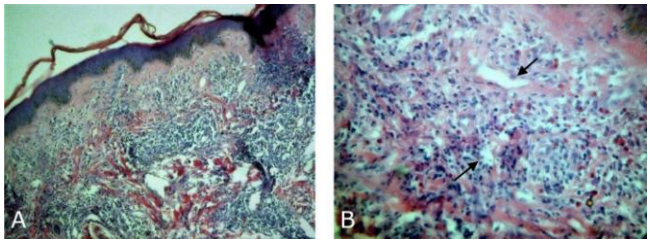

Figure 2: a) Histopathology of the skin lesion (H&E): prominent vascular proliferation; b) Epithelioid-like endothelial cells, with typical presentation of “hobnail” in the lumen of the vessels

Our treatment of choice was open spray techniques of cryotherapy with schedule of one session in every three weeks. Two freeze-thaw cycles with 15-s freezing and 1-min thawing per session. Number of treatment sessions was 3. A complete regression of skin lesions, without any recurrence during three years of follow-up was achieved (Figure 5).

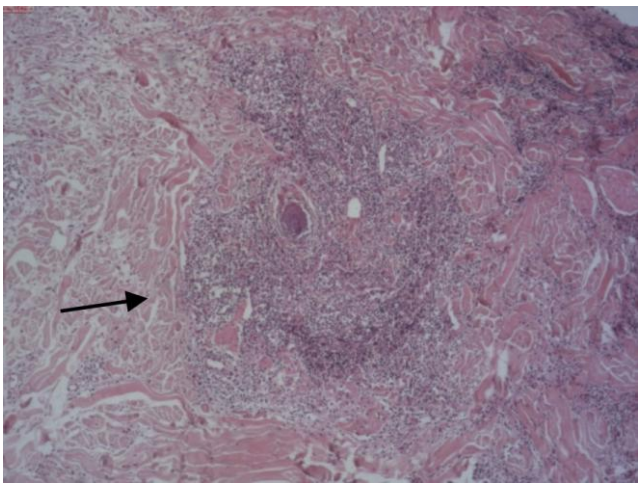

Figure 3: Histopathology of the skin lesion (H&E): polymorphous inflammatory infiltrate composed primarily of histiocytes and eosinophils with secondary lymphoid follicles formation

Discussion

Angiolymphoid hyperplasia with eosinophilia has been reported worldwide with an undetermined frequency. The pathogenesis of the disease is unclear [5]. Some authors consider ALHE as a benign neoplastic proliferation of endothelial cells [11], [12]. Others proposed a theory of unusual reactive hyperplasia of vascular tissue as a response to insect bite, trauma or infection [2]. Eosinophils could be actively involved in the pathogenesis of inflammatory reaction by production of nitric oxide and eosinophilic cationic protein [13].

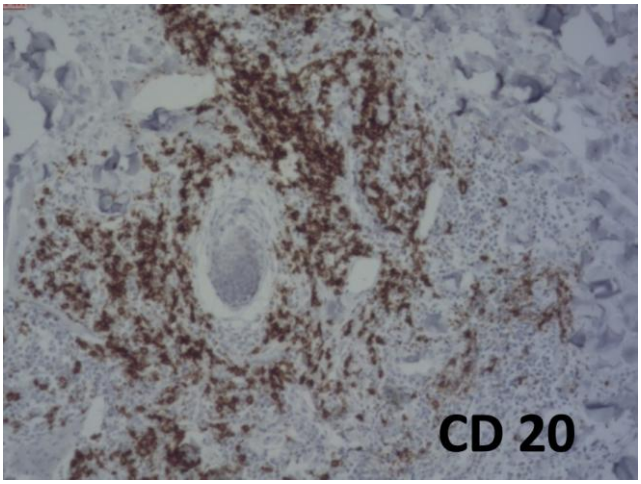

Figure 4: Immunohistochemistry: CD20 positive cells, reflecting the B-cell nature of the inflammatory infiltrate

Additional hypothesis suggest that arteriovenous shunts may play a role in the pathogenesis. In favor of the last theory, most cases of ALHE histologically show damaged and/or tortuous arteries and veins at the base of the lesion [2]. Kempf

et al., presented evidence that ALHE or its subset may represent a T-cell lymphoprolifeative disorder of low-grade malignancy [14]. This hypothesis is based on the predominance of T lymphocytes and a rearrangement in TCR receptor in some cases [14]. Therefore, immunohistochemistry could be useful in some cases. Immunohistochemistry in our patient showed CD20+ cells, reflecting the B-cell nature of the inflammatory infiltrate in dermis.

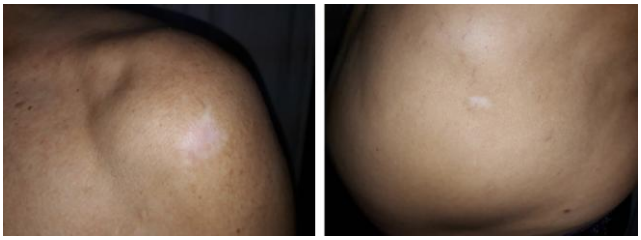

Figure 5: Clinical picture after treatment

Differential diagnosis of ALHE includes Kimura disease, cutaneous lymphoma, Kaposi sarcoma, pyogenic granuloma and cutaneous metastases [5]. Kimura disease clinically presents as large deep nodules covered by normal skin associated with adenomegaly and increased serum eosinophils and IgE [12].

In our case, the immunohistochemistry rules out all other differential diagnosis.

Diagnosis of ALHE is based on clinical, biochemical and unique histopathological findings. All these criteria were fulfilled in the presented case.

ALHE proves a great therapeutic dilemma, because there are a large variety of proposed treatments, yet there is not enough data on most of them. Many therapeutic procedures have been proposed including electrodessication, surgical excision, Mohs surgery, cryotherapy, topical or systemic corticosteroids, topical tacrolimus, imiquimod or laser therapy [11]. Surgery may be efficient in limited lesions, but recurrences are observed in 40% of the cases, due to its difficulty to determine the surgical margins [4]. Our patient actually refused surgical procedure and opted for cryotherapy, considering our explanation about the recurring nature of ALHE. However, our therapeutic approach with cryotherapy resulted in a complete regression of the skin lesions without any recurrence in the three years follow-up duration. There is no report available in the literature to date which shows long remission period after successful treatment of ALHE with cryotherapy.

In conclusion, the pathogenesis of ALHE still remains unclear and there is no consensus on the best treatment choice. Although the disease is not life threatening, it usually presents with disfiguring lesions that affect the quality of life. Further research is needed to find an effective cure and standardized therapeutic approach for this dysmorphic and recurrent condition.

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Case Report

Inverted Proximal Ileal Loop Prolapse with Ileal Rupture through a Patent Omphalomesenteric Duct: A Rare Case

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Keywords: Ileal Prolapse; Ileal Rupture; Patent Omphalomesenteric Duct; Patent Vitelline Duct

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Abstract

BACKGROUND: Prolapse of the small intestine through the umbilicus is indeed a rare presentation and is the most significant complication of the patent omphalomesenteric duct which requires pediatric surgical emergency due to its significant increase of mortality. To date, it is less than twenty cases of this presentation have been reported in medical literature. We are reporting a case of the same in an infant presenting with it on 1st week after he was delivered, but was followed by ileal rupture as well.

CASE PRESENTATION: We present a case of a patent omphalomesenteric duct with ileal prolapse and ileal rupture as its complication. It is a case of a 1-year-old infant with a history of unusual bleed-on-touch mass emerging from the anterior abdominal wall with absent umbilicus. Once his condition is stabilised, he underwent a reduction of the prolapsed bowel along with complete excision of the omphalomesenteric duct and restoration of the ileal continuity. Post-operatively he regained normal bowel function and resumed breastfeeding 5 days after surgery.

CONCLUSION: This case is an important addition to the literature about patent omphalomesenteric duct with complications of inverted proximal ileal loop prolapse and ileal rupture.

Introduction

The Omphalomesenteric duct (OMD) (vitellointestinal duct) connects the midgut to the yolk sac and provides nutrition until the placenta is established. It normally attenuates, involutes, and separates gradually from terminal part of ileum during 5th to 9th weeks of gestation [1], [2], [3], [4], [5], [6], [7]. Its persistence after intrauterine life can manifest as different pathologies called *omphalomesenteric duct remnants* (OMDR). *Patent omphalomesenteric duct* (POMD) is total incomplete obliteration of the omphalomesenteric (vitelline) duct [8].

Exact aetiology of POMD remains an enigma. None accurately addresses a direct cause of this anomalies although various teratogenic models are present in the literature, such as carbimazole or amine exposure within the first trimester of pregnancy, yet recent studies show the association between them has largely been anecdotal and still need more robust scientific evidence [9].

Various forms of OMDR is presented in Fig 1 with letter F illustrating our case [2], [7]. The OMDR occurs in approximately 2% of newborns and in 6% of these the duct remains patent, with only 20% of patent omphalomesenteric duct cases being complicated by intussusception of small bowel through the patent duct. Males are thrice more prone to be in this condition, and 73% of this case exhibit symptoms within the first 28 days of life [2], [6], [10], [11], [12], [13], [14].

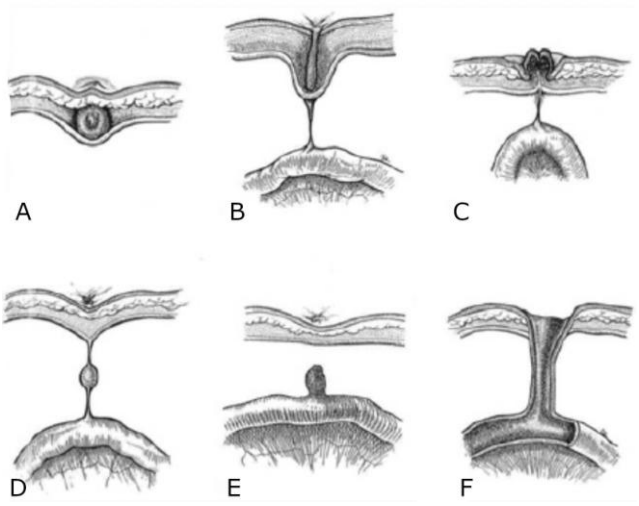

Figure 1: Various Omphalomesenteric duct remnants; A) Containing-intestinal-tissue of an umbilical cyst; B) Umbilical sinus with a band; C) Umbilical polyp covered with intestinal mucosa; D) The fibrous band containing a cyst; E) Meckel's diverticulum; F) Patent omphalomesenteric duct

Patient with patent omphalomesenteric duct can present with the anomaly itself or due to its complications secondary to the anomaly, including progressive prolapse of the omphalomesenteric duct, leading to a rupture of ileum as the compensation of high intraluminal pressure [6], [10]. In our case, the complications are ileal prolapse and intestinal perforation (rupture). This anomaly, which is known as a rare case, needs to be managed urgently for fear of gangrene of the prolapsed bowel and high risk of sepsis [3], [10], [13]. The appropriate treatment and timing of the surgery remain controversial. The principle surgical management of POMD is a reduction of the prolapsed bowel, complete excision of the omphalomesenteric duct, restoration of the ileal continuity, and umbilical reconstruction [11], [13].

At this moment we present a case report of a 1-year-old boy with patent omphalomesenteric duct (POMD) with proximal ileal prolapse and ileal perforation.

Case Report

A 1-year-old male infant was admitted in an emergency room with the chief complaint of bright red, polypoid-shaped mass emerging from the anterior abdominal wall with absent umbilicus. He was born at full term by normal spontaneous vaginal delivery to a porous 3 mother without antenatal care, helped by a midwife. The mother denied that the infant was born with the unusual abdominal mass, yet she stated convincingly the mass appeared since 1 week after delivery, with size initially 3 mm and three-looped shape.

She also noted peri-umbilical erythema, blood-mucus-containing umbilical drainage since 1 week after the infant was delivered. She also admitted the infant had recurrent fever and vomitus, yet he still passed gas and normal stool per rectally for the last one year. One day before admission, the infant was suffering from cough leading to protrusion of the red-coloured mass from the umbilicus. The mass which was initially small grew over a size of 3 cm within five hours. Five hours before admission, the infant presented feculent umbilical drainage with an absence of passing gas and faeces.

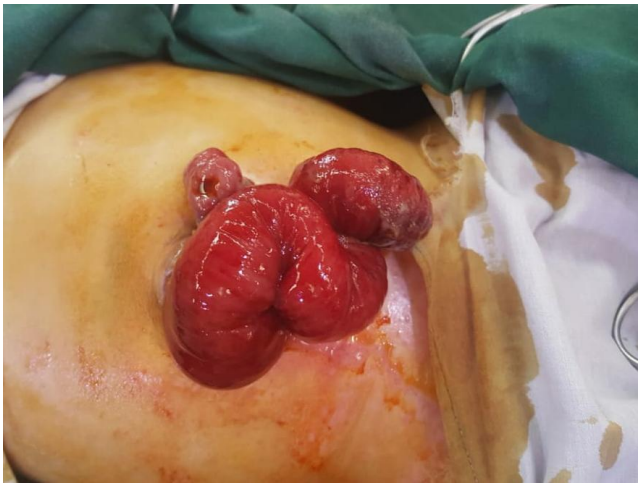

Figure 2: A Polypoid-shaped loop of intestine protruding from the umbilicus revealed prolapsed small bowel mucosa

On careful examination in the ER, the infant was pale but still comfortable and alert. Abdominal examination revealed a bright red polypoid-shaped loop of intestine protruding on the anterior abdomen (Figure 2). One of the tips of the mass was discharging feculent fluids. The mass was irreducible and bled on touch suggestive of mucosal surface. Bowel sound was normal. Anal opening was normally placed and patent.

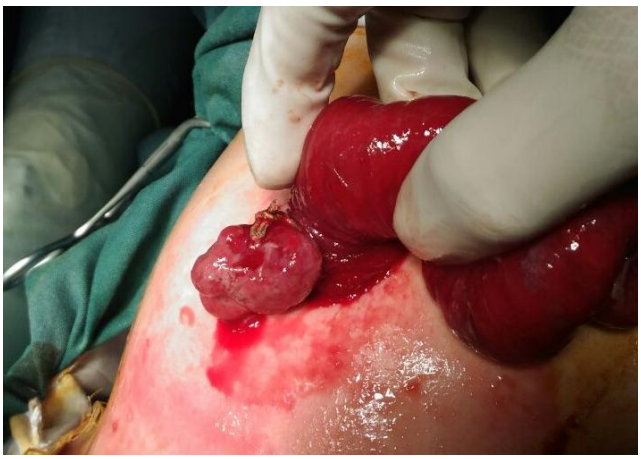

Figure 3: Prolapsed of ileum seen protruding from the umbilicus

The abdomen was neither distended nor

tender. He had passed clear urine twice, and the bladder was not palpable. Rest of the systemic examination was normal. A provisional diagnosis of the patent omphalomesenteric duct was made on clinical signs for the appearance of 5 cm duct connecting from bowel to the umbilicus and obvious feculent discharge.

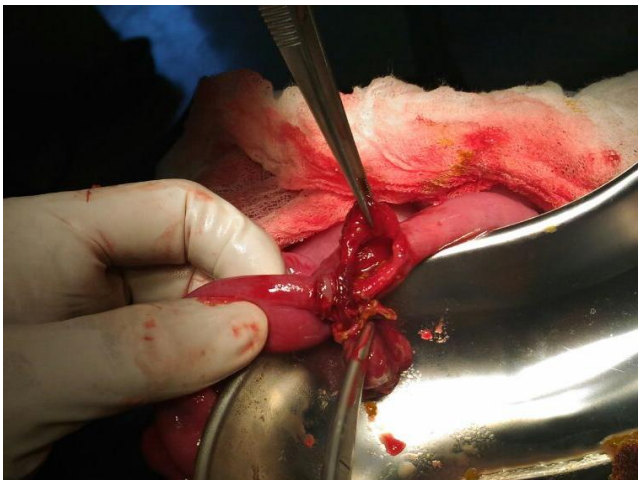

Figure 4: Intraoperative picture of the patent omphalomesenteric duct to the umbilicus with fistula seen in the picture

Blood investigations showed severe anaemia (Hb: 7.7 mg/dL) and leukocytosis (11.800/μL). To maintain the stability of the infant's condition and to prevent further complications, we performed emergency laparotomy surgery without further X-ray investigation nor ultrasound examination. The baby was initially managed and stabilised with the administration of intravenous fluid for rehydration, transfusion of 100 cc packed red cell, and intravenous broad-spectrum antibiotics cefotaxime (50 mg/kg/day).

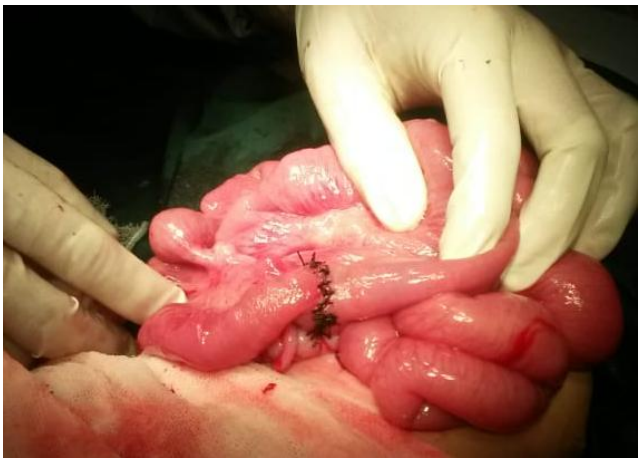

Figure 5: Intraoperative picture after resection and ileoileal end-to-end anastomosis at the site of prolapsed patent omphalomesenteric duct

A midline incision up to the abdominal cavity was made, and a 5 cm length of the proximal ileal loop that had prolapsed through a patent omphalomesenteric duct was separated from the abdominal wall by fine dissection and was meticulously reduced using milking method (Figure 3).

After a complete reduction, a defect of 5 x 5 cm rupture was found in the small intestine at the point of adherence with the abdominal wall suggesting its patency with the external environment through the umbilicus (Figure 4). The duct was released from the umbilicus. A patent omphalomesenteric duct with prolapse of ileum and ileal rupture was diagnosed.

Since there was no bowel oedema and the mucosa was healthy, ileostomy was not performed. Thus resection of the patent duct along with ileoileal end-to-end anastomoses was done (Figure 5). The infant started a small number of feeds on the 5th postoperative day. He was followed up for 3 weeks. Postoperative period was uneventful.

Discussion

The incidence of the patent omphalomesenteric duct is reported to be 1 in 5000 to 8000 newborns (approximately 2% of the population) and may begin at birth or occur within 1 to 2 weeks after delivery [6], [12]. This is suitable in our case, in which the mother admitted the appearance of unusual-red-coloured mass from the umbilicus on the first week after vaginal delivery [13]. POMD may remain silent throughout life or may present incidentally sometimes with an intrabdominal complication [5], [6].

The duct remains patent in 60% of OMDR cases and can present discharging umbilical sinus, umbilical nodule or polyp, and bleeding from the intestinal mucosa, with umbilical faecal drainage as the most symptomatic presentation of omphalomesenteric duct anomalies in developing countries [7], [14]. Another significant complication is either intussusception of the small bowel through the patent duct, which happened in 20% of the incidence of POMD or progressive prolapse of the omphalomesenteric duct, leading to polypoid-shaped bowel protrusion on the anterior abdomen which is seen in our case [5], [11].

Intussusception, volvulus, internal hernia (closed-loop obstruction) from the POMD, and a fibrous connection between umbilicus and ileum are the mechanisms of POMD causing small bowel obstruction [3], [4], [7], [10]. Meanwhile, the mechanisms of ileal prolapse through umbilicus is hypothesized by these two reasons, such as the wide mouth of the patent duct and the short distance between the patent duct and ileocecal valve in infants leading to high intraluminal pressure [2], [4], [5], [10], [12]. Besides, these conditions are exacerbated by the increased intraabdominal pressure, such as cry or cough [12]. In our case, rupture of ileum happened as

the compensation of increased intraluminal pressure caused by the end of the POMD pathogenesis, such as the small bowel obstruction. It explains the reason for infant's absence passing gas and faeces, which happened for 5 days before admission [1], [13].

Understanding the aetiology of small bowel obstruction caused by POMD without diagnostic laparotomy or laparoscopy is difficult. Abdominal plain radiographs and ultrasonography are non-specific for it. Although computerized abdominal tomography may be useful to show the band originating from the umbilicus and continuing between the small bowel loops, we did not perform them due to the lack of facilities and resources. Investigations like fistulogram were not performed as well since not only there is no need to differentiate POMD from patent urachus, but it also would not change the surgical decision in our case. In conclusion, we provisionally made the diagnosis based on history type of discharge (faecal) from the umbilicus along with clinical signs and confirmed it during laparotomy [6], [11].

Management options may include reduction of prolapsed bowel, definitive surgery such as laparoscopy or open laparotomy, wedge resection in a viable bowel, and intestinal resection in a non-viable bowel with complications of strangulation, gangrene, and perforation [4], [5], [6], [7], [10], [12], [15]. In our case, indications for emergency laparotomy are perforation and obstruction caused by ileal entrapment of the duct [15], [16]. In consideration that bacterial translocation may occur or as prophylaxis for resection, we administered broad-spectrum antibiotics although there are no controlled data about the antibiotherapy [16].

We believe this is an emergency case which must be dealt urgently due to the associated intestinal rupture caused by the prolapsed intestinal loop as any delay can lead to catastrophic consequences. In our case, despite the late referral of the patient to the hospital, we were able to stabilise the patient, performed prolapsed bowel reduction, resection of patent omphalomesenteric duct, and ileoileal end-to-end anastomoses. The infant was followed up for 3 weeks. Postoperative period was uneventful.

In conclusion, due to its rareness, this case is an important addition to the literature about patent omphalomesenteric duct with complications of inverted proximal ileal loop prolapse and ileal rupture.

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Case Report

Multiple Recurrent Acute Ischemic Strokes Treated by Thrombectomy in a Patient with Acute Pulmonary Embolism

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Keywords: Multiple mechanical thrombectomy; Recurrent large vessel occlusion; Acute ischemic stroke

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Abstract

BACKGROUND: Thrombectomy is recommended to treat for an acute ischemic stroke (AIS) patient with anterior large vessel occlusion. However, there were neither detailed guidelines nor systematic reviews of acute ischemic stroke patients having multiple times or re-occluded arteries.

CASE REPORT: In our case report, we struggled a multiple (4-times) AIS patient underwent by one intravenous r-tpA and 3 remaining of endovascular treatment of thrombectomy. Especially, the finding of both pulmonary embolism and cerebral arteries occlusion in this patient made us difficult to decide the appropriate treatment plan. The patient was considered having multiple cardiac thrombi pumping out to the brain and pulmonary vessels even in treatment with NOAC (New Oral Anticoagulant). Our priority, normally, was to recanalize the brain vessels compared to the pulmonary arteries.

CONCLUSION: In conclusion, based on this noticed case study, we want to share our experiences on the diagnosis of ischemic stroke, the strategy in treatment and prevention with anticoagulant therapy.

Introduction

Up to now, thrombectomy in the patient with acute anterior large vessel occlusion (LVO) has been recommended as the first choice of treatment for a high rate of recanalization [1]. However, as far as our knowledge, there is about 2.3% patients suffered from recurrent LVO stroke even within the first 48 hours had a very poor clinical outcome [2]. In such a special case, the indication of repeated thrombectomy is considered to be a good choice with the better result [3], [4]. In clinical practice, there are few case reports of patients with other arteries occlusion like pulmonary arteries embolism in combination with cerebral vessels thrombus. Although it is a very rare situation, it is still challenging for doctors in making a right decision. This case report aims to demonstrate our experiences in treatment of multiple recurrent acute ischeme stroke (AIS) due to occlusion of cerebral arteries associated with acute pulmonary embolism.

Case Report

A 53 years-old female patient came to our hospital at 8:45 am-75 minutes after the onset time (7:30 am), June 16th 2018 with the clinical symptoms of 3/5 left hemiplegia and dysarthria. The NIHSS (National Institutes of Health Stroke Scale) baseline was 5 and the blood pressure was 160/90 mmHg. Taking the past medical history showed that she had neither smoked nor taken oral contraceptives. The

after NCCT and CTA (Chloramphenicol Acetyl Transferase) indicated the ischemic lesions in the right insular with ASPECTS (Alberta Stroke Program Early CT Scan) baseline of 9 due to distal right M2 occlusion (Figure 1).

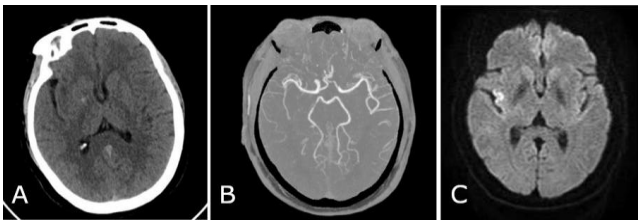

Figure 1: Imaging of the 1st stroke. A. NCCT; B. Distal R-M2 occlusion in CTA; C. MRI 24h post IV r-tPA

The patient was treated with intravenous (IV) r-tPA with a dosage of 0.9 mg/kg at 9:05 am (door to needle: 20 mins). 24 hours after treatment, she was stable with NIHSS (reduced to 2) and MRI follow-up (Figure 1) confirmed insular infarction with no extension or hemorrhage lesion. Transthoracic echocardiography and electrocardiogram (ECG) were performed without any abnormal indices. The glucose and lipid test resulted in a normal range. The patient was discharged 4 days after on June 20th 2018 with a prescription of Asprin 100 mg/day and Lipitor 10 mg/day.

Four months later, on October 14th, the patient suddenly suffered from the same symptoms (1/5 left hemiplegia and dysarthria) at home. She was transferred to our center at 7:25 am 40 mins after onset, with a GCS (Glasgow Coma Scale) of 14 and NIHSS baseline of 12. The CT (Computed Tomography) Scan revealed right M1 occlusion in combination with ipsilateral A1 thrombus. ASPECTS baseline was 8. Patients then underwent endovascular treatment (EVT) with thrombectomy by experienced neuro-interventions. The right cerebral arteries were both TICI 3 recanalization with one-time aspiration of Sofia plus 6F for M1 and one-time stent-retriever of Eric 4/24 for A1 (Microvention). All the images were illustrated in Figure 2. The clinical outcome after 24-hour follow-up was significantly improved with mild left hemiplegia 4/5 and reduction of NIHSS to 2. Peripheral Doppler ultrasound showed normal signs, but this time, using transesophageal echocardiography (TEE), we found a 3mm of foramen ovale with small right to left shunt, no thrombus found inside either left auricle atrium or pulmonary arteries. The patient was also indicated to take Xarelto (Rivaroxaban) 20 mg/day.

Unfortunately, just 1 day after hospitalization at 4:00 am on October 16th, the patient was discovered to have 2/5 left hemiplegia, and NIHSS was 10. Right M1 was re-occluded on CTA with a hyperdense sign on NCCT, and ASPECTS was 6.

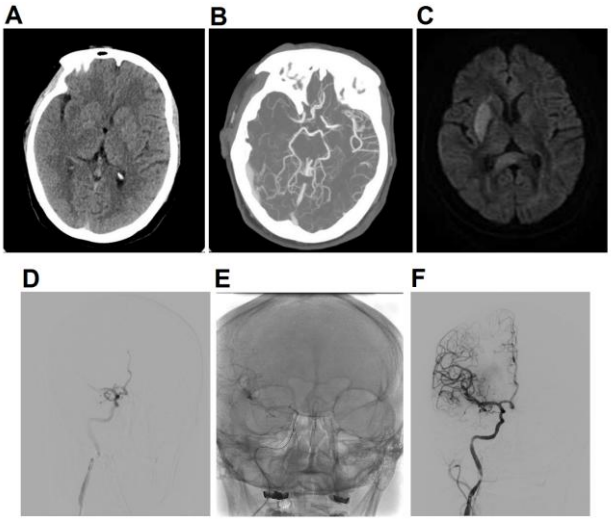

Figure 2: Imaging of the 2nd stroke. A. CT pre-procedure; B. CTA showed thrombus in both MCA and ACA; C. MRI 24h post thrombectomy; D. Angiography confirmed both MCA & ACA occlusion; E. Solumbra technique in MCA and stent-retriever in ACA; F. TICI 3 archived post thrombectomy

Figure 3 showed all the images and 2nd thrombectomy with TICI 3 archived after 1 pass of aspiration. No autoimmune disorder was found with normal results of antinuclear, antiphospholipid and anticardiolipin antibodies. The sinus rhythm was also recorded in ECG. On October 28th 2018, the patient was discharged again after 2 weeks of treatment with mRS 1 and prescription of Xarelto 20 mg/day.

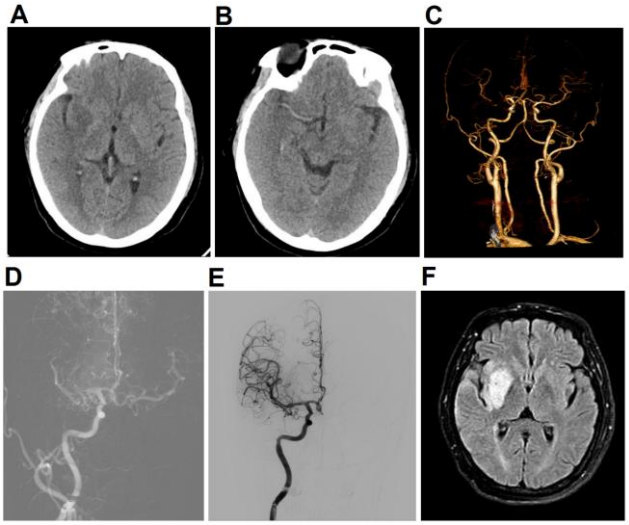

Figure 3: Imaging of the 3rd stroke. A. CT Scanner; B. R-MCA hyperdense; C. CTA confirmed R-M1 occlusion site; D. R-M1 re-occluded in DSA; E. 1 pass of Aspiration to archived TICI 3; F. MRI 24h post 2nd thrombectomy

The 4th recurrent ischemic stroke came at 6.40 am on November 7th with dyspnea and unconsciousness. Her admission was at 7:25 am – 45 mins later the onset. This time, she had right hemiplegia with a respiratory rate of 30 times/min, and SpO_2 result was only 88%. Due to these severe conditions, we indicated both cerebral and pulmonary CT Angiography for the patient (Figure 4).

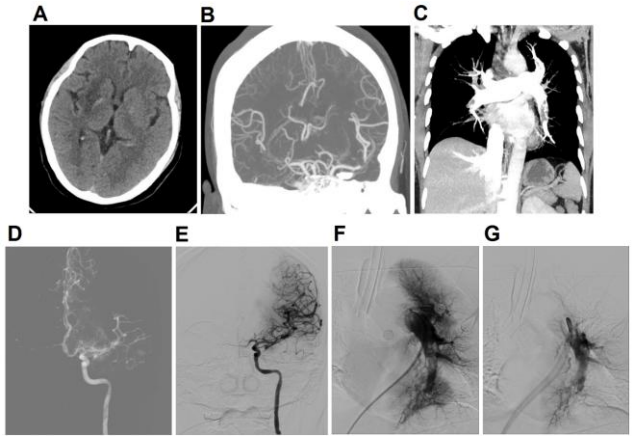

Figure 4: Imaging of the 4th stroke. A. 4th NCCT; B. CTA revealed L-MCA occlusion; C. Both pulmonary arteries thrombus identified with most one in the L side; D. Distal L-M1 occlusion in the DSA; E. TICI 3 revealed after aspiration; F. Angiography in the L-PA confirmed the diagnosis in CTA; G. L-PA after some aspirations

The results were distal left M1 occlusion in combination with pulmonary embolisms. The EVT was performed at 8:20 am with both femoral artery and femoral vein puncture by neuro-intervention team. After 1 pass of aspiration (Sofia Plus 6F – Microvention), the cerebral arteries was completely revascularized with TICI 3. Nextly, we used thrombectomy aspiration to take out most of the left pulmonary arteries embolism. The SpO_2 raised to 100% immediately thereafter. The patient then was intensively treated with a ventilator, Dobutamine and 24 hours of continuous Heparin infusion. Images following up after 1 day demonstrated no hemorrhagic lesion. Xarelto 15 mg was used twice a day. After 3 days, she became conscious without support from the ventilator and started to have rehabilitation. She then was discharged after 19 days, on November 26th, 2018. 1 month follow up showed good clinical outcome with mRS of 1.

Discussion

In this case, at the first stroke, the treatment with IV r-Tpa was successful because the occlusion just located at the small branches (distal site of right M2). The cardiac ultrasound at the first examination did not find the cardiac lesion so that we continued antiplatelet for prevention. In the 2nd stroke, she had the LVO of both right M1 and right A1. Even though the period between two strokes lasted more than 3 months meaning no indication for IV r-tPA, but we performed thrombectomy immediately without thrombolysis. As the result of the recurrent stroke, we still suspected the cause coming from a cardiac lesion. Therefore, TEE was done this time, and its results demonstrated a remnant foramen ovale. We decided to use Xarelto 20 mg/day for secondary preventative treatment, however, after only 6 hours, the patient suffered the 3rd stroke during the hospitalization due to right M1 occlusion. Imagine analysis in the CTA still supported us to continue with the second consecutive thrombectomy. This decision was similar to the ones done by other authors [3], [4], [5]. After discharge, our patient was prescribed continuously Xarelto, but she changed into Aspirin by herself without any consultant with us. Therefore, it led to her 4th stroke, meaning the 3rd stroke within only a month. In this recurrent time, she came with more severe condition (unconsciousness and unstable SpO_2) than all previous strokes. In CTA (Computed Tomography Angiography) images, not only the left distal M1 was occluded but also both pulmonary arteries embolism was identified.

In this situation, we rushed in the DSA room with thrombectomy for all two targets but aimed for the brain first because of its importance in making outcome. It was also our very first experience in ischemic stroke patient who have thrombus involved both cerebral and pulmonary arteries. Even having a successful operation with good recanalization, the patient was still in the fatal condition. Thank to our intensive care of treatment with the ventilator in combination with Dobutamine and Heparin infusion, she recovered progressively. After becoming conscious, she could breathe without ventilator support and do the rehabilitation with help. We decided to use Xarelto 15 mg 2 times/day for 3 weeks, then maintain a treatment with a dosage of 20 mg/day after discharge.

In conclusion, thrombectomy procedure treated for an ischemic stroke patient who has cerebral large vessel occlusion in combination with pulmonary arteries embolism seemed to be reasonable with an acceptable outcome.

Ethical Approval

The research was approved by the Ethics Committee of Hanoi Medical University, No 187/HĐĐĐĐHYHN on February, 20th 2016.

Informed Consent

Informed consent was obtained from the patient included in the study. The patient and her husband agreed both to participate in the treatment with understanding and allow our research group to use their studying image, data, and information in writing/publishing scientific article

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Exploring the Knowledge, Attitude and Practice Regarding Hepatitis B Infection Among Dental Students in Saudi Arabia: A Cross-Sectional Study

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Abstract

BACKGROUND: Hepatitis B infection is a universal health problem. Worldwide, 5% of health-care-related injections continued unsafe. Dentist considers being at high-risk group for cross infection. Therefore, their knowledge and practice towards Hepatitis B virus (HBV) positive patients should be at an optimal level.

AIM: The current study is aimed to evaluate and comparison of the knowledge, attitude and practice of Saudi dental students and interns towards HBV infection.

METHODS: A self-explanatory questionnaire comprising of 16 questions was designed to assess and compare students' knowledge, attitude and risk perception regarding hepatitis B infection among dental students across Saudi Arabia.

RESULTS: The response rate was 91.6% the overall knowledge of the participants was poor. The attitude was fair, with the female show a significant difference in attitude and practice (P-value < 0.05). The overall practice was good, 78.1% was vaccinated against HBV, and 73.2% stated that they regularly use personal protection equipment. The higher levels show a good attitude and practice compared with the lower levels; the difference was significant (P value < 0.05).

CONCLUSION: The overall knowledge was below average, continuous health education courses are mandatory.

Introduction

Hepatitis B infection is a universal health problem, caused by the hepatitis B virus (HBV), which is a DNA virus. The infection usually begins as subclinical or asymptomatic, except for some cases where acute symptoms are seen. Although it's a vaccine-preventable, can cause chronic infection and increase the risk of death from cirrhosis and liver cancer. Hepatitis B infection is endemic in tropical, under-developed and developing countries. Statistical data revealed that around two billion people around the world had been infected with HBV, and around 350 million people are known to be a chronic carrier for the virus. The case fatality rate is approximately one per cent [1]. According to WHO, the highest prevalence is in the Western Pacific region and the African region (6.2% and 6.1% respectively). Whereas, the WHO region of America is the least infected with a prevalence of 0.7% [2]. Saudi Ministry of Health (MOH) Statistical data have revealed that viral hepatitis is considered as second most common viral infection after chickenpox, with a high incidence rate of 19.8 per 100 thousand individuals [3]. In Saudi Arabia, the prevalence of HBV among blood donor found to be between 1.5% and 2.6% within the adult population [4].

The peak incidence of the HBV infection found to be in young adults, and most HBV infections are acquired through unprotected sex or needles sharing used by drug abusers [5]. Because of the highly sensitive virologic screening of donor blood, the risk of acquiring HBV infection from a blood transfusion is one in 2,300,000 [6]. Worldwide, 5% of

health-care-related injections continued to be unsafe [7]. This carries the risk of cross-infection in health care facilities. HBsAg is considered as a diagnostic marker for the primary infection because it is the first serological marker to appear in serum. Serological markers like anti-HBc, HBeAg and anti-HBe are used to evaluate the different state of the disease. Presence of Antibody to HBsAg (anti-HBsAg) in the serum is suggestive of withdrawal of HBsAg.

Transmission of infectious disease can occur easily in dental clinics [8]. Infections can be transmitted in the dental clinic through infected needles, direct blood contact, oral saliva, and indirect contact with contaminated instruments, operatory equipment, or environmental surfaces [9]. This can be prevented by the standard precaution that has been adopted by the U.S. Centers for Disease Control and Prevention (CDC) in 1996. This precaution should be applied by the dentist and dental team to overcome the risk of cross infection. Wearing of gloves by a dentist and dental assistant has been recommended as an essential component of cross-infection control [10], [11]. Hands are a major source of infection [11], and potentially infected blood may be retained beneath the fingernails for up to five days [12] unless there is meticulous mechanical cleansing. Serological studies in diverse parts of the world have found a higher prevalence of HBV infection with a higher possibility for transmission, among dentists, compared to the general population [13], [14].

Dental knowledge and attitude are the cornerstones in the prevention of diseases transmission. Several studies have been done to assess the level of students and dental practitioners. Keeping this in mind, the objective of this study was to evaluate the knowledge, attitude and practice behaviour of dental students and interns regarding infection control in central and Sothern Western areas in Kingdom of Saudi Arabia.

Material and Methods

The present cross-sectional survey was conducted from 15 June 2017 to 26 December 2017. A total of 789 dental students were randomly selected from the various dental cross across Saudi Arabia. A self-explanatory questionnaire comprising of 16 questions was designed to assess and compare students' knowledge, attitude and risk perception regarding hepatitis B infection among dental students across Saudi Arabia. The questionnaire was prepared in English language considering the language in which courses are taught to the student. English of the questionnaire was checked by the language expert. Face validity and content validity was assessed and evaluated by the expert in the field of research.

The questionnaire was comprised of four main domains: 1) Demography, course year of the participants, 2) Knowledge, 3) Attitude and 4) Practice of the participants towards hepatitis B infection. A pilot test was performed on 20 participants to evaluate the efficacy of the questionnaire. Only Saudi national students were selected for the survey any other nationalities were excluded.

The sample size was calculated depending on the following formula:

$$\frac{\frac{Z^2 \cdot p(1-p)}{e^2}}{1+\left(\frac{Z^2 \cdot p(1-p)}{e^2 N}\right)}$$

Where:

Z = Z value (1.96 for 95% confidence level)

p = percentage of picking a choice expressed as a decimal (0.5)

This was found to be 50% for the present study which was expressed as 0.50.

e = confidence interval, expressed as decimal (0.05)

n = total population of the region

By using the above formula, the minimum sample size was calculated to be 403.

Ethical clearance was obtained from the Institutional Ethics Committee (IEC), after a comprehensive review of the proposal. The importance of the study was explained verbally to the participants and written informed consent was obtained before the commencement of the study. Every attempt was made to maintain the confidentiality of the participants. The questionnaire was distributed among the participants during their Lecture and clinical hours; meanwhile, the research coordinates were around to answer any queries related to the questionnaire.

All the hypotheses were formulated using two-tailed alternatives against each null hypothesis (hypothesis of no difference). The entire data is statistically analysed using the Statistical Package for Social Sciences (SPSS version 21.0, IBM Corporation, USA) for MS Windows. Chi-square test was executed to compare the descriptive data. P values < 0.05 were taken as statistically significant.

Results

Out of total 789, 723 participants completed the questionnaire with a response rate of 91.63%. Among participants; 400 (55.3%) were female, and

323 (44.7%) were male. Out of the total; 19.8% (143) were interned, and 80.2% (580) was an undergraduate student. Among undergraduates; 20.6% (149), 30.6% (221), 29% (210) belonged to 4th, 5th and 6th year respectively. The age was range between (18-26) years with a mean age of 21.6 ± 4.7 years (Table 1).

Table 1: Demographic characteristic

Variables	Number (n)	Frequency (%)
Gender		
Male	323	(44.7%).
Female	400	(55.3%)
Age		
18-22	346	(48%)
23-26	377	(52%)
Year of Study		
4th year	149	(20.6%)
5th year	221	(30.6%)
6th year	210	(29.0%)
Intern	143	(19.8%)
Total	723	(100%)

Considering the knowledge score only 55 (7.6%) participant have a good level of knowledge, and about two-thirds have below the average of knowledge 64.7% (Figure 1). Regarding the knowledge, although about half of students and interns (53.8%) know that the chance for HBV transmission is more than that of HIV, while only 28.2% know the duration of HBV incubation period and only 13.6% know that most of the patients have no prominent symptoms. On the other hand, only about one-fifth of the participant was aware of how the virus is spread.

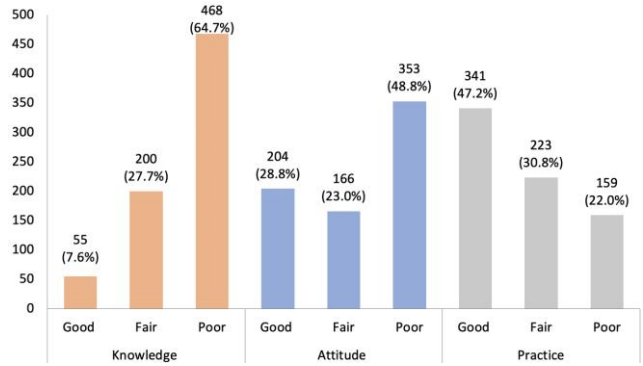

Figure 1: Overall distribution of Knowledge, Attitude, and Practice about HBV among total (n = 723) participants

Given the vaccination for HBV, two third of the participants (66.3%) know that a vaccine against HBV infection is available, but only half of them know the interval for the vaccination. On the other hand, about one third (29.9%) know that post-exposure vaccination is available. Regarding how to test for the HBV infection, 35.8% of them know that HBsAg is the confirmation test for active HBV infection.

Overall the participants presented a positive level of attitude towards HBV positive patients. Most of them consider that dentist have an ethical obligation to treat HBV patient, minority report that they don't have this obligation (8.3%). About half of them consider that HBV patient should be treated in a separate clinic. While 52.1% of participants confirmed that they go for a periodic Abs test for HBV, 57% have a strong worry about getting infected while treating a patient. Despite that, two third (64%) of participants stated that they would be able to treat patients more confidentially if they are immunised.

Meanwhile, 67.8% gave a solid knowledge about universal precaution protocol; about 73.2% stated that they use personal protection equipment (gloves and facemask) during clinical work. About one-third of them confirm that they have the professional knowledge to safely work with HBV patient. The score shows 48.8% of the participant to have a below average of attitude. Only 28.2% show a good level of attitude towards HBV (Figure 1).

When considering practice only one fifth do not receive vaccination for HBV. Almost half of the participants show a high level of practice at 47.2%. About one fifth (22%) show a low level of practice (Figure 1).

In comparing the knowledge of female to male, the female participants revealed slightly better knowledge than male participants, 7.4% of male show a good level of knowledge in comparison with 7.8% of female participants. 69.3% and 61.0% of male and female respectively show below the average of knowledge. The difference was slightly significant (P value = 0.047) (Table 2). While considering the attitude and practice, there was a significant difference between male and female participants with a P value of less than 0.001 for attitude and 0.012 for practice (Table 2).

Table 2: Knowledge, attitude, practice according to gender and year

Variable		Group	Below Average	Average	Good	P value
Knowledge	Gender	Female	61.0%	31.3%	7.8%	0.047**
		Male	69.3%	23.2%	7.4%	
	Year	4th year	62.9%	29.0%	8.1%	0.843*
		5th year	67.1%	25.5%	7.4%	
		6th year	63.8%	30.0%	6.2%	
		Intern	64.7%	27.7%	7.6%	
Attitude	Gender	Female	42.0%	26.3%	31.8%	<0.001***
		Male	57.3%	18.9%	23.8%	
	Year	4th year	51.4%	22.4%	26.2%	<0.001***
		5th year	44.1%	30.5%	25.4%	
		6th year	42.3%	19.5%	38.3%	
		Intern	44.3%	11.2%	44.6%	
Practice	Gender	Female	18.8%	29.5%	51.7%	0.012**
		Male	26.0%	32.5%	41.5%	
	Year	4th year	20.0%	38.1%	41.9%	0.166*
		5th year	22.2%	29.0%	48.9%	
		6th year	23.8%	30.1%	46.2%	
		Intern	22.8%	24.2%	53.0%	

*No significant difference; **Significant; ***Highly Significant.

When the comparison was made based on the year of study, the knowledge level was slightly better in the fourth-year students as compared to fifth and sixth-year students, with no statistic difference (P-value = 0.843). Knowledge for interns was comparable to lower level students. The attitude and practice of the student improved generally by achieving higher levels and the difference were significant for the attitude (P-value < 0.001) (Table 2).

Discussion

The result of this study reveals a higher number of female participants than male. The result also revealed that the knowledge of the dental student and the intern regarding HBV infection is generally below average. According to a study by Gayathri et al. [15] in India, and a study was done by Al-Shamiri et al., [16] in Saudi Arabia, the overall awareness of the student was fairly satisfying, which is consistent with our study results. There is a slight significant difference between the female and male in knowledge. Although one might expect that knowledge level will upgrade by year, but for this sample, the level of knowledge is higher for the lower levels compared with senior one. This could be because the junior levels study the general pathology and microbiology within their present courses. Interns showed better knowledge as compared to higher level students, but slightly lower when compared with lower level students. Improved knowledge with interns could be due to their preparation for higher studies and licensing examination.

Although most of the intern shows a low level of knowledge, they show the highest level of attitude and a good level of practice, which may indicate that they get familiar with cross-infection control measures to the level it is done routinely and that the infection control measurement is restricted. On the other hand, this brings to light the need for reinforcement of the knowledge through special courses or continuing education program. Overall, there is upgrading in the attitude score and the practice score, which may be explained by a firm regulation of infection control measurement by the infection control committees.

When considering the attitude of the students, higher level shows a good attitude comparing to low levels; the difference was significant (P value < 0.001). This might be explained by their less exposure to the clinical work compared with the higher levels. With their good level of attitude, the higher level shows the high level of good practice among the other group, although the difference was not significant. The female showed a better attitude than their male counterpart (P-value < 0.001). This was convenient with the result of the study done by Al-Shamiri et al., [16].

Since the dentist considers being at high risk to be infected by HBV [13], vaccination against HBV is an important line in the prevention of cross-infection, despite that about one-fifth of the participants do not receive HBV vaccination, this might be explained by the vaccination being a prerequisite for the clinic work. This was more than the result found by Al-Rabeah A, and Nagpal et al., which revealed that 63.5% and 64% were against hepatitis B virus respectively [17], [18]. According to the study done by Nagpal et al., [18] done at South Indian Dental College, about one third had knowledge about the post-exposure vaccination for HBV. The result of the present study coincides with the finding of above study.

A study by Vlaho Brailo' [19] found that the knowledge of the student increase by going farther in their dental studies, which is partially applicable for this study. He also stated that their knowledge was not consistent with their attitude towards treating and dealing with HBV, and HIV positive patients. This was the reverse of our study result, in which the students have a low level of knowledge and a good attitude towards the patients with HBV, which may indicate a restrict measure by the infection control committees in the dental clinics.

Jain et al., [20] in his survey among Mumbai intern found that most of the intern knows about the HBV transmission and vaccination, but few of them have knowledge about the possibility of post-exposure vaccination. His results were in the same line with this study result. Malhotra et al., [21] in his study stated that the students have suitable knowledge regarding hepatitis B, but still there was a gap in pushing their knowledge into practice. This contradicts the result from the present study; overall the students have an unsatisfactory level of knowledge with an acceptable level of attitude.

Finally, since the cross infection can be barred by infection control measurements, which is an important part of health professions, [22] most of the participants (73.2%) stated that they use personal protection equipment during clinical work. This result was less than that by Nagpal et al., [18] where 85.8% of the participants were aware of the preventive measurement about HBV.

In conclusion, this study showed that despite the low level of knowledge (the knowledge need to be improved), the attitude and practice was acceptable for the participants. This indicates that more obligatory courses regarding HBV infection, transmission and vaccination should be incorporated in the curriculum of the dental colleges. The obtain of HBV vaccination before clinical work and the boosting doses in the right intervals, besides reporting any sharp object stick should be closely monitored by infection control committees.

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The Determinants of Salivary Cotinine Concentration in Smokeless Tobacco Users

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Abstract

BACKGROUND: Smokeless tobacco products due to their high nicotine content are highly addictive and ultimately lead to an increased risk of oral cavity, laryngeal and oesophagal cancer.

AIM: This research was conducted with the aim of assessing the relationship between the salivary cotinine concentration and demographic characteristics and smokeless tobacco use for the first time in tradespeople in Chabahar, Iran.

METHODS: This descriptive cross-sectional study was conducted on 150 different tradespeople using smokeless tobacco in Chabahar who were selected through simple random sampling in 2018. In addition to the salivary cotinine measurement, data were collected using a researcher-made questionnaire with demographic and behavioural items. The data obtained were analysed in SPSS-16 using descriptive and inferential statistics.

RESULTS: The mean salivary cotinine score was 887.7 ± 180.7 in men and 611.2 ± 139.7 in women, making for a significant intergroup difference (P = 0.043). The mean salivary cotinine score was higher in those who used two or more smokeless tobacco products compared to those who used one type of tobacco, and a significant difference was observed between the type of smokeless tobacco used and the salivary cotinine score in the participants (P = 0.005).

CONCLUSION: Based on the results of the regression analysis, the type of smokeless tobacco used was a strong predictor of the concentration of salivary cotinine in the participants. It is, therefore, necessary for the government to adopt appropriate policies and take educational measures to reduce the vending and use of these substances.

Introduction

Almost one-fifth of the tobacco used in the world is smokeless form [1]. Smokeless tobacco products are highly addictive due to their high nicotine content [2]. These products also contain carcinogenic compounds such as Tobacco-Specific N-nitrosamines (TSNAs), which eventually lead to an increased risk of oral cavity, laryngeal and oesophagal cancer [3]. An increased risk of mortality due to cardiovascular diseases has also been observed among smokeless tobacco users [3]. Around 10 million people are estimated to die of tobacco use in developing countries by 2030, and this figure is higher than the figures estimated for AIDS, drug abuse, road accidents, murder and suicide [4].

There are wide ranges of smokeless tobacco products throughout the world [5]. Naswar, Gutka, Pan Parag, BT and Mawa are some of the common smokeless tobacco products among tradespeople in Chabahar [6]. All brands of smokeless tobacco that are sold for oral or nasal-use have nicotine and nitrosamines as ingredients [7]. Also, smokeless tobacco components consist of tobacco, areca nut, slaked lime, and spices [8]. The proximity of Chabahar to Pakistan and the illicit import of various smokeless tobacco products in attractive packaging has led to the wide availability of these products in the market and stores around this city [9]. The poor knowledge of tradespeople about nature and side-effects and ease of access to these substances have led to their increased use [9]. The nicotine content of a smokeless tobacco product may vary depending on its type [10]. The measurement of nicotine and its metabolite in smokeless tobacco users is therefore important for understanding its addictive potential [10].

Cotinine is a sensitive and special quantitative index for learning of the amount of nicotine absorption over the preceding few days [10]. This concentration is assessed by the rate of nicotine metabolism in body fluids [11], [12]. Saliva currently provides an appropriate diagnostic alternative to other body fluids, as it offers a cost-effective, simple and non-invasive method that does not require any particular expertise for sample collection [13], [14].

Very few studies have investigated the relationship between cotinine concentration and smokeless tobacco use behaviour in the users of these products [3], [13], [15].

The present study was therefore conducted to examine the relationship of salivary cotinine concentration with demographic details and smokeless tobacco use behaviour among tradespeople in Chabahar.

Methods

The present descriptive cross-sectional study was conducted in Chabahar, Iran, in 2018. After obtaining permission from the university ethics committee, 150 different tradespeople in Chabahar entered the study with informed consent. The study inclusion criteria consisted of using at least one type of smokeless tobacco, age 20 to 50 years, the ability to answer the questionnaire items and a trading license from the Guilds Office. The exclusion criteria consisted of age less than 20 or above 50 years, smoking cigarettes, hookah, etc., and being a seasonal tradesman. Simple random sampling was used in the study. To this end, the environmental and occupational health division of the health centre was visited and using the health records, a list of participants was extracted from 18 urban and rural comprehensive health service centres, and those eligible for inclusion in the study were selected through simple random sampling. In addition to measuring the salivary cotinine, data were also collected using a researcher-made questionnaire in two parts, including a part on demographic details and some behavioural items. The demographic questionnaire contained seven items, including age, gender, type of trade, education, marital status, use of smokeless tobacco among family members and use of smokeless tobacco among friends and peers. Behaviour questionnaire contained five items, including the type of smokeless tobacco used, age at the onset of use, the daily frequency of use, the saliva disposal method and the use of tobacco in public places.

The amounts of salivary cotinine in the 150 participants were assessed using the ELISA method and the US-made Salimetrics kit (LOT: 1710502, EXP: 2019-07-11). With the help of some facilitators, the researcher collected participants' saliva samples by spitting method. For this purpose, the candidates were asked to visit the center in a fasting state from one hour before the test and to refrain from alcohol consumption 12 hours before the test and to rinse their mouth properly before the test samples were to be taken (for 10 minutes), and then spit into a test tube via a glass funnel for at least five minutes. At least 5 ml of saliva were collected from each candidate, and the samples were delivered to the laboratory of the comprehensive health service centre of Polan (a village in Chabahar County) with the cold chain maintained and daily. At the laboratory, the salivary samples were frozen at -20°C in an ultra-low temperature freezer. Finally, after these steps were over, all the samples were tested together according to the kit's specific instructions.

The data obtained from the questionnaire were analyzed in SPSS-16 using descriptive statistics (frequency, mean and standard deviation) and inferential statistics (the Kolmogorov-Smirnov test for assessing the normality of the distribution of salivary cotinine, the ANOVA and independent t-test for assessing the relationship of the salivary cotinine concentration with the demographic variables and use behaviors, and the regression analysis for predicting the concentration of salivary cotinine through the variables related to tobacco use). The level of statistical significance was set at $P < 0.05$.

Results

The majority of the participants were male (59.3%), and Most of them were in the 20-30 age group (66.7%). The use of at least one type of smokeless tobacco was reported as 11.3% among the family members and 81.3% among close friends (Table 1).

Table 1: Participants' demographic details

Variable		Count	Percentage
Age (years)	20-30	100	66.7
	30-40	40	26.7
	40-50	10	6.6
Gender	Male	89	59.3
	Female	61	40.7
Marital status	Single	55	36.7
	Married	95	63.3
Trade type	Confectionery	7	4.7
	Drapery	22	14.7
	Supermarket	20	13.3
	Auto repair	17	11.3
	Hairdressing	35	23.3
	Dressmaking and embroidery	9	6
	Wholesale	22	14.6
	Other (hoteling, restaurant, carpentry, etc.)	18	12
Education	Illiterate	19	12.7
	Primary School	25	16.7
	Junior High School	23	15.3
	High School and Above	83	55.3
Use of one type of smokeless tobacco products among family members	Yes	17	11.3
	No	133	88.7
Use of one type of smokeless tobacco products among five close friends	Yes	122	81.3
	No	28	18.7

Dividing the results by gender, Naswar was the most frequent type of smokeless tobacco used by the participating women (83.6%), but most of the participating men (67.42%) used a combination of two or more smokeless tobacco products. A significant relationship was found between the type of smokeless tobacco used and gender (P = 0.000). Dividing the results by age at the onset of use, approximately 86% of the participating men and women had started tobacco use before and after the age of ten. A significant relationship was also observed between age at the onset of use and gender (P = 0.000). Concerning the frequency of use, the majority of the participants (53.33%) used these products more than five times per day. Overall, 70% of the participants spit their saliva into public pathways in an unhygienic manner after consumption. Furthermore, the majority of the participants (62%) used these products in public places. A significant relationship was also found between this variable and gender (P = 0.007) in this study (Table 2).

Table 2: Participants' smokeless tobacco use behaviour

Tobacco Use Behavior		Total Count (percentage)	Male Count (Percentage)	Female Count (Percentage)	P-Value
Type of smokeless tobacco	Pan Parag	2 (1.33)	2 (2.24)	0	0.000
	Gutka	19 (12.66)	11 (12.36	8 (13.12)	
	Naswar	66 (45)	15 (16.86)	51 (83.6)	
	Mawa	1 (0.66)	1 (1.12)	0	
	A combination of two or more	62 (41.34)	60 (67.42)	2 (3.28)	
Age at the onset of use	Below 10	85 (56.67)	77 (86.52)	8 (13.11)	0.000
	Above 10	65 (43.33)	12 (13.48)	53 (86.89)	
Frequency of daily use	Less than 5 times	70 (46.67)	41 (46.07)	29 (47.54)	0.869
	5 times and more	80 (53.33)	48 (53.93)	32 (52.46)	
Disposal of saliva	Hygienic	105 (70)	63 (70.79)	42 (68.85)	0.857
	Unhygienic	45 (30)	26 (29.21)	19 (31.15)	
Use of tobacco in public places	Yes	93 (62)	63 (70.79)	30 (49.18)	0.007
	No	57 (38)	26 (29.21)	31 (50.82)	

The mean score of salivary cotinine was 666.5 ± 119.3 ng/ml in the 30-40 age group, which is higher than that in the other age groups. No significant relationship was observed between the salivary cotinine score and age group (P = 0.295). The mean salivary cotinine score was 887.7 ± 180.7 ng/ml in the women and 611.2 ± 139.7 ng/ml in the men, which makes for a significant difference (P = 0.043). In terms of marital status, the mean salivary cotinine score was higher in the single than the married participants, although not significantly (P = 0.69). The mean salivary cotinine score was the highest in the auto repair business, but the difference was not significant (P = 0.1) between the various businesses (Table 3).

Table 3: The relationship between the demographic variables and the concentration of salivary cotinine in smokeless tobacco users

Variable		Cotinine (ng/ml)		df	F	P-Value
		Mean	SD			
Age (years)	20-30	666.5	119.3	2	1.23	0.295
	30-40	831.5	18.5			
	40-50	112.2	22.7			
Gender	Male	887.7	180.7	148	3.26	0.043
	Female	611.2	139.7			
Marital status	Single	1073.0	132.5	148	0.78	0.69
	Married	642.0	364.0			
Trade type	Confectionery	310.5	150.5	9	1.66	0.1
	Drapery	642.0	364.0			
	Supermarket	831.5	18.5			
	Auto repair	887.7	180.7			
	Hairdressing	660.0	118.3			
	Dressmaking and embroidery	333.5	220.5			
	Wholesale	22.5	528.7			
	Other (hoteling, restaurant, carpentry, etc.)	370.6	220.5			

The mean salivary cotinine score was 1068.5 ± 131.2 ng/ml in the participants who used two or more smokeless tobacco products, which is higher than in those who used other forms of tobacco and a significant difference was thus observed between the type of smokeless tobacco used and the salivary cotinine score (P = 0.005). The mean salivary cotinine score was higher in the participants who had started using smokeless tobacco before the age of ten (760.0 ± 207.6 ng/ml), but no significant relationship was observed between age at the onset of use and the mean salivary cotinine score (P = 0.746). The mean salivary cotinine score was lower in those who had reported their frequency of use as less than five times per day compared to those who had reported their daily use like 5 times and more; however, the difference between these variables was not significant (P = 0.776; Table 4).

Table 4: The relationship between tobacco use behaviours and the concentration of salivary cotinine in smokeless tobacco users

Variable		Cotinine (ng/ml)		df	F	P-Value
		Mean	SD			
Type of smokeless tobacco	Pan Parag	703.5	73.5	4	3.93	0.005
	Gutka	501.0	18.5			
	Naswar	627.0	160.3			
	Mawa	4.0	18.0			
	A combination of two or more	1068.5	131.2			
Age at the onset of use	Below 10	760.0	207.6	148	1.33	0.746
	Above 10	719.3	72.2			
Frequency of daily use	Less than 5 times	790.0	42.8	148	0.118	0.776
	5 times and more	662.0	133.3			

The results of the regression analysis showed that, of the six subscales (gender, trade type, marital status, type of smokeless tobacco used, age at the onset of use and daily frequency of use), only one subscale (type of smokeless tobacco used) predicted the concentration of salivary cotinine significantly (P = 0.001); that is, with every standard deviation of increase in the score of the type of smokeless tobacco used, the score of the concentration of salivary cotinine increased by 0.66 standard deviations (Table 5).

Table 5: The prediction of salivary cotinine concentration through the demographic variables and tobacco use behaviour using the multivariate regression analysis

Predictor Variable	B	SE	Beta	T	P-value
Constant	-47.7	246.1	-	-0.19	0.84
Gender	97.4	90.9	0.13	1.07	0.28
Trade type	6.3	10.4	0.05	0.6	0.54
Marital status	-30.9	57.9	-0.04	-0.53	0.59
Type of smokeless tobacco used	98.4	27.9	0.33	3.53	0.001
Age at the onset of use	-9.3	81.7	0.01	-0.11	0.9
Frequency of daily use	45.2	56.1	0.06	0.8	42

Discussion

The present study was conducted to assess the relationship of the concentration of salivary

cotinine with demographic variables and smokeless tobacco use behaviours for the first time among tradespeople in Chabahar. Another exclusive feature of the study is that it assessed all types of smokeless tobacco products commonly used in Chabahar. The results obtained showed that auto repair businesses had the highest mean salivary cotinine score (888 ± 181ng/ml), while the confectionery trade had the lowest score (311 ± 151 ng/ml); however, the difference between the businesses was not significant in this respect (P > 0.005). In a study conducted by Ferketich et al., [16], the mean salivary cotinine score was 581 ± 364 ng/ml in full-time workers and 389 ± 264 ng/ml in part-time workers.

In this study, the mean salivary cotinine score was very high in both men and women (749.5ng/ml) compared to the other studies on the subject [15], [16], [17], [18], [19].

According to the results, the mean salivary cotinine score was higher in the participants who used two or more types of smokeless tobacco products compared to those who used only one type, and a significant relationship was observed between the type of smokeless tobacco used and the salivary cotinine score (P < 0.005). Naswar was the most commonly-used type of smokeless tobacco (84%) by the women, while the men most commonly used a combination of two or more different types (67%). One of the reasons for this difference between the genders could be the social norms of the society under scrutiny, as women's use of other types of smokeless tobacco products (such as Pan Parag, Gutka, Mawa, BT, etc.) is not acceptable in the cultural fabric of the society. The mean salivary cotinine score in people who used two or more different types of smokeless tobacco was lower in other studies compared to in the present study –revealing an inconsistency between findings [3], [16], [20].

The mean salivary cotinine score was higher in those who had started using smokeless tobacco before the age of ten compared to those who had started after the age of ten (760 ± 208 ng/ml vs 719 ± 72 ng/ml), but there were no significant relationships between the age at the onset of use and the mean salivary cotinine score (P > 0.005). In other words, those who had started using smokeless tobacco from a long time ago had a higher mean salivary cotinine score. The longer duration of use might affect the salivary cotinine concentration in people who use these products. In the study by Ferketich et al., [16], the mean salivary cotinine score in people who had started using smokeless tobacco before the age of ten was 610 ± 387 ng/ml, which is less than that found in the present study.

According to the present findings, the mean salivary cotinine score was higher in people whose daily use of smokeless tobacco products was less than five times compared to in those who used these products more than five times per day (790 ± 43 ng/ml vs 662 ± 133 ng/ml), but the difference between these variables was not significant (P > 0.005). In Ferketich's study [16], the mean salivary cotinine score was higher in those whose daily use of smokeless tobacco was more than 48 times compared to those whose daily use was less (620 ± 400 ng/ml vs. 483 ± 321 ng/ml), which disagrees with the present findings in this regard. This disparity of findings could be due to the self-reporting nature of that study (i.e. false reports of the frequency of use), which is often one of the main challenges in research in various fields of science.

The mean salivary cotinine score was 888 ± 181 ng/ml in men and 611 ± 140 ng/ml in women, which suggests a significant difference between the genders (P < 0.005). One of the reasons for this difference could be the type of product used by the two genders. The most commonly-used product in women was Naswar (84%), while most of the men used a combination of two or more products (67%), which explains the much higher concentration of salivary cotinine in men (who used two or more types of smokeless tobacco products) compared to women (who only used Naswar) in the present study. In a study conducted by Huque et al., [3], the mean salivary cotinine score was 399 ng/ml in men and 361 ng/ml in women, which shows no significant differences between the genders in terms of the concentration of salivary cotinine, as inconsistent with the present findings. The results obtained by Asha et al., [21], however, nearly agree with the present findings.

In the present study, the mean salivary cotinine score was higher in the single compared to the married participants (1073 ± 133 ng/ml vs 642 ± 364 ng/ml); however, the difference between the two groups was not significant (P > 0.005). In Ferketich's study [16], the mean salivary cotinine score was 364 ± 254 ng/ml in the single and 562 ± 319 ng/ml in the married people, which disagrees with the present findings, since it shows a higher mean concentration of salivary cotinine in the married compared to the single people and also a significant relationship between marital status and the concentration of salivary cotinine. In a study conducted by Binnie et al., [22], the mean salivary cotinine score was higher in the married compared to the single people, which disagrees with the present findings. The disparity of findings between the present study and the two cited studies could be due to the false reports of marital status or type of tobacco products used.

The results of the regression analysis in the present study showed that, out of the six subscales of demographic variables and tobacco use behaviors, only one subscale, i.e. the type of smokeless tobacco used, predicted the concentration of salivary cotinine significantly (P < 0.001); that is, with every standard deviation of increase in the score of the type of smokeless tobacco used, the salivary cotinine concentration score increased by 0.66 standard

deviations. In the other studies, too, this variable could predict the concentration of salivary cotinine in the participants [3], [16]. In a study conducted by Lorina et al., [20], subscales including age, the frequency of daily use and age at the onset of use predicted the concentration of salivary cotinine in tobacco users.

One of the limitations of this study is the self-report nature of data collection about smokeless tobacco use behaviours, such as the frequency of use, age at the onset of use, type of smokeless tobacco used, etc.

In conclusion, according to the results of the regression analysis, the type of smokeless tobacco used is a strong predictor of the concentration of salivary cotinine. It is, therefore, necessary for the government to adopt appropriate policies and educational measures to reduce the vending and use of these substances. Similar studies are recommended to be conducted with larger sample sizes on other groups of the users of these products.

Informed Consent

Informed consent was obtained from all individual participants included in the study.

Ethical Approval

The Ethics committee of the Shahid Sadoughi University of Medical Sciences-Yazd approved this study. Ethic code: IR.SSU.SPH.REC.1396.105.

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Relationship of Benzene Exposure to Trans, Trans-Muconic Acid and Blood Profile of Shoe Workers in Romokalisari Surabaya, Indonesia

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Abstract

BACKGROUND: Benzene is a hazardous ingredient for health. Benzene is used as a latex glue solvent in the shoe industry.

AIM: The purpose of the study was to analyse the relationship between benzene exposure with trans, trans-muconic acid (t, t-MA) and the blood profile of shoe workers in Romokalisari Surabaya.

METHODS: The study was a cross-sectional design conducted in the shoe industry in Romokalisari Surabaya with some subjects of 20 shoe workers. Data collection was carried out by measuring benzene levels conducted at 8 measurement points in Surabaya Romokalisari, taking workers' blood, measuring body weight and conducting interviews with respondents. Data were analysed using correlation tests.

RESULTS: The results showed that there was no relationship between benzene levels with t, t-MA (p-value = 0.205), there was no relationship between benzene Risk Quotient (RQ) and t, t-MA (p-value = 0.271) and there was no relationship between the Excess Cancer Risk (ECR) and blood profile of workers in Romokalisari. However, there were some abnormal blood profile parameters due to exposure to benzene although it was small.

CONCLUSIONS: It seems another factors such as length of work, nutritional status, duration of exposure, weight, and frequency of exposure have a considerable contribution in the determination of intake of xenobiotic ingredients in the body to cause health effects especially blood profiles.

Introduction

The footwear home industry sector is one of the informal industrial sectors whose influence is strong on Indonesia's development with a total value of US $ 1.51 million which contributes 3% of the world's demand for footwear products [1], [2]. The process of making shoes in the informal shoe industry is always accompanied by factors that contain health and safety risks so that they are very vulnerable to biological hazards, chemical, physical and psychological hazards [1]. The hazardous substances present in the glue are volatile organic compounds (benzene, toluene, and xylene) which fall into the volatile VOC (Volatile Organic Compound) category [3].

Unfortunately, large and small industries use toluene and benzene as a mixture or reagent although it was hazardous material [3]. Benzene is a toxin with higher health risk and included in the category of carcinogens A1 (a confirmed human carcinogen)

while toluene and xylene are included in the category A4 (not classifiable as a human carcinogen). This means that benzene is a carcinogen in humans whose termination of the bond is more difficult when compared to toluene and xylene [4].

Benzene is used as a latex glue solvent in the shoe industry [5], [6]. Benzene vapour in a low concentration could cause poisoning to humans if it were inhaled continuously. The negative effect of benzene such as bone marrow damage that occurs latently due to the metabolite reaction of benzene epoxide [7].

Benzene that enters the body will undergo phase I biotransformation with the cytochrome P450 2E1 (CYP 2E1) enzyme to become benzene epoxide, a compound that is unstable and will undergo oxidation to form trans, trans-muconaldehyde and then t, tMA) that will be released through urine. Relevant and sensitive indicators for measuring exposure and doses of benzene that enter the body, one of which is to use biological indicators (biomarkers), namely t, t-MA contained in the urine because it has a sufficient half-life that is 6 hours unlike benzene in the blood has a short half-life in the blood so the time taken for sampling is also very short [8].

The study conducted in Pakistan showed significant results from increasing the number of total leukocytes, lymphocytes, eosinophils, and monocytes as well as decreased platelet counts and neutrophil counts in shoemaking workers when compared to groups control [9]. Also, research conducted in Thailand about the workers exposed to benzene can alter haematological parameters with a significance of changes namely in decreasing haemoglobin, hematocrit and eosinophil count with trans, trans Muconic Acid (t, t- MA) in urine as a body biomarker [10].

Research in Indonesia, one of them shows that child labour in the Cibaduyut shoe industry in Bandung is threatened by various types of respiratory tract infections, bronchitis, liver and or kidney damage, even leukaemia [5]. Research in the same place conducted on showed that the results of tests carried out on the concentration of benzene exposure with blood profiles obtained several significant variables, namely haemoglobin level, erythrocyte levels, and eosinophil levels [11]. Other studies show that the working environment with adequate benzene vapor content (0.138-6.271 ppm) in the industrial gluing section of Tasikmalaya City sandals is able to provide a health effect that is strengthened by nearly 70 percent of workers experiencing respiratory problems and feeling dizzy due to long periods of steam [1]. Another study shows non-cancerous and cancerous effects due to benzene exposure for leather shoe industry workers in Pulogadung Small Industrial Center (PIK) [12].

The purpose of this study was to analyse the relationship between benzene exposure with trans, trans-muconic acid and the blood profile of shoe workers in Romokalisari Surabaya.

Material and Methods

This study was an observational study with a cross-sectional approach in Romokalisari Surabaya (home industry in Tambak Oso Wilangun Village) with seven home industry locations conducted during October-November 2016. The research subjects were all shoe craftsmen in Tambak Oso Wilangun Village with a total of 20 people with female and male workers who met the inclusion criteria, namely not menstruating, not pregnant, not taking certain drugs/anaesthesia and alcohol and in good health. The variables are toxicity score benzene, t, t-MA level, and blood profile.

Benzene measurements were carried out at 8 points in 7 work locations during the day (12.00-14.00 WIB) with the consideration this time is the peak time of work in using glue, the temperature is still quite high so that it can cause benzene in the glue to evaporate quickly so that the device can be caught air suction (vacuum pump) [12]. Benzene measurements are carried out by experts from the laboratories of the Surabaya City Health and Safety Technical Implementation Unit (UPTK3). Sampling of urine (samples of trans-trans Muconic-Acid) was carried out by asking the study subjects to accommodate 50 ml of urine in the urine pot that was provided and taken after the expiration period lasted about 7-8 hours while for measurement t, t-MA was carried out by the method Mass Spectra Liquid Chromatography by Prodia Surabaya laboratory. The method was NIOSH Methode 1501 with a sample taken by benzene vapour carried out by Gas Chromatography (GC)/FID & HC Analyzer. Set GC injector as 225°C, detector set in 225°C, the initial column in 50°C, hold time 3.0 minutes then 15°C/minutes to 200°C. Then, measured benzene levels will be compared to exposure limit standard of benzene according to REL NIOSH 2005 (normal if < 0.01 ppm) and Ministry of Manpower/Transmigration Indonesia No. 13 of 2011 (normal if < 0.5 ppm). Blood collection was done by taking 2-3 cc of venous blood from the workers and then examined using a Blood Cell Counter with blood cell examination performed using a haematology analyser.

Blood count is a measured hemoglobin level compared to the standard reference of male hemoglobin between 13.2-17.3 gr/dL = normal, and female hemoglobin levels between 11.7-15.5 gr/dl = normal, measurable hematocrit levels compared with references between 40-52% = normal, measured leukocyte levels were compared with reference values between 3,800-10,600 µ/L = normal and calculation of

percentage results by referring to standards namely 50-70% neutrophils, 2-4% eosinophils, basophils 0-1%, lymphocytes 25-40%, and monocytes 2-8% [13].

Data collection was carried out by interviewing respondents using questionnaires and observations related to the characteristics of respondents and measurements of body weight and height. The analysis in this study was carried out by conducting correlation analysis using Spearman and Pearson (α = 0.05).

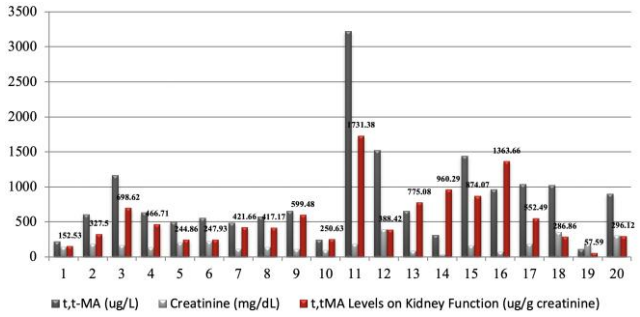

Figure 2: Trans Levels, Trans-Muconic Acid Workers in Romokalisari

Results

Measured Benzene Levels at Research Sites (ppm)

Based on Figure 1 shows that there are 2 locations where benzene exposure exceeds the benzene threshold value according to Ministry of Manpower and Transmigration Regulation No. 13 the year 2011 (> 0.05 ppm) which is the location V of 0.9129 ppm and location VI is 2.333 ppm [14]. Meanwhile, workers gender has the same percentage (50% female and 50% male).

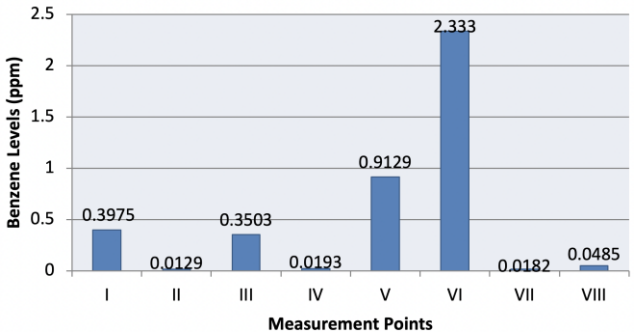

Figure 1: Measured Benzene Levels in the Study Area (ppm)

Trans, Trans Muconic Acid

Figure 2 shows the levels of trans, trans-muconic acid urine of the study subjects and correction of creatinine (µg/g creatinine) was carried out. The figure shows the highest t,t-MA level of 1,731.38 µg / g creatinine, which is the respondent at the 7th point of work location, while the lowest t,t-MA level is 57.59 µg / g creatinine, the respondent is at the 8th work point. From Figure 2 also shows that as many as 8 research subjects had levels of t, t-MA were not normal or outside the threshold / Biological Exposure Indices (BEI).

Blood Profile of Shoe Workers

Table 1 shows the levels of each blood profile parameter that is the subject of the study, namely the shoe craftsmen in Romokalisari Surabaya.

Of all subjects, the highest haemoglobin level was 16.4 g/dL, and the lowest was 9.6 g/dL, the highest hematocrit level was 46.5%, and the lowest was 27.7%, the highest leukocyte level was 15.430 µ/L, and the lowest level was 5,250 µ/L, the highest eosinophil level was 17% and the lowest was 0%, the highest neutrophil level was 93% and the lowest level was 37%, the highest basophil level was 1% and the lowest was 0%, the highest lymphocyte level was 46% and the lowest by 22% and the highest monocyte level by 11% and the lowest level by 3%.

Table 1: Blood Profile of Shoe Workers in Romokalisari

Sample (M/F)	Hemoglobin (g/dL)	Hematocrit (%)	Leukocyte (uL)	Eosinophil (%)	Basophil (%)	Neutrophil (%)	Limphocyte (%)	Monocyte (%)
1 (M)	15.8	45.9	7270	8	0	48	34	10
2 (M)	14.9	42.5	8370	3	0	64	27	6
3 (M)	15.8	46.5	5250	4	1	54	34	7
4 (M)	15.2	43.1	5320	5	0	58	28	9
5 (M)	14.9	42.9	7030	6	0	50	37	7
6 (F)	12.9	39.4	9050	3	0	45	46	6
7 (M)	15	44.5	11310	6	0	57	28	9
8 (F)	9.6	27.7	8700	4	0	64	26	6
9 (M)	16.4	46.5	9920	2	0	54	37	7
10 (F)	14.2	41.5	9130	7	0	61	26	6
11 (F)	12.8	38.1	8800	3	0	67	24	6
12 (F)	12.8	35.7	6690	2	0	61	29	8
13 (F)	13.3	39.6	8050	17	0	53	22	8
14 (M)	14.6	41.9	8720	4	0	60	26	10
15 (F)	13	37.6	15340	7	0	93	4	3
16 (F)	13.7	40.3	9620	4	0	65	24	7
17 (M)	14.2	40.3	8300	3	0	63	27	7
18 (M)	14.8	43.5	7510	7	1	37	45	10
19 (F)	15.1	42.9	15430	5	0	58	26	11
20 (F)	14	41.8	9830	4	0	53	37	6

Table 1 also shows the blood profile of the subjects in the normal category or not. Categorisation of grade values from each blood profile refers to the reference guidelines for standardised reference values by ISO 15189: 2012 regarding the specific requirements of medical laboratories used by the Surabaya Parahita Laboratory as partners in examining these blood profiles. Of the 20 research subjects, 19 respondents had normal haemoglobin levels, and only 1 respondent had a low haemoglobin level, as many as 19 respondents had normal hematocrit levels and as many as 1 respondents had low hematocytes, 17 respondents had normal leukocyte levels and 3 respondents had high leukocyte levels, as many as 11 respondents had normal eosinophil levels, as low as 1 respondent and as many as 8 respondents had high eosinophil levels, as many as 16 respondents had normal neutrophil levels, 3 respondents had low neutrophil levels, and as many as 1 respondents had high neutrophil levels, 20 respondents have normal basophil levels, as many

as 14 respondents had normal lymphocyte levels, low as many as 4 respondents and as many as 2 respondents had high lymphocyte levels, and as many as 14 respondents had normal monocyte levels, and 6 respondents had high monocyte levels.

Relationship of Benzene Exposure to Trans, Trans-Muconic Acid

Table 2 shows that there is no correlation between benzene exposure and t,t-MA because the *p-value* > 0.05 (*p-value* = 0.205).

Table 2: Relationship of Benzene Exposure to Trans, Trans-Muconic Acid Shoe Workers in Romokalisari

Variable	*p-value*	*r*	N
Concentration of Benzene Trans, Trans-Muconic Acid	0.205	0.295	20

Intake of Benzene

The formula used to determine the intake of benzene toxin in the body is(14):

$$\text{Intake of benzene} = \frac{\text{CxRxtExfExDt}}{\text{Wb x tavg}}$$

Description:

C = benzene concentration (mg/m^3)

R = respiration rate (m^3/h)

tE = exposure time (hour/day)

fE = average exposure in a year (day/year)

Dt = exposure duration (years)

Wb = weight (kg)

Tavg = average of benzene exposure (non-carcinogen)

= 30 years x 365 days/year

Risk Quotient (RQ)

The formula used to find out the RQ is [15]:

$$\text{RQ} = \frac{Intake}{RfDatauRfC}$$

Risk characteristics for non-cancer effects can be known by sharing the value of Noncancer Intakes with RfC or RfC values with the assumption that:

1. If the RQ value ≤ 1 indicates an indication of the possibility of the risk of noncarcinogenic health effects, it must be maintained so that the numerical value of RQ does not exceed 1.

2. While RQ > 1 shows an indication of the possibility of risk of non-carcinogenic health effects and the need for control efforts [15].

The respondent's RQ value is as follows:

Table 3: Value of Risk Quotient Benzene in Romokalisari

Respondent	Intake Non-carcinogen	Risk Quotient
1	0.004084	0.480471
2	0.003391	0.398941
3	0.004022	0.473176
4	0.008403	0.988588
5	0.010251	1.206
6	0.003022	0.355529
7	0.004593	0.540353
8	0.000481	0.056588
9	0.009434	1.109882
10	0.006952	0.817882
11	0.228851	26.92365
12	0.0288	3.388235
13	0.155914	18.34282
14	0.171152	20.13553
15	0.078304	9.212235
16	0.112532	13.23906
17	0.120726	14.20306
18	0.175	20.58824
19	0.38811	45.66
20	0.181243	21.32271

Table 3 shows that most of the RQ shoe workers in Romokalisari are > 1 that is 12 respondents and 8 other respondents the RQ value is < 1.

RQ relationship with t, t-MA

Correlation test results show that there is no correlation between benzene RQ and t, t-MA workers in Romokalisari because the p-value is > 0.05 (0.271).

Table 4: Relationship between Risk Quotient Benzene and Trans, Trans-Muconic Acid Workers in Romokalisari

Variables	*p-value*	*r*	N
Trans, Trans-Muconic Acid RQ Benzene	0.271	0.258	20

Excess Cancer Risk (ECR) with a Blood Profile

When calculating cancer risk, lifelong exposure is needed. CSF (Cancer Slope Factor) value uses the value set by US-EPA of 0.055 mg/kg. Day. In calculating the health risk characteristics for the effect of cancer is the ECR which is used is the CSF value.

ECR values or risk characteristics for cancer effects can be obtained by multiplying the value of cancer intake by CSF values by the formula [15]:

ECR = Carcinogenic Intake (Ik) x CSF

Assuming the results of the ECR calculation are:

If the ECR is -4 10-4, then the concentration of benzene exposure to workers is not at risk of causing carcinogenic advisory effects.

If ECR > 10-4, the concentration of benzene exposure to workers can cause carcinogenic health effects.

The ECR value for the 20 respondents is as follows:

Table 5: ECR Value of Shoe Workers in Romokalisari

Respondent	Intake Carcinogen	ECR
1	0.00175	0.00009625
2	0.001453	0.000079915
3	0.001724	0.00009482
4	0.003601	0.000198055
5	0.004393	0.000241615
6	0.001295	0.000071225
7	0.001969	0.000108295
8	0.000206	0.00001133
9	0.004043	0.000222365
10	0.002979	0.000163845
11	0.098079	0.005394345
12	0.012343	0.000678865
13	0.06682	0.0036751
14	0.073351	0.004034305
15	0.033559	0.001845745
16	0.048228	0.00265254
17	0.05174	0.0028457
18	0.075	0.004125
19	0.166333	0.009148315
20	0.077675	0.004272125

Table 5 shows that the ECR value of all shoe workers in Romokalisari is > 10-4, which means that exposure to benzene at work sites can cause carcinogens in the worker's body.

ECR relationship with Blood Profile

Table 6: ECR relations with Blood Profile Workers in Romokalisari

Variables	*p-value*	*r*	N
Hemoglobin ECR	0.723	0.085	20
Hematocrit ECR	0.629	0.115	20
Leukocyte ECR	0.734	0.081	20
Eosinophil ECR	0.734	-0.081	20
Basophil ECR	0.644	0.110	20
Neutrophil ECR	0.697	-0.093	20
Limphocyte ECR	0.776	0.068	20
Monocyte ECR	0.254	0.268	20

Table 6 shows the results of the correlation analysis between the ECR and the blood profile of shoe workers in Romokalisari. Based on the results of the correlation test, it was found that there was no correlation between the ECR and the blood profile of shoe workers in Romokalisari.

Safe Duration of Exposure (Safe Dt)

In this study risk management was carried out in the form of reducing the duration of exposure to shoe workers in Romokalisari.

Safe Dt Non-Carcinogen

Calculation of safe DT non-carcinogen namely [15] :

$$\text{Safe Dt} = \frac{RfCxWbxtavg}{CxRxtExfE}$$

The safe Dt value of shoe workers in Romokalisari is as follows:

Table 7: Safe Value of Non-Carcinogen Workers in Romokalisari

Respondent	Safe Dt (Year)
1	70.8
2	50.1
3	33.8
4	40.5
5	29.9
6	47.8
7	55.5
8	53.0
9	22.5
10	29.3
11	1.6
12	2.4
13	2.3
14	1.1
15	2.2
16	2.0
17	2.2
18	1.2
19	0.4
20	0.7

From the data above shows the duration of safe exposure for each shoe worker in Romokalisari Surabaya, that is each worker is different. It can be seen that shoes workers 1 to 10 has a safe duration of exposure to get non-carcinogen effects from benzene exposure for decades. Unlike the 11th to 20th shoe workers who have a safe duration of exposure to get non-carcinogenic effects from benzene exposure very little time (< 3 years).

Safe Dt Carcinogen

Calculation of safe DT non-carcinogen namely(14):

$$\text{Safe Dt} = \frac{Wb\ x\ tavg\ x\ ECR}{C\ x\ R\ x\ tE\ x\ fE\ x\ CSF}$$

The safe Dt value of shoe workers in Romokalisari is as follows:

Table 7: Safe Value of Carcinogen Workers in Romokalisari

Respondent	Safe Dt (Year)
1	3.53
2	2.50
3	1.69
4	2.02
5	1.49
6	2.39
7	2.77
8	2.65
9	1.12
10	1.46
11	0.08
12	0.12
13	0.12
14	0.06
15	0.11
16	0.10
17	0.11
18	0.06
19	0.02
20	0.03

From the above data shows the duration of safe exposure to the carcinogen effect of benzene exposure for each shoe worker in Romokalisari Surabaya is that each worker is different. It is seen that shoe workers 1 to 10 has a safe duration of exposure to get carcinogen effects from benzene exposure < 4 years. In contrast to the 11th to 20th shoe workers who have a safe duration of exposure to get a carcinogen effect from benzene exposure very

little time is < from 1 year.

Discussion

Benzene exposure measurement results in the study location showed several measurement points that benzene levels exceeded the threshold limit value (TLV) of benzene in the workplace according to the regulation of the minister of workforce No.13/MEN/X/2011, namely the normal limit of benzene levels of 0.5 ppm [14]. The measurement point for which the benzene TLV exceeds the standard NAV is at 2 measurement points, namely at points 5 and 6 which are 0.9129 ppm and 2.333 ppm respectively.

From the measurement results showed that there was a very large difference between the measurement points below and above the benzene TLV such as the difference in benzene levels between location 2 which had the lowest benzene levels and locations 5 and 6 with the highest benzene levels. This difference is influenced by several factors such as the number of shoes produced, the method/method of work done by workers, the type of raw material used in the production process and the presence of adequate ventilation.

The number of shoes produced by workers is proportional to the amount of glue used. The more shoes that are produced, the more glue is used. At the location of the highest NAV, shoes are 35-40 Kodi/week or around 700-800 pairs, while the location below the TLV produces 37 Kodishoes/week. At the highest location, TLV uses 9-10 kg of yellow glue and 6-7.5 kg of white glue to produce shoes, while at the location below the NAB, 8 kg of yellow glue and 5 kg of white glue is used.

The high level of benzene at the work site is due to a large amount of glue used during the production process. When the glueing process of the shoe occurs, the evaporation of glue in the air will be greater which can affect benzene levels in the air. Moreover, if the glue container is not closed again after use as is often the case at point 5 when observing. This is in line with another research shows a large amount of shoe production in line with the use of glue which is quite a lot is one of the causes of high levels of benzene in the leather shoe industry in PIK Pulogadung [5].

Based on observations in the field, almost all study locations lack air exchange or ventilation especially at locations 5 and 6. In locations 5 and 6 the condition of the window/ventilation is closed, the air exchange only relies on a fan. Also, in locations 5 and 6 the working conditions were not sufficient because of a large number of equipment and raw materials for making shoes that were not neatly arranged.

Biological monitoring (biomarker) is one of the monitoring conducted to evaluate the degree of exposure to workers that have been absorbed into the body of the worker [16]. Benzene in urine is one of the most important biomarkers for benzene and trans exposure, trans-muconic acid is the most sensitive and specific metabolite for benzene exposure because it can be detected in urine for benzene exposure with a concentration of < 1 ppm [8]. T-level, t-MA tolerated by the human body is < 500 µg/g creatinine [17].

Based on the results of the correlation test, it was shown that there was no correlation between benzene exposure and t level, t-MA shoe workers in Romokalisari. From the description of the levels of t, t-MA workers and benzene concentrations in the work environment showed that from 20 respondents, only 3 respondents were in benzene concentrations above the benzene threshold in the work environment and most of the workers had normal t, t-MA levels. In general, benzene that enters the body has 2 possibilities, namely metabolised in the body and then cause acute and chronic effects or excreted/excreted into urine metabolites, in this case, the metabolite t-MA [8].

A metabolite of t, t-MA urine is only used as a biological marker for people exposed to benzene. The manifestation of an individual exposed to benzene is seen from the metabolite. The level of t, t-MA in urine is the result of the metabolism of benzene, the more benzene inhaled, the greater the benzene that enters the body and is excreted [6]. The results of the correlation test analysis in this study indicate that there is no significant relationship between benzene RQ and t, t-MA. This research is not in line with the research which concludes that there is a relationship between RQ and t, t-MA in workers in Tambak Oso Wilangun, Surabaya. The results of the study did not show a significant relationship because workers with benzene RQ > 1 mostly had normal tt-MA levels [18].

In general, benzene that enters the body has 2 possibilities, namely metabolised in the body and then cause acute and chronic effects or excreted/excreted into urine metabolites, in this case, the metabolite t, t-MA [19]. Based on the results of a study that shows no correlation between RQ and t, t-MA means that benzene that enters the worker's body is mostly metabolized into the body. Also, out of the 20 shoe workers studied, only 3 workers working in the work environment whose benzene exposure exceeds the benzene threshold value, thus making the majority of t, t-MA normal workers. Worker nutrition can also influence the absence of a relationship between benzene RQ and t, t-MA levels. Some studies stated that there is a less form of t, t-MA metabolites in urine if the person has higher enzyme activity CYP 21. Also, the normal nutritional status of workers will cause the metabolic process in the body

to run normally [20].

It can be seen that the results of the calculation of cancer risk level for each worker have a result of ECR > 10-4 calculation, the results explain that the exposure time is currently shoe workers in Romokalisari who have cancer health risks. There are several epidemiological and clinical studies that prove that long-term benzene exposure causes leukaemia, so it is classified as a carcinogenic substance in humans (Group 1) by the IARC [19]. Research conducted on crude oil mining workers found a decrease in leukocytes and platelets in workers, this is due to workers who are exposed to high benzene continuously 24 hours and long lasting which indicates a chronic haematological effect of their work [21].

The results showed no association between benzene concentrations with levels of t, t-MA urine, benzene RQ with t, t-MA and ECR with blood profile of research subjects, although there were abnormal levels of t, t-MA from the study subjects and there are also some abnormal blood profile parameters, but overall the levels of t, t-MA and blood profile of the study subjects were normal. This is due to hematologic disorders (pancytopenia, aplastic anaemia, thrombocytopenia, granulocytopenia, and lymphocytopenia) which are associated with benzene in the workplace occurring chronically [6].

In this study, there was no relationship between ECR and all blood profile parameters. This is indicated by only a small percentage (3 respondents) of the study subjects who were at the work location/measurement point with benzene levels above the benzene threshold with blood profile levels that were outside the normal values such as only in lymphocyte and blood monocyte levels. Also, most of the abnormalities of blood levels exist in the study subjects at the work site/measurement point with benzene levels below the threshold with blood profile levels outside of normal, i.e. haemoglobin levels, hematocrit, leukocytes, eosinophils, and neutrophils. From this, there are many factors that make no relationship between these variables, in other words, the variable benzene in the air is not a determining factor about blood profile abnormalities in the subjects of the shoe craftsmen in this study.

There are other factors such as length of work, nutritional status and others that can contribute to the absence of a relationship between ECR variables and blood profile. This is in line with the theory of Louvar and Louvar in determining exposure assessment regarding the amount of chemical intake received by individuals, where the factors of work duration, duration of exposure, weight, and frequency of exposure have a considerable contribution in determination of intake of xenobiotic ingredients in the body so that they can cause health effects [22]. In addition to this, the Agency for Toxic Substance and Disease Registry states that the presence of hematologic disorders (changes/abnormalities) in blood levels from the presence of benzene chemicals in the work environment occurs chronically, which requires a very long time to see the existence of the disorder [6].

The results of research conducted on petroleum industry conclude benzene levels in an inhaled work environment by employees (workers) (< 1 ppm) did not appear to be significantly at risk of causing haematological anaemia with a work period of 3-16 years [23]. In addition to the research conducted in a study of benzene exposure in the work environment in South Korea showed there was no significant relationship between haematological parameters and benzene air levels [24].

The concentration of benzene exposure to shoe workers in Romokalisari Surabaya is affected by the air condition at the location of the workers, and many of the materials are not in the form of shoe glue used for the production of shoes. From the calculation of the duration of safe exposure to get non-carcinogen and carcinogen effects it can be seen that some workers have a safe duration of exposure to get very large carcinogen and non-carcinogen effects that reach up to decades, but the parts again have a safe duration of exposure to get very small carcinogenic and non-carcinogenic effects that only reach < 1 year.

Like workers 19 and 20 have a safe duration of exposure to get non-carcinogenic effects only 0.4 and 0.7 years and have a safe duration of exposure to get the effect of carcinogens only 0.02 and 0.03 years. This is proportional to the RQ and ECR values of each worker. The 19^{th} and 20^{th} workers have a value of RQ > 1 and ECR > $10^{-4,}$ so it has a risk to get carcinogen and non-carcinogen effects faster than other workers. This is because the 19th and 20th workers are shoe workers who are at work locations with the highest benzene levels, namely locations 5 and 6 so that workers are exposed to more than other workers.

In conclusion, there is no correlation between benzene levels and t, t-MA, there is no relationship between RQ and t, t-MA and there is no relationship between the ECR and all the profile parameters of the blood workers in Romokalisari. Safe duration exposure of workers varies and is directly proportional to the RQ and ECR values of each worker. Although there is some effect found some abnormal blood profile parameters due to exposure to benzene. However it is so small. The caused for this difference with other studies such as length of work, nutritional status, duration of exposure, weight, and frequency of exposure have a considerable contribution in the determination of intake of xenobiotic ingredients in the body to cause health effects especially blood profiles.

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Data Availability

The manuscript data used to support the findings of this study have been deposited in the Relationship of Trans Kadar, Trans-Muconic Acid (Tt-Ma) In Urine with Hematological Profile In Coco Spbu WorkersPertamina Mor V with accessed on http://repository.unair.ac.id/45529/on Airlangga University.

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Dark or Bright Half of the Moon: A Qualitative Study Exploring the Experience of Iranian Heart Failure Patients Regarding their Quality of Life

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Abstract

BACKGROUND: Heart failure (HF) is a major public health problem in different societies and has numerous impacts on quality of life (QOL).

AIM: The present study was carried out with the aim to explore the experience of HF patients regarding the negative effects of the disease on their QOL.

METHODS: In this qualitative exploratory study data collection was performed through face-to-face, semi-structured, in-depth interviews with 19 patients with HF, who were selected through purposive sampling method from April to September 2017. Data analysis was carried out based on the framework analysis method.

RESULTS: The negative consequences of HF on QOL emerged in the form of 6 main themes including symptoms, disease complications, cognitive impairment, psychological distress, functional limitations and economic problems. Most of the participants (14 out of 19) assessed their QOL as well or very well.

CONCLUSION: The majority of the patients in this study, despite the many negative impacts of HF, had a high QOL that could indicate their satisfaction and effective coping with HF by creating a positive outlook and the perceived positive effects of the disease.

Introduction

Heart failure (HF) *is a major* and growing *public health* problem worldwide. Twenty-six million people in the world suffer from HF [1]. In Iran, patients with HF account for 3.3% of the population [2]. This condition is caused by myocardial injuries, ischemic heart diseases, hypertension and diabetes. It can also be caused by cardiomyopathies, valvular heart diseases, myocarditis, infections, systemic toxicity and cardiotoxic drugs. HF is a chronic progressive condition in which the heart cannot meet the metabolic demands of the body [3]. The 1-year mortality has been reported in 20% of HF patients, and 5-year mortality has been reported in 59% of men and 45% of women [4]. Patients experience physical and emotional symptoms such as dyspnea, fatigue, oedema, sleep disorders, depression and chest pain, which can disrupt their quality of life (QOL) [5]. Maintaining a good QOL is as important as survival to patients with HF. QOL in patients with HF is lower than the general population and those with other chronic diseases [6], [7]. The prognosis of this condition is worse than that of many malignant diseases, and its unpredictable future is considered as one of its main features [8]. Health status deteriorates progressively due to the heart's deficiency in pumping out blood, which is characterised by acute episodes of the decompensated symptom.

Therefore, patients require frequent, long-term and costly hospitalisations [9].

Moreover, the available evidence suggests that changes in patients' health status may not necessarily affect QOL [10]. According to the World Health Organization (WHO), QOL means one's perception of his or her position in life in the cultural context and value systems in which one lives and is related to one's goals, expectations, standards and concerns [11]. As a dynamic, subjective and multidimensional concept, the QOL does not reflect the physiological status of patients; therefore, in similar clinical situations, patients have different perceptions of QOL [5], [12]. Our search for the perceived negative effects of HF on QOL in Iran showed that no research had been conducted in this area. However, Understanding the negative effects of HF on QOL will lead to the provision of effective supportive interventions, increased health promotion and reduced burden of disease in patients and their families. Since qualitative studies enable the researcher to perceive the experiences of the participants to the highest possible extent [13], the current study has been conducted using this method. The present qualitative study aimed to explore the perceived negative effects of HF on QOL from the perspective of patients with HF and their self-assessment of QOL.

Methods

Study Design and Participants

This study had a qualitative exploratory design. Purposive criterion sampling was initiated and continued until data saturation. The inclusion criteria consisted of informed consent, the confirmation of HF diagnosis and the ability to speak Farsi. The exclusion criterion was severe psychiatric or cognitive problems. Out of the 19 participants in this study, 14 patients were admitted to Imam Reza Hospital, consisting of those who had referred to the HF subspecialty clinic. The other 5 participants had referred to a specialised HF outpatient private clinic in Mashhad, Iran.

Data collection

The data was collected through face-to-face, individual, semi-structured, in-depth interviews. All of the interviews were conducted by one of the researchers (H.H) in the Cardiovascular Research Center in a quiet room, far from any noise. The interview questions (topic guide) consisted of: Would you please explain about your life after being affected by HF? Has HF affected your QOL? What are the negative effects of HF on your QOL that have led to your disappointment? What is the greatest negative effect of HF on your life? What is the most important limitation caused by HF in your life? What would you rate your QOL from zero to ten? Please explain why you rated your QOL this way? After obtaining the participants' permission, the interviews were recorded using a voice recorder. After conducting each interview, the researcher responsible for conducting the interviews would write down all the field notes and the recorded voices at the first possible chance. They were written word-by-word; first, handwritten, and then, typed. All the interviews were conducted from April to September 2017, and each one lasted 35-90 minutes, with an average of 60 minutes. The individual and clinical features of the participants were extracted from their medical records.

Data analysis

In this study, the analytic process based on the framework method presented by Ritchie and Spence has been implemented [14], [15]. The data analysis consisted of 5 major stages; familiarisation with the interviews, developing a thematic framework, indexing, charting and interpretation and mapping [14]. In this study, the process of analysis consisted of a series of separate phases, based on the independent cooperation of at least two researchers by whom a consensus was reached. The research team reviewed the transcribed interviews and field notes over and over, and in some cases listened to the participants' voices to become familiarised with data, obtain a general perspective and immerse in the data, and therefore, became aware of the main ideas recurring in the data. In this way, the preliminary thematic framework was designed based on the interviews, prior thoughts and literature, and was discussed among researchers in a series of iterative meetings. Then, the corresponding author read the transcribed interviews line-by-line to mention the themes introduced through the topic guide. The text of the interview was individually coded by two researchers [(H.H) and (S.M)]. The coding was appropriately adjusted after a consensus was reached by the two above-mentioned researchers. The reliability of the final coding scheme was monitored and verified by a clinician (F.V), a health science researcher (M.M) and a sociologist (SS.M) through recording an accidental sample. Then, the transcribed interviews were entered through the codes' links into the index thematic framework. Thus, a chart was created for each theme, in which the interviews were placed in rows and the related sub-themes were placed in columns [16]. In the phase of interpretation and mapping, the researchers discussed the significance of the nature of the themes and how themes can be described and interpreted in regard with the research objectives, theories and the related models. The data were analysed manually without using the software. To increase data credibility, the researchers read the transcripts over and over and immersed in the data for a long time. Inserting the

interview quotations in the findings reflect the credibility and dependability of the study. All research processes, from developing the study to the discussion of findings, have been carried out through the collaboration of at least 2 researchers. The researchers' triangulation led to a decrease in the preconception risks or dominant prejudices in collecting data and results; thus, resulting in the study's trustworthiness.

Moreover, analytical triangulation or peer debriefing was used in the data analysis process. One of the researchers who was an expert in the field evaluated and verified the data analysis process. Data transferability was increased by providing the specification of the participants' profiles and research environment, as well as considering the heterogeneity of the samples. To verify the validity of the data analysis results, 6 of the participants were contacted to ensure that the results were consistent with their experiences (member check).

Ethical Consideration

The Ethics Committee of Gonabad University of Medical Sciences, Iran, provided the necessary ethical approval for the study (Reg. No: IR.GMU.1395.18). All participants signed an informed consent form, were made aware of the study's objectives, and were assured that their data would remain anonymous. They were also informed of their right to withdraw from the study at any time.

Results

The mean age (SD) of the participants was 59.5 (12.4) years, and the mean duration of the disease was 5.2 years. The mean (SD) of ejection fraction was 23.1% (8.3). Most participants were men (68.4%) and retired (42.1%). The mean (SD) of education degree was 11.4 (5.7) years of study.

Table 1: Themes and subthemes of the negative effects of heart failure on the quality of life

Main themes	Subthemes			
Symptoms	Fatigue	Shortness of breath	Sleep deprivation	
Disease complications	side effects of medications	Comorbidities		
Cognitive impairment	Memory loss and concentration impairment	Decision-making problems		
Psychological distress	Depression	Anxiety		
Functional Limitations	Loss of independence	Loss of sex	Loss of jobs	Loss of social activities
Economic problems	Inability to pay medical expenses	Inability to pay living expenses		

From the transcripts, 98 initial codes were obtained. These codes were then summed up into 6 themes as well as 15 subthemes. The 6 themes emerged which addressed the negative effects of HF on QOL were symptoms, disease complications, cognitive impairment, psychological distress, functional limitations and economic problems (Table 1).

Symptoms

This theme includes the subthemes of fatigue, shortness of breath and sleep disorders. Most patients stated that HF has caused fatigue and reduced their energy levels. A *63-year*-old man said: "*The most important negative effect of HF is fatigue. Sometimes, I feel I do not even have the power to raise a cup of tea" (p9, retired).* Most of the participants also complained of shortness of breath. A *62-year*-old woman said: *"While doing activities, I get out of breath" (p11, housewife)*. A participant in Functional Class IV said: *"I suffer from shortness of breath even while talking" (p18, 37-year-old, higher education)*. Also, many patients were having difficulty falling asleep and remaining asleep. A 69-year-old man commented: *"As I am retired, I sleep during the day, so I cannot sleep a wink at night and stay up till morning" (p6).* They blamed their sleep deprivation on anxiety, orthopnea, sleeping during the day and overnight insomnia, as well as frequent overnight urination and the need to go to the washroom repeatedly. Therefore, some of them took hypnotics.

Disease complications

This theme includes the subthemes of the side effects of medications and comorbidities. One-third of the participants complained about the side effects of the medications*: "I resent my medicine box. This much medicine hurts you" (p1, 63-year-old, retired).* Generally, the participants felt depressed about a large number of tablets and the combination of drugs they were prescribed because they did not realise the purpose of pharmacotherapy. HF is usually accompanied by comorbidities such as diabetes, hypertension and kidney disease. *"Every moment, I think blood is clotting in my vessels; my blood pressure is* fluctuating ..." (*p11, 62-year-old, housewife*).

Cognitive impairment

This theme includes the subthemes of memory loss, concentration impairment and decision-making problems. About a quarter of the participants were experiencing cognitive impairments such as memory loss and concentration impairment. *"Since the time I was affected by HF, I have forgotten where my things are" (p17, 48-year-old, housewife).* A *41-year-old* woman said: *"I need to read something several times to comprehend the material" (p13, higher education).* These patients are unable to concentrate for a long time, and they do not enjoy simple activities such as reading. Patients also need

to optimise shared decision-making interventions, and they are unable to make appropriate decisions on their own*: "I am not as strong as I used to be in decision-making, and I usually regret my decisions later on" (p17, 48-year-old, housewife*). Decision-making problems lead to impairment in self-care, failure in the timely reporting of the symptoms of disease severity, disability, frequent hospitalisation, decreased QOL and increased mortality rate, which indicates the importance of evaluating cognitive impairment in patients with HF.

Psychological distress

This theme includes the subthemes of depression and anxiety. Fatigue, shortness of breath and unpredictable life-threatening acute crises in HF lead to psychological distress. The unpredictable nature of HF in some participants, especially in women, had caused depression. A *62-year*-old woman *said: "I easily burst into tears. I am hopeless...." (p11).* Another participant said: "*When I have chest pain, not only do I feel depressed, but I also have to convince others that it is nothing important" (p7, 69-year-old, higher education, retired*). Patients without an implantable cardioverter-defibrillator (ICD) were afraid they would have a heart attack again. An anxious patient said: "*I am afraid of loneliness. I am constantly stressed" (p1, 63-year-old, retired*). The bad mood had negative effects on QOL. Depression and anxiety in patients with HF are associated with a greater number of symptoms. Treatment of depression and anxiety in patients with HF might significantly improve QOL.

Functional limitations

This theme includes the subthemes of loss of independence, sex, job and social activities. About one-third of the participants had experienced the negative effect of HF on their independence; "*I lack the freedom to act that I am supposed to have in my daily life, and I need help to do my daily tasks" (p4, 69-year-old, housewife*). Two male participants complained of sexual dysfunctions such as erectile dysfunction and libido reduction. A 57-year-old diabetic man stated: "*The most important limitation of HF is sexual issues"* (p3, retired); in contrast, a 41-year-old woman said: "*Our sex frequency has decreased to a great extent, but I have no problem because my husband and I are not much excited about it*" (*p13*, *higher education*). Loss of job and reduction in income had negative effects on QOL. Most male participants complained that they were not able to do professional work. A *68-year*-old man *said:* "*If you do not work, you will be like a dead person, and time will not pass" (P2, retired)*. Compulsory job resignation was a bitter experience for younger men. A *37-year*-old man said: "*I used to be a teacher. I felt like I was an emperor in the classroom. At present, I am not satisfied, because I lost my empire"* (p18, higher education, unemployed). Being deprived of social activities, such as entertainment, hobbies, traveling, sports and participating in parties had a negative impact on the QOL of some of the participants; *"That I cannot walk and exercise much, has made me feel that I lack something" (p4, 69-year-old, housewife) and "It makes me upset that I am always at home" (p15, 63-year-old, housewife).* A *69-year*-old man said: *"I wish I could travel and do sightseeing more frequently, but I cannot*" (p6). Medications (*diuretics*) for controlling HF-related symptoms do not allow patients to be socially active.

Economic problems

Treatment costs and loss of a job can have negative effects on the participants' QOL. A *65-year*-old man said: *"Treating HF, the provision of medications, pacemaker implantation and frequent hospitalisation, especially for those who are retired with low retirement incomes and are not able to work, is very costly and affects the QOL negatively*" (p10). Some patients seemed to need financial support but did not receive any special aid. Also, the results showed that patients with low socioeconomic status found it difficult to cope with HF. It seemed that the participants who were satisfied with their economic situation gave a higher score to their QOL. The uncertainty about the ability to cover treatment costs had made more than one-third of the participants worry about providing money to cover the costs of renting a house, transportation, urban services, clothing and their children's education, and it had even influenced their nutritional costs. A *41-year*-old woman said: "*I do not shop the way I used to any more. I am scared I may be hospitalised and be short of money" (p13, employee).*

The patients' self-assessment of their QOL

The patients' self-assessment of their QOL showed that most participants (14 out of 19 people) rated their QOL as well or very well. A large proportion of these patients believed that their high QOL was the result of economic security. A 69-year-old man said: "*Thank God ... I am satisfied with my life, and I do not think I have a problem. I rated my QOL 9 at the moment and rated it 10 before being affected by the disease ... If I did not have a good economic condition, I would rate it 2*" (*p7, higher education, retired*). A 71-year-old anesthesiologist rated his QOL 8 before and 7 after the disease; "*if I had economic problems in my life, I would rate my QOL 4" (p14, 71-year-old, higher education, employee*). In addition to economic security, many participants attributed their high levels of QOL to their satisfaction with the social support they received from their family especially their spouse. A 67-year-old man said: *"My wife babies me, we have got a lot closer to each other ... If she were*

*not there for me, the score of my QOL would drop from 9 to 3 or 4" (*p8*,* retired*)*. Also, half of the participants believed a healthier lifestyle and religious-spiritual growth to be effective in their high QOL. A *63-year*-old man said: *"My score of QOL was 5 before my HF, and is 7 after it. The reason behind it is that I quit smoking and drug addiction, and this improved my life in many ways" (p1, 63-year-old, retired)*. A 57-year-old man associated his QOL score after his illness to being close to God (p3, retired). In this regard, a 47-year-old man said: "*The score of my QOL was 5 before my illness and is 8 after it. The reason behind it is paying attention to religious issues, healthy eating and reduced stress" (p19, employee).*

Discussion

The results of this study present many perceived negative effects of HF on patients' QOL, but having a good QOL in most of them show that they had found ways to endure, adapt, and accept the negative effects of HF. The majority of the patients reported they had symptoms, so limitations in daily life due to symptoms were affecting their QOL negatively. The most frequently reported symptoms were fatigue and breathlessness. The symptoms of HF such as dyspnea and fatigue are reported in 80% of the patients. Worsening symptoms are the main cause of hospitalisation [5]. Symptoms were a major influence on QOL [17]. Sleep deprivation can contribute to the exacerbation of symptoms. Patients with HF sleep more in the daytime compared with healthy people. Symptoms such as orthopnea and depression associated with HF may contribute to shortened sleep time and impaired sleep efficiency [18]. The side effects of polypharmacy had negatively affected the QOL of these patients.

Moreover, they had problems due to their lack of ability to distinguish between the side effects of the drugs and HF symptoms — side effects such as frequent urination and drug-related impotence affected QOL [19].

Many patients expressed fear of medication as it made them feel iller and caused hypotension and gastrointestinal dysfunction. Comorbidities such as diabetes and arthritis were common. Most of the time, their presence alongside HF was assumed as a double burden. HF affects older individuals, who have extensive comorbidity. Non-cardiac comorbidities are highly prevalent in older patients with HF and strongly associated with adverse clinical outcomes [20]. In this study, most participants evaluated their QOL as satisfactory in spite of experiencing the negative effects of symptoms and multiple complications of the disease. It seems most of them had accepted the symptoms and complications, and did not consider such disorders to be important because they had changed their lifestyles and organised their daily lives using their resources and abilities. Bosworth showed that the patients had been coping with the disease and lived well despite the unpredictable nature of HF [21]. Ekman arrived at similar results, stating that it seemed that the medical diagnosis of HF was not important for some patients [6].

Nevertheless, the results of this study do not confirm the results of some previous studies with a quantitative approach reporting a low QOL in patients with HF [7]. The reason behind this difference may be that many of these studies have measured and reported the pre-determined dimensions associated with the assessment of the general health status rather than the QOL. The 36-item Medical Outcomes Study Short Form (SF-36), which is the most commonly used generic QOL instrument, mainly assesses health status rather than QOL; therefore, it cannot reflect QOL.

About a quarter of the participants had been negatively affected by cognitive impairments, which is consistent with the results of numerous qualitative studies that have reported concentration and memory impairment in patients with HF [21], [22]. Since self-care depends on having the ability to learn, perceive, interpret, respond and make decisions, it is difficult for patients with cognitive impairment to take care of themselves [23]. The importance of this issue has also been reflected in the literature that has suggested the urgency of further research on cognitive impairment in HF [24]. The most common psychological distress that had negatively affected the QOL of some of the participants in this study was depression and anxiety; this finding was in agreement with that of other related studies [25], [26]. Such feelings lead to lack of coping with the illness [27] and have negative effects on QOL in HF [28]. The results of other studies indicate that 30 to 40% of the patients with HF experience emotional distress such as depression. However, the meaning of QOL in HF is more about being able to be happy and enjoying spending time with the significant other, rather than not having emotional distress [5], which indicates why most participants enjoyed a high QOL despite the negative effects of HF. Depression in HF is associated with the fear of developing physical symptoms such as shortness of breath and functional limitations. Fear and anxiety can also lead to the denial of the symptoms of the illness and, as a result, lack of timely referral to the doctor. It seems essential that the periodic assessment of the psychological distresses of patients with HF be considered and its results are taken into consideration in therapeutic interventions.

The severity of symptoms could compromise functional capacity. HF is a progressive chronic disease, and the patients experience worsening health status following the exacerbation of fatigue and shortness of breath in the process of the disease. This poses an ever-increasing constraint in daily activities, leading to the patients' lack of independence. A small

number of participants believed sexual issues to be the most important negative effect of HF. In a correlational study, three-quarters of the patients with HF reported that both their inclination and their sex frequency had decreased, and in the remaining one-quarter of the subjects, sexual activity had stopped, but these changes had not affected their marital relationship [29].

Regarding functional limitations, some participants, especially men who had gone through the acute phase of the disease, were willing to resume their professional work and social activities, but despite their good physical and mental state, they relaxed at home and did not work. Evidence suggests that after having gone through the acute phase of the disease, people with HF can resume their normal life and career, but this is often neglected [30]. The results of a review of some qualitative essays show that one of the most prominent effects of HF on daily life is social isolation and loss of social activities of the affected following physical limitations due to experiencing shortness of breath and fatigue, diet, taking medications and diuretics (the increased amount and frequency of urination), fear and the lack of communication with family and friends [31]. Therefore, the affected people participate in recreation and leisure and travel less frequently, so they experience the feeling of being imprisoned at home [32]. Economic problems were found to be one of the most important negative factors affecting QOL. The majority of the participants in the study stated that despite the negative effects of HF on their economic situation, they had received no systematic support from any organisations. Considering the increasing burden of cardiovascular diseases and the effects of social and financial support on the treatment of HF, financial support programs such as the full coverage of drug and hospitalisation costs by contributors can be considered as a part of the plan for improving QOL in these patients [5].

Ferrans believes that QOL means having a good feeling about the important parts of life from the perspective of individuals, and what is important for some people at a stage of life may be unimportant to others [33]. This can explain the good feelings of most patients about the significant parts of life and why HF has not been able to blemish their satisfaction with life. Calman notes that the QOL consists of reviewing the current situation and determining the distance between the individual's existing situations (the individual's present experience) and his or her expectations [34]. On this basis, it can be concluded that the people who believed their QOL was good had adjusted their expectations based on their abilities. Consistent with the results of this study, the results of two other qualitative studies have shown QOL to be good and very good in more than half of the patients with HF, and good and excellent in patients with serious disabilities [5], [35]. According to the participants in the study, HF has led to spiritual growth, religious growth, appreciation, a healthier lifestyle, a positive outlook, self-strengthening, getting closer to one's family and receiving more support from them. This way, they reduced the negative effects of HF and tried to maintain their QOL through appreciating the positive effects of the illness and creating a positive outlook. One of the limitations of this study was related to the geographic constraints of this exploratory study. Another limitation was that all the participants were Muslims.

The fact that the majority of the patients had a good QOL despite the many negative effects of HF may reflect their satisfaction with life and their effective coping with HF through creating a positive outlook and perceiving the positive effects of the disease. Therefore, healthcare providers can significantly help patients in coping with HF and increasing their QOL by focusing on the possibilities and the positive effects of HF as well as handling the limitations and negative effects. Moreover, some of the participants had also experienced post-traumatic growth; some of them claimed that their QOL had even improved after being affected by HF. Accordingly, an investigation into the positive effects of the disease and post-traumatic growth in HF by enthusiastic researchers can help lighten the "bright half of the Moon".

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Public Health

Prevalence of Risk Factors for Diabetes Mellitus and Hypertension Among Adult in Tabuk - Kingdom of Saudi Arabia

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Abstract

BACKGROUND: Cardiovascular disease (CVD) is a common but chronic condition that can cause death, and is seen as a substantial source of disability and health costs. A balance between prevention and intervention, as is the case with other infectious diseases, is the best way to stem the increasing burden of CVD.

AIM: This study assesses the prevalence of diabetes mellitus, hypertension, in a sample from the University of Tabuk.

METHODS: A cross-sectional study was done in 2018, with 120 employees and students at the University of Tabuk (in Tabuk City, Saudi Arabia), for those over age 20 (60 males and 60 females) from different faculties and departments at the university.

RESULTS: Assessment was done with 120 participants to assess the prevalence of hypertension and diabetes mellitus, at the University of Tabuk, in which the prevalence is actually higher than in most cities of Saudi Arabia. Ten percent of participants are hyperglycemic and 10% are developing problems in that area, with no significant difference between males and females.

CONCLUSION: This study shows that cardiovascular risk factors will influence those with major health disorders in the future. Consequently, a nationwide campaign encouraging wholesome eating, better lifestyles, as well as physical activity, is a healthcare priority.

Introduction

Cardiovascular diseases (CVD) are one of the most common chronic diseases that cause death and are a substantial source of chronic disability and health costs [1]. The major identified risk factors for CVD include high blood pressure, high blood glucose, serum lipid abnormalities, smoking, obesity, low fruit/vegetable intake, and physical inactivity. Studies have indicated that these risk factors are widespread globally.

Most developing countries in the Middle East countries are in a stage of epidemiologic transition, which is the transition from infectious to chronic diseases [2]. These countries are also experiencing a major transition in the lifestyle patterns regarding the nutritional habits, physical activity and certain habits (such as smoking). In Saudi Arabia, there is little information about the influence of changes in lifestyle such as the reduction of physical activity and dietary habits on the prevalence of diabetes mellitus, hypertension and hypercholesterolemia. Consequently, these changes are occurring rapidly in Saudi Arabia.

CVD can be prevented if the community acquires appropriate information, education and communication on possible risk factors. Most of the risks are attributable to lifestyle and behavioral patterns and can be changed [3]. Therefore, determining the burden of risk factors for CVD in the population helps to design and implement promotional

and preventive measures. In the developing world, a wide gap exists between the reality of the chronic disease burden and response to it. If the emergence and prevention of risk factors are left undirected, the growth of the problem will continue accelerating [4], [5].

A systematic Literature review carried out by Colosia, et al., (2013) [6] the link between Diabetes Mellitus and Hypertension is discussed. It was found that the patients of diabetes mellitus are very much likely to have hypertension as well. It was identified that diabetes and hypertension are correlated with each other in a sense that their root cause is obesity. Through literature search of different databases the correlation of hypertension and obesity with type II diabetes has been investigated.

The article titled carried out by Saeed, *et al*., (2011) [7] encompasses the different variables related to prevalence, control, awareness and treatment of hypertension. During the study with sample population, the predictors identified are gender, environmental setup, social environment, physical activity and obesity. Though the prevalence of hypertension is found to be high in sample population but awareness and control practices are minimal which needs the introduction of a proficient awareness program to control the prevalence in Saudi Arab population.

Another significant study titled *Prehypertension among young adult females in Dammam* carried out by Koura et al., in 2012 [8]. This study investigates the prevalence of hypertension in young females through studying the sample population from four universities in Dammam. By conducting questionnaire 13.5% females, have the condition of prehypertension and they were unaware of it. The sources of this prevalence are physical inactivity, obesity, and unhealthy lifestyle.

A study *Diabetes Mellitus in Saudi Arabia* carried out by Al-Nozha, *et al.,* in (2004) [9] identified prevalence of the Diabetes Mellitus in the Saudi people among both genders as well as rural and urban communities. It covers the environmental and physiological causes and factors which resulted in the occurrence of disease. Furthermore, study also identifies different health risk associated with diabetes majorly Coronary Artery Disease (CAD)

Another study *Prevalence of diabetes mellitus in rural Saudi Arabia* carried out by Fatani, Mira & El-Zubier in 1987 [10] focuses on the occurrence of the Diabetes Mellitus in the rural areas of the Saudi Arabia. This study deals with the different prevalence and underlying reasons of diabetes in a different environmental setup like rural and urban areas. Through statistical analysis of BMI and blood glucose level in relation to the economic and social condition of the different sample population, the effect of environmental factors have been studied.

In the study *Therapeutic Management of Hypertension and Hyperlipidemia in Type-2 Diabetes Mellitus Patients in Southwestern Region of Saudi Arabia: A Pharmacist Perspective* carried out by Khan, Venkatachalam, Alakhali, Alavudeen, Ck & Ansari in 2014 [11] encompasses the hyperlipidemia in the patients of diabetes mellitus, and provides the identifications of the ways to manage the occurrence and the treatment of hyperlipidemia. Through different therapeutic treatments, systolic HTN can be controlled better in comparison to diastolic.

The article *Prevalence of Obesity and Some Related Attributes among Umm Al-Qura University Female Students in Makkah, Saudi Arabia* (2013) by Abdelhafiz, et al., [12] is about the occurrence of the disease in the female university going healthy females of the society. In the year of 2009-10, 224 students were selected from Umm Al-Qura University, and values of weight, glucose level, BMI, waist-hip ratio were collected. Statistical analysis showed that prevalence of obesity is observed to be 25% due to different social, physical and psychological factors.

Methods

A cross-sectional study was conducted with 120 employees and students at the University of Tabuk, Umluj City kingdom of Saudi Arabia during 2018. Subjects were aged 20 or more years (60 males and 60 females) from different faculties of the University.

The cross-sectional design facilitates observations of some subset of the population at the same time with respect to the independent variables (Polit and Beck, 2004) [13]. A correlation study is an efficient and effective design for collecting large amounts of data regarding certain phenomena (Polit and Beck, 2012) [14].

The sample size was subjected to a power analysis using a power estimation of 0.80 and a medium effect size for a two-tailed test with alpha = 0.05 as the minimum. The adequate sampling size for the study was 120 respondents.

Accordingly, a random sample consisting of employees working in both the male and female campuses were recruited for personal interviews using a questionnaire, clinical examination and body/laboratory measurements. The largest division was by gender. These groups were sub-categorized into four age groups. There were equal numbers in the gender categories, but males and females are not equally numerous in the overall population; their comparative proportions vary with age.

We developed a structured self-administered questionnaire with 25 questions and employing a 4-

point Likert scale to evaluate participants age, sex, level of education, socioeconomic status, presence of risk factors for cardiovascular diseases (family history and pre-existing medical conditions like diabetes mellitus, hypertension and hyperlipidemia). The Cronbach's alpha score was 0.825.

Blood pressure measurements were conducted in the morning upon participant's arrival at the study sites. Participants were advised not to eat or drink anything before measurements, and we ensured that the participants had not consumed coffee or smoked before coming to the study because this may affect blood pressure measurements. Blood pressure was measured using a mercury sphygmomanometer (KBM, sm-500, Japan). Two blood pressure readings were taken on the upper left arm with the participant in a seated position after at least 5 to 10 minutes of rest. The average of the two readings was used in this analysis. Hypertension is defined as ≥ 140/90 mmHg in accordance with the WHO Classification of Hypertension (NIH, 2008) [15].

A blood sample was collected by a qualified phlebotomist. Blood samples were separated within 6 to 8 hours of specimen collection and stored at (2-8°C). These were batch tested by a senior technician. The instruments were calibrated daily based on standardized procedures. Fasting blood glucose, total cholesterol, triglycerides, HDL-cholesterol, and LDL-cholesterol levels were measured using spectrophotometer (Optima, sp-300, Japan). Participants were diagnosed with diabetes mellitus if they had fasting blood glucose level of ≥ 126 mg/dl while fasting blood glucose between > 100 and < 125 was considered impaired fasting blood glucose level (CDC, 2011). High cholesterol was defined as ≥ 240 mg/dl. High triglyceride levels were defined as ≥ 200 mg/dl. Low HDL-cholesterol was defined as < 35 mg/Dl for men and < 40 mg/dl for women. The LDL-cholesterol was estimated using the Friedewald formula [i.e., LDL = total cholesterol-HDL-(TG/5)] [16], [17], [18]. High LDL-cholesterol was defined as ≥ 160 mg/dl.

Different methods of data analysis were performed including descriptive analysis and inferential statistical analysis. Descriptive statistics for the demographic data and other questionnaire items were presented using means, standard deviations and P-values. A variety of statistical tests were used to identify the differences between groups such as the independent sample t-test and analysis of one-way variance (ANOVA ≤ 0.05). The sample size was calculated to identify the main outcome variables being measured as well as the instrument used to measure those variables and the anticipated differences between groups (Gerrish and Lacey, 2010) [19].

A medium effect size was expected because this is a very common practice in the field of study [20]. It has been argued that obtaining more responses from the survey site increases its statistical power [21].

Ethical approval for the study was obtained from the research ethical committee of the University of Tabuk on Sep. 20, 2018, Number: S-1439- 0028. Each participant was provided with an information sheet that clarified the aim of the study, the rights of the participants, the assessment process, the process for completing the survey and the use of the collected data. The information sheet also stated that all information provided by the participants would remain anonymous and that privacy would be guaranteed. The information sheet also provided contact details for any queries. The participants were advised about the voluntary nature of participation in the study and informed that they had the option to refuse without penalty or loss of benefit(s). Also, it was made that the study findings would be presented in a format that makes it impossible to identify the participants.

Results

The first group was 20–35 years and contained 20.8% of the subjects. The second group was 36-45 years and contained 21.7% of the subjects. Most participants were in the third group (46-55 years) and fourth age group (> 55 years) with 33.3% and 24.2%, respectively. Moreover, 62.5% of the participants were Saudis and 37.2% were non-Saudis.

Table 1: Participants' distribution regarding to demographic characteristics of the risk factors associated with development of diabetes and hypertension

Demographics	Frequency	Percent
Gender		
Male	60	50%
Female	60	50%
Age		
(20-35)	25	20.8%
(36-45)	26	21.7%
(46-55)	40	33.3%
above 55	29	24.2%
Level of education		
School	24	20%
Diploma	22	18.3%
Undergraduate	13	10.8%
Graduate	61	50.8%
Job Position		
Employees	58	48.3%
Students	62	52.7%
Nationality		
Saudi	75	62.5%
Non Saudi	45	37.5%
Marital Status		
Single	28	23.3%
Married	80	66.7%
Divorce	7	5.8%%
Widowed	5	4.2%

General blood pressure mean for systolic and diastolic was 126.6/85.2 ± 13.9/11.2 mmHg; there were steady additions with both ages. Triglycerides and total cholesterol levels were measured on all subjects. The overall percentage of abnormal cholesterol level was 17.5%. Mean total cholesterol level for female participants was significantly higher than for males (160.1 vs. 157.3, p < 0.047). The mean

values of total cholesterol found here are mostly higher than those levels stated earlier from the general Saudi population [22] and some other studies on Americans and Europeans [23] but comparable to some other studies done somewhere else [24].

Fasting sugar testing showed that 10% of the participants were hyperglycemic, and 10% were on the borderline with no significant differences between males and females. Thus, this research shows an increase in the prevalence of diabetes mellitus versus previous Saudi studies.

Triglycerides and total cholesterol levels were measured on all subjects. The overall percentage of abnormal cholesterol level was 17.5%. Mean total cholesterol level for female participants was significantly higher than for males (160.1 vs. 157.3, $p < 0.047$). The mean values of total cholesterol found here are mostly higher than those levels stated earlier from the general Saudi population [22] and some other studies on Americans and Europeans [23] but comparable to some other studies done somewhere else [24].

Table 2: Frequency and Percentage of Laboratory Blood Tests

Laboratory Blood Tests	Frequency	Percent
Blood Pressure		
Less than 140/90	95	79.2%
More than 140/90	25	10.8%
Fasting Blood Sugar		
70-99 mg/dl	96	72.5%
100-126 mg/dl	12	10%
More than 126 mg/dl	12	10%
Cholesterol		
Less than 180 mg/dl	99	82.5%
More than 180 mg/dl	21	17.5%
Triglycerides		
Less than 200 mg/dl	98	81.7%
More than 200 mg/dl	22	18.3%

Table 3 presents the frequency and percentage of the HDL level among male and female participants. According to the Table 3, 16.6% of the participants had HDL level less than 35 mg/dl. The abnormal HDL level among male participants was higher than females (20% vs. 13.3%, respectively). ANOVA analysis between the different age groups with LDL shows that F and P-values were 6.68 and < 0.001 respectively. In addition, analysis amongst different age groups with HDL for women demonstrates that F and P-values were 3.95 and .013 correspondingly. This shows a significant relationship and demonstrates that there is an impact of age on the LDL and HDL for female participants. On the other hand, analysis among different age groups with HDL for men indicates a non-significant relationship with F and P-values of 1.80 and 0.157 respectively (Table 4).

Table 3: HDL Frequency and Percentage Differences between Male and Female

HDL	Male		HDL	Female	
	Freq.	Perc.		Freq.	Perc.
More than 35 mg/dl	42	80%	More than 40	52	86.7%
Less than 35 mg/dl	12	20%	Less than 40	8	13.3%

Table 4 present statistical analysis with ANOVA values for results of the relationship between age with blood pressure, fasting blood sugar, cholesterol and triglycerides. Table 4 shows that blood pressure ANOVA analysis among different age groups had F and P-values of 3.62 and 0.015, respectively. This is a significant relationship and demonstrates that there is an impact of age on blood pressure. Table 4 also showed that ANOVA analysis between different age groups with fasting blood sugar. Here, F and P-values were 2.30 and 0.081 respectively, which demonstrate the non-significant relationship between different age groups and fasting blood sugar. Detailed ANOVA analysis information of the research participants, including the relationship between age with cholesterol and triglycerides, is presented in Table 4.

Table 4: ANOVA Analysis with Age Groups

	F	P-value
Blood Pressure	3.62	0.015
Fasting Blood Sugar	2.30	0.081
Cholesterol	4.62	0.004
Triglycerides	3.99	0.009
LDL	6.68	< 0.001
HDL for male	1.80	0.157
HDL for female	3.95	0.013

Table 5 displays statistical analysis with Independent Sample T-Test values for findings for the relationship between job with blood pressure, fasting blood sugar, cholesterol and triglycerides. The Independent Sample T-Test analysis between students and employee groups for blood pressure had T and P-values of 2.72 and 0.046, respectively (Table 5). This shows a significant relationship and demonstrates the impact of job on blood pressure. Detailed Independent Sample T-Test statistics of the research participants-including the relationship between job and diabetes, cholesterol and triglycerides-are shown in Table 5. Matrix among hypertension and diabetes mellitus with lipid profiles using Pearson's Product-Moment correlation.

Table 5: T-Test of participants regarding their job related to hypertension, diabetes and hyperlipidemia

	T	P-value
Blood Pressure	2.72	0.046
Fasting Blood Sugar	2.93	0.035
Cholesterol	2.76	0.048
Triglycerides	2.6	0.05

It is clear from Table 6 that there was a strong positive correlation between hypertension and triglycerides, cholesterol, HDL for male, HDL for female and LDL ($r = 0.765^{**}$, $r = 0.790^{**}$, $r = 0.896^{**}$, $r = 0.746^{**}$ and 0.845^{**} at P-value < 0.001). In addition, all lipid profiles correlated positively with diabetes mellitus. In fact, the correlation between hypertension and lipid profiles was stronger than the correlation.

Table 6 shows the distribution of waist to height ratios over the recommended (> 0.5) level in 16.8% of the Participants, which represents a risk for cardiovascular diseases and diabetes. Also, a positive family history of diabetes, hypertension, or both was found in 26.7% of the participants.

Table 6: Correlation between diabetes mellitus and lipid profiles

		Triglycerides	Cholestero	H.D.L. Male	H.D.L. female	L.D.L.
Hypertension	Pearson Correlation	0.765**	0.790**	0.896**	0.746**	0.845**
	P value	<0.001	<0.001	<0.001	<0.001	<0.001
Diabetes Mellitus	Pearson Correlation	0.560**	0.555**	0.592**	0.478**	0.574**
	P value	<0.001	<0.001	<0.001	<0.001	<0.001

** Correlation is significant at the 0.01 level (2-tailed).

About 43.3% of the participants habitually watched television or played video games for more than 3 hours a day, every day, which is regarded as a risky sedentary habit. Risky dietary habits included the consumption of carbonated drinks or sugary fruit juices daily (36.7%). Also 17% of the Participants admitted to having food outside the home, particularly fast food from branded outlets, on more than 3 days of the week instead of consuming homemade food.

Table 7: Distribution of the risk factors associated with development of diabetes and hypertension among participants

Risk factors	No. participants (n = 120)	%
Waist-to-height ratio (WHR > 0.5)	14	16.8%
Positive family history for diabetes and/or hypertension	32	26.7%
Mostly outside food intake	21	17.5%
Sedentary activities for more than 3 hours per day	52	43.3%
Carbonated drinks/fruit juice	44	36.7%

Discussion

This study measured the prevalence of CVD risk factors. The study was validated with the WHO STEPS rules and testing strategies that included a straightforward and irregular examining method. This study is the first of its kind in the nation to attempt all three segments of WHO STEPS in a group setting. The low response rate might influence the genuine dispersion of the risk factors, and the results should be translated with caution. The prevalence of hyperlipidemia is growing markedly and it is one of the main contributors to the occurrence of several diseases due to its pathophysiological connection to other cardiovascular risk factors such as diabetes mellitus and hypertension [25].

The predominance of hypertension (10.8%) is simlar to that in Butajira, Ethiopia [26] and a meta-analysis (25 studies) from 10 sub-Saharan African nations [27]. Our value is lower than in Addis Ababa, Ethiopia (Tesfaye, 2008) [26] and systematic review of studies in Europe and North America (Wolf-Maier et al., 2003) [28]. The conceivable purposes behind the distinction could be varieties of study populaces in socio-demographic and economic characteristics. The prevalence of hypertension was more than two times higher in urban than rural zones. This is consistent with discoveries in Ethiopia (Tesfaye, 2008) [26] and the systematic review of sub-Saharan studies (Addo *et al.,* 2007) [28]. No significant differences were observed between males and females in terms of hypertension-this contrasts with the Ethiopian study (Tesfaye, 2008) [26] and review of European and North American researches (Wolf-Maier et al., 2003) [28]. However, the results in a review of sub-Saharan Africa did not indicate reliable distinction crosswise over nations (Addo *et al.,* 2007) [26].

This research indicated that hypertension affects less than a quarter of the participants, which agrees with an earlier national study (Al-Nozha *et al.*, 2007) [9]. Of note, our mode age is 10-15 years young than that in corresponding studies in developed nations [29]. This demonstrates the worldwide burden of hypertension is extensive and expanding suggesting the need for urgent intervention. This study demonstrated the significant relationship of hypertension with age in both genders and with national and global studies in many populations the different geographic, social, and financial attributes [30], [31].

Globally, the prevalence of diabetes mellitus will be a rise of 42% from 2003 to 2025. The prevalence in the Gulf area is highest in Bahrain (25.7%) and Oman (16.1%) [32], [33]. This research indicated a further escalation in the prevalence of diabetes mellitus in association with earlier studies carried out in Saudi Arabia. The dramatic rise in the diabetes mellitus prevalence can be clarified by the disorder of advanced years. About 29% of the participants over 55 have diabetes. Studies carried out in Saudi Arabia have diverse age-specific prevalence rates. This study shows an increase in diabetes mellitus prevalence in the elderly. Indeed, this relationship between diabetes and age is consistent with earlier studies [34], [35].

The prevalence of diabetes mellitus (10%) and borderline diabetes (10%) is similar to the results of Al-Nozha, *et al.,* in 2004 [34] that recognized the prevalence of diabetes mellitus in the Saudi population in both males and females. Another study by Fatani, *et al.,* (1987) [10] reported the rate of diabetes mellitus in rural Saudi Arabia-both studies were consistent with this study. Worldwide, the prevalence of diabetes mellitus is similar in males and females, but it is slightly higher in females at < 60 years of age and in males < 60 years old. This was not detected in this research. Hyperlipidemia is reaching advanced prevalence rates in Saudi Arabia. Moreover, these findings show that hypercholesterolemia (HC) (total cholesterol < 180 mg/dl) and hypertriglyceridemia (HT) (total triglycerides < 200 mg/dl) are prevalent health problems that affect around 18% of the Saudi community. This increases the risk for Coronary Artery Disease (CAD) and other disorders related to excess lipids. Clearly, the HC and HT prevalence increase with age. This finding suggests that CAD will soon be the main health problem. Increasing physical activity, a reduction in obesity and implementing healthier nutrition habits should be of considerable

interest to Saudi Arabian citizens.

A cross-sectional national epidemiological study was carried out in Saudi Arabia involving of 4539 Saudi participants over the age of 15 years (Al-Nuaim, *et al.,* 1996) [36]. The prevalence of HC (5.2-6.2 mmol/l) was 9% and 11% for all male and female participants, respectively (P = 0.74). The prevalence of HC (> 6.2 mmol/l) was 7% and 8% for male and female participants, respectively (P = 0.52). However, the HC in our study was 17.5% in both male and female participants. Thus, our study indicated a further increase in the prevalence of HC. Other principal risk factors explored in this study showed that the waist-to-height ratio (WHtR) was more than the recommended limit in 16.8% of the participants. Many studies have supported the use of WHtR inclusively in identifying at risk populations with prevalence ranging from 10-23%.

This study also revealed that 38.9% of the participants had a positive family history for diabetes and hypertension which independently is a strong predictor of its occurrence in later years. This finding is supported by studies that points to an overwhelming genetic predisposition towards this disease and consanguinity as one of the primary reasons for it. In fact, the World Health Organization has declared physical inactivity to be one of the top five risk factors for premature mortality. Dietary choices like preference for food outside the home (fast food, restaurant meals, etc.) was found among 11.5% of the participants, coupled with a higher intake of carbonated drinks or sugary fruit juices (36.4%) signaling a high risk for obesity and overweight among participants, in this study.

In summary, the prevalence of hypertension and diabetes mellitus found in the Umluj City, University of Tabuk, is higher than the prevalence stated in most cities of Saudi Arabia, Attar (2015) [37]. The prevalence of cardiovascular risk factors is higher than in other previous Saudi studies Abdelhafez, (2013) [12]. Despite having a younger population, Tabuk Umluj City had a high prevalence of cardiovascular risk factors. Indeed, even higher than European and North American countries. This implies there will eventually be an increase in the prevalence of hypertension and diabetes a as the population ages unless preventive policies are presented. The data suggest that cardiovascular risk factors influence individuals and will be major health disorders in the future. Consequently, a nationwide campaign encouraging healthy diet and lifestyle should be promoted.

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Review Article

Application of Imaging Technologies in Breast Cancer Detection: A Review Article

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Abstract

One of the techniques utilised in the management of cancer in all stages is multiple biomedical imaging. Imaging as an important part of cancer clinical protocols can provide a variety of information about morphology, structure, metabolism and functions. Application of imaging technics together with other investigative apparatus including in fluids analysis and vitro tissue would help clinical decision-making. Mixed imaging techniques can provide supplementary information used to improve staging and therapy planning. Imaging aimed to find minimally invasive therapy to make better results and reduce side effects. Probably, the most important factor in reducing mortality of certain cancers is an early diagnosis of cancer via screening based on imaging. The most common cancer in women is breast cancer. It is considered as the second major cause of cancer deaths in females, and therefore it remained as an important medical and socio-economic issue. Medical imaging has always formed part of breast cancer care and has used in all phases of cancer management from detection and staging to therapy monitoring and post-therapeutic follow-up. An essential action to be performed in the preoperative staging of breast cancer based on breast imaging. The general term of breast imaging refers to breast sonography, mammography, and magnetic resonance tomography (MRT) of the breast (magnetic resonance mammography, MRM).

Further development in technology will lead to increase imaging speed to meet physiological processes requirements. One of the issues in the diagnosis of breast cancer is sensitivity limitation. To overcome this limitation, complementary imaging examinations are utilised that traditionally includes screening ultrasound, and combined mammography and ultrasound. Development in targeted imaging and therapeutic agents calls for close cooperation among academic environment and industries such as biotechnological, IT and pharmaceutical industries.

Introduction

Cancer is the cause of one in eight deaths worldwide, and about 12 million new cases are diagnosed yearly. In spite of using aggressive treatment strategies, the rate of mortality has remained high and thus; there need more studies about developing new approaches to cancer management [1], [2], [3]. One of the major tools used in comprehensive cancer care is medical imaging. It brings many advantages including monitoring capability in real time, no need to tissue destruction, minimal-invasive procedure, and usability over broad periods and size ranges that are usually needed in pathological and biological processes. The main factor in reducing mortality and cancer management costs is an early diagnosis. Leonard Fass [4] study showed that imaging through symptomatic disease management and screening plays an important role in cancer management. This is shown schematically in Figure 1.

Another tool used in all stages of cancer management is biomedical imaging. It can be used in prediction, staging, screening, prognosis, biopsy guidance for diagnosis, plan of treatment, therapy guidance, therapy response recurrence and palliation [5], [6], [7], [8], [9].

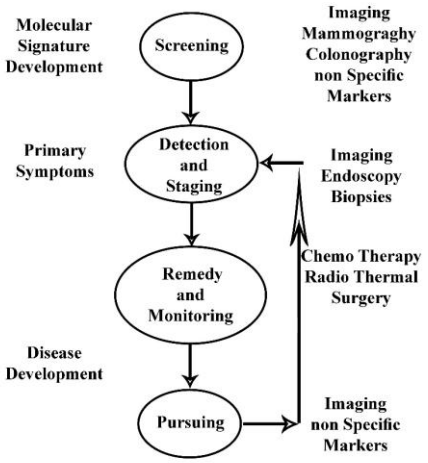

Figure 1: The role of Imaging in cancer management [4]

Translational studies link fundamental science to clinical applications; therefore, resulted in significant health consequences [3], [10] (Figure 2). With regards to the wide range of usage in the management of patients with cancer for diagnosis, staging, therapy planning, monitoring, and surveillance, imaging considered as an essential component of clinical cancer protocols [11]. Current clinical imaging techniques have some limitations such as using of computed tomography (CT) or magnetic resonance imaging (MRI) for studying in vivo biological changes in relation with prognosis, carcinogenesis and therapy response [12].

Full spatial mapping of gene expression and molecular activities within cells and tissues are required to get health benefits of understanding the genome and proteome. To this end, structural and functional imaging of molecules is vital. To recognise the existence of cancer, the tumour stage, its aggressiveness and response to therapy, imaging biomarkers are under development [13]. Several medicinal treatments are under development, classified as anti-hormonal, cytotoxic, immunotherapeutic and molecularly targeted. The molecularly targeted treatments include angiogenesis inhibitors, multi-targeted tyrosine kinase inhibitors, cell cycle inhibitors, signal transduction inhibitors, apoptosis inducers and epigenetic modulators [14]. Several targeted agents have been developed as cancer markers comprising of αvβ3 integrin, carcinoembryonic antigen (CEA), epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), prostate stimulating membrane antigen (PSMA), somatostatin receptors, MC-1 receptor, folate receptors and transferrin receptors. In vitro imaging methods (i.e. imaging mass spectrometry (IMS)) by their ultra-high resolution can be used to specify the spatial distribution of proteins, peptides, and drugs in tumour tissue specimens. The main focus of this review is the clinical imaging methods [8].

Developments in our ability to analyse molecular processes, such as gene and protein expression, cellular and molecular biochemistry, make it possible of a better understanding of breast cancer biology and recognition of new therapies for patients [15]. Measuring biological processes in vivo without disturbing them, helps in better characterisation of tumour biology. It also lets us evaluate the way biological, and cytotoxic therapies change key pathways through which tumour responds and generate resistance. Molecular imaging can carefully characterise the properties of the tumour, and biological processes hence play an important role in clinical care from detection to staging, breast cancer knowledge, evaluation of therapeutic objectives, and assessment of responses to various therapies [16].

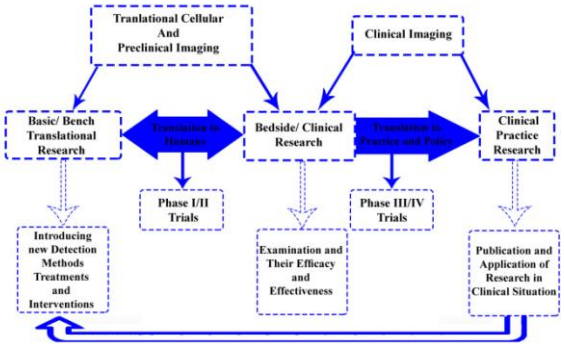

Figure 2: Map of translational research based on practice. (The working model of bench-to-bedside translational research is described in this diagram); Forward Translation: Molecular explorations translated into the clinical application; Reverse Translation: Scientific questions arise from relevant clinical findings resulted in research progress

The elasticity of cancer tissues is lower than normal tissues. This difference in elasticity makes ultrasound elastography suitable to the differential diagnosis of prostate cancer, liver fibrosis and breast cancer [17], [18]. Lots of clinical imaging systems work based on the different response of body tissues and fluids to electromagnetic radiation. But ultrasound works through frequency variation, dispersion and reflection of acoustic waves. The interaction of ultrasound and tissues makes it able to illustrate tissue elasticity. Another application of ultrasound is in thermal therapy, and its mediating ability of gene expression and gene transfer is well known [19]. Imaging of pancreatic masses, lymph nodes, adrenal and submucosal tumours by endoscopic ultrasound elastography is possible and can avoid fine needle aspiration biopsies [20].

In their study about breast cancer imaging techniques, Poplach et al., [21], [22] stated that imaging techniques based on non-ionizing electromagnetic radiation such as microwave imaging spectroscopy and near-infrared spectroscopy among others had been mainly investigated for breast imaging. However physical properties of imaging systems involving sensitivity, temporal and spatial resolution are different. The most sensitive clinical

imaging techniques are PET and nuclear medicine which their sensitivity varied from nanomole/kilogram to picomole/kilogram. The sensitivity of X-Ray systems such as CT is millimole/kilogram whereas the sensitivity of MR is around 10 μmol/kg [23].

Because cancer is a disease with multiple factors, imaging has to display the variant mechanisms and stages of pathogenesis. Tumour volume characteristics can only show response delay to therapy and can't indicate the metabolism and other parameters. Thus its application alone as a measure of disease development is not inadequate. That's the reason for using multiple imaging methods for managing cancer. Multiple imaging techniques such as PET/CT incorporates the benefits of PET (i.e. metabolic sensitivity) and CT (i.e. temporal and spatial resolution). Integration of imaging techniques such as SPECT/CT, PET/CT, PET and MR and ultrasound and MR in the future will require developing standard approaches to perform longitudinal studies about the response to therapy [25].

At last, integrating various imaging technologies will lead to diagnostic orthogonality. It is supposed that the combination of such information not only provides a mean to validate individual technologies in the future but also forms a part of the diagnostic process, particularly for prediction, early detection and early therapy response of the disease [17]. In this review, the most recent technologies in the world's newest research studies are introduced. Also, new scientific methods for the examination, diagnosis and evaluation of benign and malignant tumours are presented.

Digital imaging systems

Full field digital mammography is one of the helpful digital imaging systems for breast cancer diagnoses. It has several privileges over film-based techniques for screening of breast [26] including increased dynamic range, improved sensitivity for dense breasts, lower dose, digital archiving, computer-aided detection/diagnosis, telemedicine, tomosynthesis, softcopy review, 3-D visualisation techniques and reducing breast compression pressure. Overlapping and obscuring malignancies by normal structures like glandular tissue in the breast, particularly those placed deep in the breast is a potential constraint of 2D mammograms. This can cause cancers to be skipped in the scan.

On the contrary, overlapping normal tissues can be misunderstood with tumours in an X-ray image and caused extra imaging, performing unessential biopsies, higher costs of healthcare and patient anxiety. The combination of tomosynthesis and digital mammography can result in lower false negatives and increasing true positives [27]. Also, using 3D X-ray techniques along with tomosynthesis can reduce breast compression. Other 3D methods to produce stereoscopic images are available. Digital X-ray images of the breast taken at two different angles by about eight degrees' difference can produce stereoscopic mammograms. The radiologist through these images can see the breast interior structure in three dimensions and make better distinguish between benign and malignant lesions. Accordingly, one can say that this technique has a greater detection rate and lower false positives compared to regularly 2D mammography [28]. In the other side, a higher number of images in this technique increases the risk of exposure high radiation dose.

Jong [29] stated that Contrast-enhanced mammography is an investigational technique that uses iodinated contrast agents. This technique is built on the principle that the quick growth of tumours necessitates enhanced blood reserve across angiogenesis. If the compression device is not activated, then contrast should be administered. Angiogenesis regions resulted in the accumulation of the contrast agent. Diekmann and Bick [30] uttered that contrast-enhanced mammography with tom synthesis is an imaging contrast distribution technique in breast tissue. There are two methods to evaluate images. The first one is to look for maximum iodine concentration in the image, generally 1 minute after injection. The regions with high absorption demonstrate the growing activity of tissues, and likely the malignant ones. The kinetic analysis technique can track iodine contrast agent flow inside of a tissue region and outside of it. The speed of wash-in and wash-out of iodine in malignant cancers is high, while iodine uptake of benign tissues is slow, took about 5 min in this study. This is identical with perfusion imaging by MRI using gadolinium-based contrast agents.

Dual-energy contrast mammography has the ability of better breast lesions diagnosis at lower radiation doses than non-contrast enhanced mammography, but this is not identified for contrast-enhanced MRI and needs to be evaluated. Combination of tomosynthesis with contrast-enhanced mammography may lead to better detection of preliminary and secondary lesions and also the potency to monitor therapy [31], [32].

To enhance lesion detection efficiency, computer-aided detection (CAD) is used. Dual-energy techniques have some advantages include removing the structural noise, contrasting media that magnify the area around the tumour, and improving lesions detectability. CAD aims to facilitate recognise lesions, particularly in locations where obtaining a second reading is difficult. CAD is more suitable for recognising micro-calcifications but less effective for breast masses. Competent breast cancer experts who are capable of differentiating benign lesions from malignant ones can better utilise CAD [33]. CAD has great sensitivity for breast cancer detection on initially and short-term follow-up digital mammograms. Reproducibility for true positive CAD marks is

significantly higher than for false positive ones [34]. Recently, a mega-study composed of 231,221 mammograms showed that CAD improves the efficiency of the single reader and increases sensitivity just with a little increment in recall rate [35].

Increased sensitivity and specificity is possible using dual modality systems that combine ultrasound and X-ray systems [36]. Mammography has no sensitivity in imaging young compact breasts because the encompassing fibroglandular tissue decreases the manifestation of lesions. Moreover, screening by ultrasound can considerably increase the detection rate of small cancers. Also it can represent considerably more cancers at a smaller size and lower phases compared to physical examination alone, which identifies hardly any cancers. Increasing breast compression that is naturally greater in older women with dense breasts significantly declines the mammographic sensitivity for breast cancer. The detection sensitivity of full field digital mammography systems for dense breasts is better than film-based systems. The American Cancer Society recommends that even women with a good health condition should continue screening mammography [37].

Breast Cancer Magnetic resonance imaging (MRI)

The application of magnetic resonance systems in cancer detection, monitoring therapy response, staging, least-invasive therapy guidance and biopsy guidance has been documented [38]. Imaging techniques using in cancers are based on diffusion-weighted imaging, perfusion imaging using contrast agents, exogenous spectroscopic imaging with hyperpolarised contrast agents, endogenous spectroscopic imaging, blood oxygen level determination (BOLD) imaging, magnetic resonance elastography, relaxivity-based imaging with and without contrast agents [39].

The first investigators who used MRI for breast cancer examination were Ross et al., [40]. Today, breast MRI is considered as a supplementary technique along with ultrasound and mammography. So, MRI technology of the breast has greatly improved by the extension of advanced surface and gradient coils, contrast agents, parallel imaging and novel fast imaging sequences. Simultaneous imaging of both breasts are possible using new surface coils, allows indicating involvement of the contralateral breast. Breast MRI sensitivity for breast cancer detection is higher than ultrasound or mammography. Because of high expenses, hard accessibility, and high false positives of MRI, it is not widely used as a screening test for breast cancer, but in special cases [41]. Breast MRI is not based on ionising radiation. As a result, it is suggested in the repeated screening of patients with high risk of radiation-induced DNA mutations. Breast MRI is applied to screen women with very dense breast tissue, women with a family background of breast cancer, or women with silicone implants that may conceal pathology in mammography. It can also be used for seeking recurrence in patients with scar tissue. The American Cancer Society has strongly confirmed using MRI to identify contralateral disease extension and lymph node involvement in breast cancer [37].

Because of its ability to show multi-focal tumours, chest wall involvement, retraction of the skin and lymph node metastases, MRI is the most accurate instrument for the local staging of breast cancer. Compared to other methods, it performs better in imaging invasive lobular carcinoma. Magnetic resonance imaging due to its ability in easier analysing of pathological response as well as a low rate of reoperation needed for positive margins is preferable than mammography and ultrasound [42]. This suggests one important role of MRI, i.e. facilitate decision-making about performing conserving surgery or mastectomy.

Strong paramagnetic nature of gadolinium makes it possible to alter the magnetic situation of hydrogen atoms inside water molecules. High concentrations of gadolinium components due to susceptibility effects resulted in local changes in the magnetic field. Dynamic studies of wash-in and wash-out are possible using contrast-enhanced MR. The maximum effect is accrued after rapid intravenous injection during the first passage of a bolus of contrast agent. This creates darkness on gradient echo T2*-weighted images in highly perfused areas of tissues. But tissues with a high contrast agent uptake appear bright on T1-weighted images.

Takayoshi et al., [43] argued that myocardial perfusion imaging could be used in the assessment of two-sided micro-calcifications following by stereotactic vacuum-assisted biopsy. They further stated that it could illustrate the existence of malignant micro-calcifications seen on mammography. Naoyo Nishida et al., [44] declare that evaluating neo-angiogenesis requires using dynamic contrast MRI with gadolinium-based contrast agents that is related to microvessel density, histopathology and response to chemotherapy [45], [46], [47]. The time/signal intensity graphs at the maximal enhancement site for each progression lesion are achieved closely. Three types of time/signal intensity curves identified by Riham H. et al., [48] as below:

(I) continual enhancement curves are normally related to benign lesions.

(II) Curves with a quick contrast uptake following with a plateau state can be representative of both benign and malignant lesions.

(III) Curves with a quick contrast uptake and wash-out are usually pertained to malignant lesions.

Using agents that block angiogenesis, MR perfusion imaging can be used directly and indirectly to monitor therapy. Because of the potential ability of

anti-angiogenic therapy in temporarily normalising the abnormalities in structure and function of tumour vascular system, it can terminate angiogenic blood vessels. Due to the less permeability of normalised blood vessels, their efficacy in drug and oxygen delivery is better. Pericytes that are vascular smooth muscle cells has an essential role in blood vessel formation. Further, they can help the normalised vessels to remain strong [49]. The strong vessels can diminish the intravasation of cancer cells, hence decrease the risk of haematogenous metastasis. Severity time curves for various breast tissues are illustrated in Figure 3.

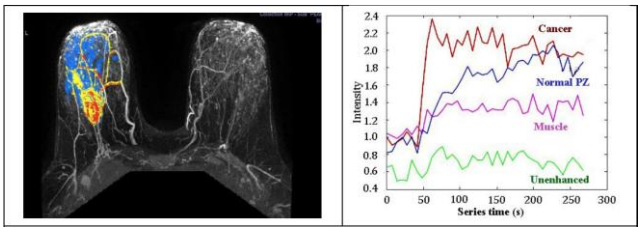

Figure 3: Time/severity curves of breast MR contrast uptake (derived from; Riham H. et al., [48])

American Breast Imaging Reporting and Database system [50] has declared that enhancement of heterogeneous or rim is an indication for malignancy and irregularly shaped speculated masses. Nunes [51], on the other hand, stated that a nonmass asymmetrical enhancement with a segmental or regional pattern could be considered as a powerful symptom of in situ ductal carcinoma. Liberman et al. [52] reported in their study that non-enhancing septa or smooth borders which are observed in many fibroadenomas suggest benign lesions. They further stated that small lesions with the size of smaller than 5 mm often have no clinical significance.

Diffusion-weighted imaging Method

Diffusion-weighted imaging (DWI) MRI formerly used in cytotoxic oedema detecting in stroke. Today, it is also used to measure Brownian movement (the diffusion of water molecules). It is expected that this technique can be used for the identification of tumours and metastases as well as classifying breast lesions as benign or malignant [53]. Both quantitative and qualitative information on the changes developed at a cellular level can be obtained using DWI MRI. This information shows the effect of cell membrane integrity and tumour cellularity. Thanks to recent advancements, this technique is extensively applied for tumour assessment inside the body and have developed whole body DWI. Here is an example of osseous and soft tissue metastatic lesions; Figure 4-1 and 4-2 illustrate a 42 years old female with breast cancer and massive metastatic nodal spread along some parts of her body that was presented with lower back pain, right sciatica [54].

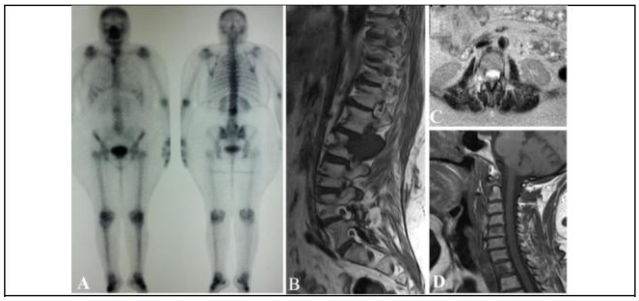

Figure 4-1. An instance case of metastatic breast cancer; A) The anterior and posterior projections of the patient, focal zones of hot uptake at D1, L2 and right aspect of the manubrium sterni are shown in a bone scan; B) Sagittal T1W image of the dorsolumbar spine. A sizable hypointense marrow lesion can be seen in the right posterolateral aspect of the L2 vertebra.

Further, a pedicle with expansion and extra-osseous soft tissue component are demonstrated; C) Axial T2W image at L2 level. Right sided infiltrative process with intra and retrospinal soft tissue extensions are shown; D) Sagittal T1W image of the cervical spine. Hypointense metastatic lesions at C5 and D1 are demonstrated

According to Galbán et al., [55], tumour tissues cause a disruption in water molecule diffusion, as a result, the rate of apparent diffusion constant (ADC) reduces, resulted in high signal in DWI images. Positive response to therapy is represented by a rise in ADC. The number of killed cells is directly related with increase of water ADC after therapy. Water liberation into the extracellular space in result of cell necrosis is a possible cause of this relationship.

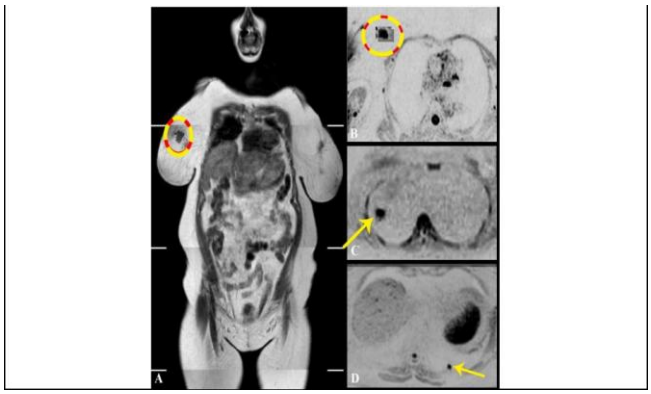

Figure 4-2: The previous case of metastatic breast cancer; A) WB-MRI coronal T1W image. The primary breast lesion can be seen at upper inner quadrant of the right breast. It is showed by dark signal and irregular outlines; B) Corresponding axial inverted DWI. Breast lesion are shown as a flared out zone of evident diffusion restriction. The mean ADC value of the zone is 0.91 x 10-3 mm2/sec; C) & D) Axial inverted DWI at different levels. The pulmonary nodules are shown as rounded focal zones of diffusion restriction at lateral segment of right middle lung lobe C) and posterior segment of left lower lung lobe D) giving a low mean ADC value ranging between 0.97 and 1.03 x 10-3 mm2/sec

Buijs et al., [56] stated that response evaluation of metastatic breast cancer to chemoembolization is possible by ADC Measurement. ADC values of normal tissues would not change, whereas this value for tumor tissue showed an increase after transarterial chemoembolization. In the other hand, a full response cannot be obtained in solid

tumors using MRI, even if volume changes viewed with contrast-enhanced. Response to radiation therapy in the cases with High DWI MRI may be possible [37]. The response of tumours having a high diffusion constant due to their wide necrotic region is worse. Palpation is applied as a part of the clinical diagnosis of prostate, thyroid, breast and abdominal pathologies. It determines the rigidity of a region compared to the nearby tissues. The regions with increased ADC are obtained by partial responders. Whole-body MRI is comparable with PET/CT and scintigraphy in the identification of sclerotic metastases, which are usual processes in breast and prostate cancers as well as multiple myeloma. Currently, PET/CT is applied for soft-tissue metastatic disease, while diffusion-weighted MRI methods have the potential to be used in the future.

Magnetic resonance elastography

Magnetic resonance elastography (MRE) by transmission of shear waves and imaging their propagation using MRI, measures the mechanical characteristic (stiffness) of soft tissues [57]. It is a non-invasive medical imaging technique. The stiffness of diseased tissues is often more than the normal tissues around it. This is done while the application of acoustic waves by adjusting motion-sensitive phase contrast MRI sequences. The range of acoustic waves frequencies is among 100 Hz to 1 kHz. Propagation of shear waves is a function of the shear modulus of the tissue [58]. MRE produces images of shear wave propagations with variable wavelengths. Some researchers based on these criteria have investigated the application of MRE in breast cancer detection as a constant method of detection. They reported that; breast cancer tissues are much harder than healthy fibroglandular tissue. Physicians used this characteristic for screening and diagnosis of many diseases, through palpation. MRE, through a non-invasive and objective way, calculates the mechanical parameter while elicited by palpation, [59], [60], [61].

Apoptosis/Receptor imaging

Binding agents to cell surface proteases that attract phagocytes to dying cells are also used to direct imaging of apoptosis. To perform optical and nuclear medicine imaging, Annexin V has been utilised. Zhao et al., [62] reported the using of targeted super-paramagnetic iron oxide (SPIO) to perform receptor imaging. This is a practical method for breast cancer imaging. Artemov et al., [38], for example, used targeted iron oxide to image tyrosine kinase Her-2/neu receptor in breast cancer cells. The targeted agent used to MR contrast was Streptavidin-conjugated super-paramagnetic nanoparticles. Boosted T2 MR contrast were generated for Her-2/neu-expressing cells via directing these nanoparticles to receptors which previously classified as a biotinylated monoclonal antibody. The expression level of Her-2/neu receptors governs the contrast of MR images. The level of expression attained independently using fluorescence-activated cell sorting (FACS) analysis. One of the limitations of MRI for direct imaging is its sensitivity but could utilize in pre-clinical imaging. Such target-mediated imaging is successfully applied in nuclear medicine imaging, include imaging of breast cancer tumours [63], [64]. In this method, the molecular expression pattern of tumours is targeted. Synthesised target ligands composed of antibodies, peptide analogues, nanobodies and affibodies can target those molecules (for example, enzymes, transporters and receptors) overexpressed on cancer cells. They stick to the target with high affinity and specificity (Figure 5).

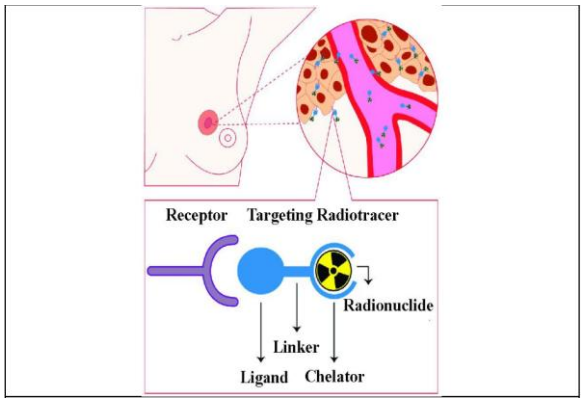

Figure 5: Schematic of receptor targeted nuclear imaging. Ligands can bind those targets that are overexpressed on breast cancer (BC) cells. These ligands can be joint to a chelator, mostly by a linker

Magnetic Resonance Spectroscopy

An additional technique to improve the accuracy of a breast MRI study is Magnetic resonance spectroscopy (MRS). Following conventional MRI imaging of a breast, a chemical “spectrum” is evaluated in a suspicious region to provide additional information about the chemical content of that region. This information can be used to distinguish benign from malignant masses, and to determine the faster respond of a mass to chemotherapy treatments and MRI alone [65]. The molecules such as hydrogen ions or protons are analysed in MRS, but the more common method is Proton spectroscopy. Localising tumours, detecting a response to therapy and directing biopsies in the breast are the potential role of Spectroscopic imaging and 3D MR proton spectroscopy [66]. Integrating MR spectroscopic imaging with MR anatomic imaging lets to localise the spatial position of metabolites. The main phospholipid in cell membranes is Choline. It is known as a potential marker of cell division and helps in phosphatidylcholine formation. According to Chung-Ho et al., [67], the present of carcinogenesis in human breast epithelial cells causes membrane choline phospholipid metabolism to be changed progressively. Yeung et al., [68] in their study about the application

of proton MR spectroscopy in preliminary observations and specifying of histopathologic subtypes in axillary node metastases of breast cancer, noticed that high choline levels are related to increased lymph node metastases and invasive ductal carcinomas of the breast. An example of proton spectroscopy for a breast lesion on MRI and the corresponding MRS spectrum is illustrated in Figure 6 [69].

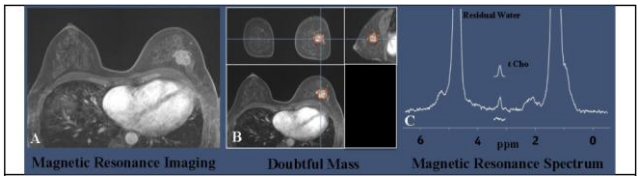

Figure 6: Imaging of a breast lesion using MRI anatomic image and proton spectroscopy. (Derived from; Haddadin et al. [69]); A) Magnetic resonance spectroscopy derived from a post-contrast gradient echo MRI of a 51-year-old woman with invasive ductal carcinoma; B) Detected Areas with doubtful masses; C) The resonances of corresponding water and lipid suppressed spectrum acquired

Bartella and Huang [70] further showed that monitoring and predicting response to chemotherapy as well as reducing the number of breast biopsies is accessible, provided that choline levels are used to differentiate benign from malignant tumours. Change monitoring in these metabolites can help to observe therapy response or malignant transformation. Owing to the low concentration of these isotopes inside tissues, the obtained signal is weak, and also, acquisition of signals takes very long times. As a result, clinical application of spectroscopy of endogenous has been limited [71], [72].

Breast Ultrasonography

Ultrasound is an imaging examination. Application of ultrasound in the detection of tumours in the breast, thyroid, prostate, pancreatic, liver, uterine, ovarian, and kidney is very common and is considered as a common diagnostic imaging method. During performing the ultrasound test, sound waves with high frequency passed across the breast and turned into images that are shown on a display screen. Ultrasound is used to complete other screening tests and is not a screening test for breast cancer by itself [73]. When observing an abnormality on mammography, or when felting it by physical examination, the best method to identify whether the irregularity is rigid (for example, a benign fibroadenoma or cancer) or fluid-filled (such as a benign cyst) is performing an ultrasound test. Ultrasound cannot be used to determine if a solid lump is cancerous. Visualisation of lesions is enhanced in volume ultrasound. Another main application of ultrasound is to guide biopsies. With no need to ionising radiation, ultrasound is suitable for follow up studies to check for recurrence [74]. Some of the recent developments in ultrasound include ultrasound elastography, targeted microbubble contrast agents, photoacoustic imaging and locally activated ultrasound [73], [75].

Breast Scintimammography

Specialising imaging systems would be an area of developments in the context of nuclear medicine in future. Development of specialised gamma cameras, for example, could help in imaging of breast and axillary nodes by the use of scintimammography. This is because of the need to prevent dispersing from extra-mammary sources and has a significant role in breast imaging using radiotracers. It is also the prevailing effect when imaging near the chest wall by the use of mammoscintigraphy [76]. Another technique for breast imaging is Scintimammography. It is used to identify cancer cells in women breasts who have abnormal mammograms, or those with post-operative scar tissue, dense breast tissue, or breast implants. Radiopharmaceuticals images for scintimammography are taken using traditional gamma cameras that are also called a large field of view cameras [77]. Due to the large passive area at the edge of these camera detectors that prevents it from imaging breast tissue closing to the chest wall, scintimammography is generally performed either in patient's supine position, and the camera takes a lateral image of the breast, or in the prone position so that the breast hangs freely. Therefore, with no breast compression, the sensitivity for detecting smaller lesions is decreased [78], [79], [80]. Breast Mammography and scintimammography of carcinoma used in the scintimammography in a woman are shown in Figure 7. A woman in the figure received a small amount injection of a radioactive substance which is absorbed by cancer cells. A gamma camera is used to image the breasts [81]. To overcome the restrictions of traditional scintimammography, specific breast-specific gamma camera imaging (BSGI) systems have been developed. The cameras using in these systems have a narrow field of view, causing improved flexibility of movement and increased resolution in comparison to conventional gamma cameras [76].

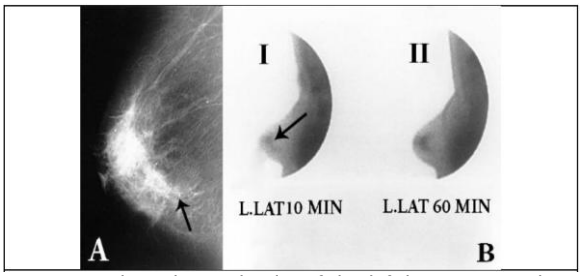

Figure 7: Ductal carcinoma in situ of the left breast, comedo, solid and cribriform type [81]. A) Mammography, medio-lateral projection. Microcalcifications (transparent arrow) behind the nipple; B) Scinti-mammography, left lateral projection: 99m Tc-(V) DMSA at 10 min and 60 min (i-ii)

Brem et al., [79] argued that the sensitivity of

a specific gamma camera to detect breast cancer in patients with two-sided mammograms is comparable to MRI. Sweeney and Sacchini [82], on the other hand, believed that this superiority is because of using breast-specific gamma camera imaging system. Some recommendations for scintimammograph application in recent studies are as follows:

- In patients having lesions with a low probability of malignancy who referred for biopsy.

- In patients with breast cancer who their disease is confirmed to detect axillary lymph node metastases.

- As an additional test to mammography in patients who have palpable masses or suspicious mammograms to distinguish benign and malignant breast lesions.

- In patients whom mammography showed the benign probability, and are recommended for repeating mammography (in 3-6 months).

- In patients whose, dense breast tissue is found on mammography but is hard to evaluate it on mammography.

Conclusion

One of the techniques utilised in all stages of cancer management is multiple biomedical imaging. Imaging as an important part of cancer clinical protocols can provide a variety of information about morphology, structure, metabolism and functions. Application of imaging technics along with other detective apparatus including in vitro tissue and fluids analysis would help clinical decision-making. Mixed imaging techniques can provide supplementary information used to improve staging and therapy planning. Imaging aimed to find minimally invasive therapy to make better results and reduce side effects. Some of the research areas with the potential of clinical use in the next decade include gene therapy expression, targeted imaging of receptors, and cancer stem cells. Further development in technology will lead to increase imaging speed to meet physiological processes requirements. Development in targeted imaging and therapeutic agents calls for close cooperation among academia and industries (biotechnological, IT and pharmaceutical industries).

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Review Article

Maternal Mortality and Maternal Health Care in Nigeria: Implications for Socio-Economic Development

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Abstract

BACKGROUND: Even though maternal mortality, which is a pregnancy-related death is preventable, it has continued to increase in many nations of the world, especially in the African countries of the sub-Saharan regions caused by factors which include a low level of socioeconomic development.

AIM: This paper focuses on cogent issues affecting maternal mortality by unpacking its precipitating factors and examining the maternal health care system in Nigeria.

METHODS: Contemporary works of literature were reviewed, and the functionalist perspective served as a theoretical guide to examine the interrelated functions of several sectors of the society to the outcome of maternal mortality.

RESULTS: It was noted that apart from the medical related causes (direct and indirect) of maternal mortality, certain socio-cultural and socioeconomic factors influence the outcome of pregnancy. Also, a poor health care system, which is a consequent of weak social structure, is a contributing factor.

CONCLUSION: As a result, maternal mortality has debilitating effects on the socioeconomic development of any nation. It is therefore pertinent for the government to improve maternal health and eradicate poverty to ensure sustainable development.

Introduction

Maternal mortality has been on the increase in recent time with detrimental effects on the socioeconomic development of the nation. According to the World Health Organization [1], approximately 830 women die every day from preventable causes related to pregnancy and childbirth. More worrisome is the fact that 99% of all maternal deaths occur in developing countries [1].

Maternal mortality refers to deaths due to complications from pregnancy or childbirth. Even though, the United Nations International Children Emergency Funds [2], reported that from 1990 to 2015, the global maternal mortality ratio declined by 44 per cent – from 385 deaths to 216 deaths per 100,000 live births, according to UN inter-agency estimates. This translates into an average annual rate of reduction of 2.3 per cent. While impressive, this is less than half the 5.5 per cent annual rate needed to achieve the three-quarters reduction in maternal mortality targeted for 2015 in Millennium Development Goal 5. More worrisome is the fact that maternal mortality, known to be the loss of lives of women in their maternity stage due to pregnancy complication, is classified among preventable deaths [3]. Maternal and infant mortality rates are social indicators used to measure the development of any country, and the situation in Nigeria is of great concern [4]. In spite of resolution and adoption of the Sustainable Development Goals (SDGs), an effort by the United Nations enacted at the end of the Millennium Development Goals (MDGs) timeline in 2015. Part of its major task is the improvement of the health of pregnant and nursing mothers (maternal health) and reducing maternal and child death by 2030 [5]. Despite this global commitment, the loss of women's lives resulting from complications during pregnancy

has been on the increase in most sub-Saharan African countries [3]. In Nigeria for instance, maternal mortality accounts for 59,000 deaths of women annually [6], [7], [8]. Arguably, Nigerian women are 500 times more probable to lose their lives in childbirth when compared to most advanced nations of the world [9]. He further noted that Nigeria is ranked second after India in global maternal incident rate and the worst in Africa. Furthermore, Nigeria's maternal mortality is reported to be 545 per 100,000 births [7], [5]. The prevalence of maternal mortality in Nigeria has become very disturbing as every birth procedure becomes a potential incidence, from the report above, there is at least one case of maternal mortality in every 20 live births. This challenge may not be unconnected to the nation's poor maternal health care system.

Flowing from above, maternal and child mortality is a serious concern to the government and all interested stakeholder, and as such, it has become a vital issue for research. This study, therefore, aimed to unravel the concept of maternal mortality within the Nigerian context, unpack it precipitating factors and bring to the fore the debilitating effects especially in areas of socioeconomic development. The improvement of the maternal healthcare system in Nigeria cannot be overstretched, and it is a vital aspect of sustainable development.

Conceptual Clarification

Maternal mortality

Maternal mortality refers to any loss of a woman's life resulting from pregnancy complication or death within 42 days after childbirth, notwithstanding the period or site of the pregnancy, emanating from issues that are linked or escalated by the management of the pregnancy but not from accident or incidental factors [10]. There are other known factors aside medical conditions responsible for maternal mortality in Nigeria-these factors include but are not limited to social, economic and cultural factors, which have a direct influence on maternal mortality [11]. Interestingly, maternal mortality in most of the rural areas in Nigeria is caused by other precipitating factors that are non-medical. These factors range from poverty, low level of education or absence of it, prohibited food, low purchasing power and certain harmful cultural beliefs and practices; more so, with the introduction of user charges in state and federal-owned hospitals, high percentage of women, especially in the rural areas, now patronize faith clinics and traditional practitioners as alternative health care [10].

Maternal morbidity

This refers to the disease/illness experienced by pregnant women. Many times, this often results in an inability to function properly and in many situations affects the 'victim's economic, social and fertility roles [12]. In sub-Saharan Africa, the danger of death during pregnancy or childbirth is known as maternal mortality is 175 times higher when compared to other developed nations of the world, and risk for pregnancy-related illnesses and adverse consequences after child delivery is much higher [2]. Suffice to say therefore that when a woman dies from any preventable childbirth complication, a total of 500,000 deaths is recorded per year within the sub-Saharan African region [13], [2].

Maternal health care

This is the overall wellbeing of women at the stage of pregnancy and children below age 5. Maternal healthcare is comprehensive as it includes educational, social, nutritional services as well as medical care during and posts pregnancy. As has been observed, some reasons have been attributed to why many pregnant and nursing mothers chose not to make use of appropriate antenatal and postnatal cares [13]. Some of these reasons can be cultural, hereditarily related to the social, economic and political developments. This implies that both the natural environment-biological-and the social environment perform powerful and critical functions in healthcare utilisation behaviour of women across most African societies. Cultural factors, therefore, are a major determining factor influencing health care utilisation behaviour of pregnant women in Nigeria [13].

Theoretical framework

This paper will be guided by the functionalist theory. The theory supports the fact that for a successful pregnancy/maternal outcome, there must be a conscious working together of all the interrelated stages of pregnancy ranging from family planning, pregnancy, delivery to the postpartum days. Also, the need for a good maternal health care system in ensuring a positive outcome of pregnancy is very important.

Functionalist theory

The general assumptions of the functionalist view hinge on the fact "that society can be explained as a whole unto itself" [14]. To the classical theorists like Comte, Spencer, and Durkheim, human society is likened to an organism that has different but interrelated parts with a functional prerequisite for adaptation and survival. So for a society to survive, the different parts (social institutions) must work and

adapt to each other. The functionalists perspective are more refined in their thinking as they attempt to view the society as a system with interconnected and interrelated structures which form a whole indicating that the existence of the societies requires that specific aspects of the social world must work in tandem for the smooth running of the society [14].

Notable among these contributions is the work of Talcott Parsons which has left an indelible mark in the area of sociological thinking [14]. Parsons attempted to build concepts that would assist in the organisation of the perceptions of the social world. In his analysis, any society or social system has four basic requirements namely Adaptation, Goal attainment, Integration and Latency with the acronym (AGIL). First among the functional prerequisites is an adaptation which refers to the association between the social system and its environs. It is worthy to note that for the society to stay alive, social systems must have some measures of control mechanism over their environs. Food, clothing and housing must be available to meet the physical and social prerequisites of citizens in the society. The second functional prerequisite, Goal attainment refers to the need for all members in the social order to set goals towards the direction of social events. The responsibilities of governments are not only to set objectives but also to assign resources to accomplish them. In a nutshell, the financial or monetary status is controlled and directed by rules and regulations passed by the governments. The third functional prerequisites, Integration refers to the bringing together and joint adjustments of the societal systems.

A typical example is when conflict arises, it is settled by the judicial system thereby not leading to the dissolution of the social system. The fourth functional prerequisites, Latency meaning the constant upkeep of values, norms established in the social world. Social organisations that execute these responsibilities consist of the educational system, religion and the family [15].

The functional prerequisites of a social system are connected to social and cultural imperatives. Also, the complexity of social institutions under the rubric of the process of socialisation exhibits the integrative responsibilities of the social system. Meanwhile, the values and norms serve to motivate social action grouped as part of the cultural system.

Theoretical imperatives and the state of maternal healthcare in Nigeria

Flowing from the above, maternal mortality and maternal health care can be understood about pregnancy outcome and maternal health care in Nigeria. Pregnancy outcomes in women are a result of numerous aspects ranging from cultural, social and economic. Also, the reproductive period of women is a function of different but related activities-biological and socio-cultural. For instance, every stage of pregnancy must be well planned and require adequate attention to avoid pregnancy complications before, during and after birth. As noted by the functionalist view, failure at any stage of the pregnancy may affect the outcome of the pregnancy. If there is no proper family planning, this might lead to unwanted pregnancy, and abortion may be considered which may terminate the life of the woman. Also, the health care sector is a major component of maternal well-being of women of childbearing age. A good health care system will promote maternal health thereby reducing maternal mortality. This position is supported by [16], in his estimation of maternal death in Nigeria; over 70 per cent of its causes were linked to five main pregnancy difficulties: infection, haemorrhage, obstructed labour, unsafe abortion and hypertensive disease of pregnancy. In Nigeria, poor access to healthcare facilities and under-utilisation of effective and efficient reproductive healthcare services significantly contribute to the high level of maternal mortality [16].

In the view of Akokuwebe and Okafor (2016), the Nigerian health sector is confronted with the most daunting crisis of maternal morbidity, during pregnancy and post-delivery. With government spending about 70 per cent to 80 per cent of the nation's available resources for healthcare at the point of need of the citizens, the social system cannot perform in a different and better way to deliver better health outcomes. In addressing health care needs and improving the issue of maternal mortality, funds should be available and spent on healthcare delivery at all cost [17]. The key indicator of maternal health is primary healthcare. Among women in Nigeria, the social epidemiology of disease includes but it is not limited to haemorrhage, or complications of childbirth, like postpartum infections. Most of these diseases are highly avoidable. Despite the huge sum of money budgeted or dispensed for health care system in Nigeria, the reality remains that these funds do not get to be used to the purpose for which it was committed and as such the pending challenges within the health care system persist [17]. It is therefore imperative that the healthcare delivery system needs a transformation that will address the inherent corruption that syphon resources meant to lead to efficient and effective delivery of services within the sector. Primary health care should be duplicated most especially in the rural areas of Nigeria to allow pregnant mothers to have access to free health care services or at least, quality and affordable health care services. There is also a need for government to commit more finance into the healthcare system as the current investment in health is minimal compared to other advanced countries of the world [14]. A well-organised health insurance scheme at the community level can increase substantial resources to provide healthcare to women.

Methods

The secondary was used in work with existing data from the various international health organisations like the WHO, UNICEF, NHDS and so on. This is necessary because of the wider scope of the study.

Maternal Health and Women's Reproductive lifecycle

Women's reproductive period is a very crucial period and spans several stages from the pregnancy, antenatal, childbirth or delivery, postnatal period and family planning stages [4]. It is rightly believed because even though there is an expectation for a positive outcome, many times it results in death. Reproductive/Maternal health indicates that women can go through pregnancy and child delivery safely and that reproduction is carried to a favourable outcome. This however remains a mirage in Nigeria because, the patriarchal system (male domination) expects the man to be adventurous and risky without any recourse to his actions, while the woman bears the brunt of the consequences such as sexually transmitted infections like HIV/AIDS, reasons being that she is in no position to discuss or negotiate the use of condom or family planning methods [4].

Consequently, Nigeria is noted as one of the countries with severe cases of maternal mortality in the world [18]. As posited by Nwokocha (2012), different stages of the women reproductive lifecycle must be given due attention for a smooth pregnancy process. He further proposed modules on different stages of maternal health/reproductive lifecycles. The modules as highlighted by Nwokocha (2012) are at this moment discussed below.

Family Planning

This module is very crucial to the maternal outcome because it enables the couple to decide some issues. It enables the couple to plan and avoid unplanned pregnancy properly.

Pregnancy

This module according to Nwokocha (2012) is aimed at establishing the fact that pregnancies are supposed to be planned and not a product of circumstances. Circumstantial pregnancy has been the issue for many illiterate couples, especially those in rural areas of Nigeria and other sub-Saharan Africa countries. This module is programmed not only to emphasise the linkage between family planning and pregnancy but also to highlight individual and household expectations during pregnancy.

Antenatal care services

The focus here is on timely registration for antenatal care, choice of location, accessibility and quality of service. Also, important is the attitude of both the caregiver and care seeker in achieving maternal wellbeing.

Child Delivery

Irrespective of the seeming experience level about childbearing among women, it is still imperative to further enlighten them on some important but neglected issues around childbirth [19]. Services of certain medical specialist are therefore needed to handle these various issues.

Postnatal period

After childbirth, many individuals are likely to become complacent towards important postpartum practices may be due to excitement or lack of knowledge of the health implications. It has however been noted that postpartum death contributes largely to maternal death [19].

Beyond postnatal

According to Nwokocha (2012), breastfeeding, for some women is a form of contraceptives, while for others, it is a different thing entirely. ''Therefore, to manage fertility beyond the postpartum period requires insight, perseverance and diligence which should be carefully taught'' (Nwokocha, 2012:22). This module involves relevant factors that border on coitus, intake of dietary and avoiding sex without offending your spouse among others.

Maternal Health Care in Nigeria

According to Okeke *et al.,* (2016) Women and their health have largely been influenced by the African traditional culture. Owing to the patriarchal nature of most of these African societies, diverse inequities are being perpetrated against women. ''It is not just what is done to women, but what is not done for them'' [4]. Maternal health according to [6] is defined as a state of total physical, mental and social wellbeing and not just the nonexistence of illness or infirmity in all issues that has to do with the reproductive age of women. Furthermore, with peculiarity to the African societies, maternal health would include the ability to "exercise reproductive rights of family planning and access to basic focused antenatal care, without the encumbrances of patriarchy, financial or geographical inhibitions impacting on her overall health'' [4].

Maternal health care services include an

extensive scope of health services mothers are given before pregnancy, during pregnancy, delivery and post-natal. Maternal health care services, therefore, comprise pre-natal care, childbirth and postnatal care.

However, in Nigeria and other parts of African societies, certain cultural practices continue to affects maternal wellbeing, and by consequent, the children. As noted by Nwokocha (2008) among Ibani people of Rivers State, women are not allowed to come out during certain festivals as doing so will amount to severe punishment, not minding her health condition even when she is pregnant. Also, in the northern part of the country where the purdah system is practised, women's moving about is somehow restricted to prevent them from being seen by other men asides their husbands. This has implications on their maternal health as some will frown at male health practitioners attending to their wife during delivery. They would rather prefer female health practitioners to attend to them. The Oro festival among the Yorubas in the southern part of Nigeria is another cultural activity that impinges on women freedom of movement, especially during emergencies.

Another issue affecting maternal health in Nigeria is the poor state of infrastructure, systemic failure and inability to access health care services especially the rural dwellers. The main challenge facing pregnant and nursing women in Nigeria is that of poor organisation and inability to access maternal health services.

Maternity care in Nigeria is divided into three stages namely primary, secondary and tertiary care levels [19]. Primary healthcare centres are established in all the 774 local government areas of Nigeria. It is expected that pregnant women should receive antenatal care, delivery and postnatal care in the primary health centres closest to them. In case of pregnancy difficulties they are referred to secondary care centres, under the management of state government, or tertiary centres, managed by the federal government [19].

According to Akokuwebe and Okafor (2016), pregnancy-related death is the main most critical health problem that medical practitioners and Nigerians are faced with. Many of the Nigerian populace, especially women who are of the low socioeconomic background are constantly at the verge of being a victim of maternal mortality. This condition makes them prone to sickness, infirmity and in most cases result in death due to their inability to access good health services, especially reproductive health services. It is important for women in their reproductive age to have unhindered access to quality reproductive health services and be empowered to make decisions on issues of family planning [14]. Having unhindered access to comprehensive reproductive health care, will increase women's chances of survival during pregnancy, give them the opportunity of having healthy children and enable them to have a balanced family and work life.

However, the health care system in Nigeria is bedevilled with the challenges of quality service delivery, poor attitudes of staff to patients, lack of expertise, lack or poor equipment, and shortages of essential drugs. Erratic and epileptic power (electricity) and water supply and the health sector as a whole are in a state of comatose. Nigeria healthcare system was ranked by 187th among 190 United Nations member states [1].

According to a study conducted in 2003, it was shown that only 4.2 per cent of public facilities in Nigeria are of internationally acceptable standards for crucial obstetric care [20]. Almost two-thirds of all women in Nigeria deliver outside of health facilities and without medically skilled attendants present [21]. The poor outcome of Nigerian health care is not unconnected with the nation's bad leadership and poor governance. Kleptomania nature of the political system is at its peak, deeply entrenched corruption with a high level of impunity, making developmental goals which encompass the provision of quality and affordable health for Nigerian citizens inconclusive.

Cultural factors influencing maternal mortality

According to Salami & Taiwo (2012), certain cultural practices have been observed as responsible for incidences of maternal and infant mortality in Nigeria and other parts of the sub-Saharan African societies. In the same vein, Nwokocha (2003) cited in Elem and Nyeche (2016) has discovered in a study conducted among Ibani people in Rivers State on pregnancy outcomes that incidences of maternal mortality rate are on the increase and outcome of pregnancy (which could either be positive or negative) is affected by socio-cultural factors.

For instance, some cultures abhor forbids pregnant mother from eggs and snails consumption which is needed for the supply of nutrients to the mother and baby during pregnancy. This may lead to incidences of high iron-deficiency or anaemia that consequently leads to death if not well managed. Also, women are saddled with numerous tasks of reproduction, home management and community building, among others. They are defined by their reproductive function, coupled with the very many household chores of providing for and keeping the home front. They enjoy no special attention or care during pregnancy which makes many of them tired and experiences fatigue during pregnancy.

However, what is required of maternal mothers at this time are improved nutrition, rest, and focused ante-natal care as well as moral and financial support [4]. Unfortunately, these experiences are very rare for many Nigerian women. Hence maternal mortality has become a public health issue as statistical evidence shows that at every moment, a

woman dies from difficulties of pregnancy, bringing the maternal mortality rate for Nigeria to 3200 per 100,000 live births [7]. It is further stated that these figures are worse in the Northern part of Nigeria, raising the tension that a process, such as pregnancy, can be a life-threatening process [4].

Socioeconomic factors influencing maternal mortality

The social and economic factors affecting maternal mortality are interconnected and interrelated with the financial power of women, inadequate access to wealth and economic resources as well as difficulty in getting well-paid employment. Wealth, especially in terms of finances acquired by women, is spent on the family rather than themselves. The present global economic crises has compounded the plight of many people with increase in the cost of healthcare services and facilities coupled with lack of proper funding of the health sector by government (World Health Organization recommends that 5 per cent of government funding should be for health) but most countries spend 1.5 to 4 per cent [14].

The right to use maternal health services is hooked on quite a lot of factors about responsiveness, social and economic status, socio-cultural philosophies and practices to mention a few [23]. It has been observed that maternal education is significantly associated with fertility and maternal health [24].

In Nigeria, a high percentage of pregnant women do not receive adequate care; this is as a result of lack of services in the residence they live, or inability to afford the services because they are too expensive. A significant proportion of women do not make use of services because they do not like how care is provided or because health services are not delivering high-quality care. Furthermore, cultural philosophies, patriarchal nature of the country and insubordination of women in the society can prevent a pregnant woman from getting the care she needs. To improve the quality of maternal health, gaps in the capacity and quality of health systems and barriers to accessing health services must be identified and tackled at all levels.

Effects of maternal mortality on socioeconomic development

The effects of maternal mortality on socioeconomic development cannot be overemphasised. Maternal mortality which in most cases is as a result of low level of socioeconomic development is also a major factor hindering sustainable development. Maternal mortality remains a major indicator used in measuring the level of development of a society and the performance of the healthcare delivery system [14]. They further posited that maternal mortality remains the main focus of developmental agenda in sub-Saharan Africa (SSA) countries. The goal is to tackle the causes by adopting a paradigm of development that is more inclusive for sustainable transformation and development. At present, maternal health in Nigeria is incapable of achieving long-lasting development in Nigeria as a result of poverty, massive corruption, misplaced priority, and neo-liberal policies of government leading to social and economic dislocation of families [14]. In finding solutions to fight high maternal mortality in Nigeria, there is a need to provide a framework for development strategies by the government and relevant stakeholders. There is also a need to transform maternal health by eradicating poverty amongst disadvantaged groups in the rural areas and incorporating sustainability considerations. In developing countries, especially in Nigeria, maternal health is a major concern. Without the achievement of maternal health, sustainable development will remain elusive for any country. A high percentage of women in Nigeria who are poor are very vulnerable to illness, disability and even death [3]. This is as a result of lack of access to comprehensive health services, especially reproductive health services. A situation which currently put Nigeria as one of the highest in maternal mortality rates in the developing nations [21].

According to Akokuwebe and Okafor (2016), the implications of poor maternal health and high maternal mortality among others include [14]:

• The failure of Nigeria to meet up with Millenium Development Goals (MDGs) by 2015 in the sustainable and transformation agenda.

- High level in maternal death or maternal health-related concerns, affect women's ability to fully participate in the labour force that will enhance sustainable development.

- High maternal mortality results in low life expectancy in the population pyramid for women in the reproductive age (15-49 years) who are the main drivers of sustainable development in any country.

- Budgetary allocation year-in-year-out to combat maternal health rather than diversifying resources to other sectors for developmental purpose.

- High incidence and prevalence of maternal mortality equal to the high incidence of single parents and orphaned children, which is a threat to sustainable development.

In conclusion, the rates of maternal and infant mortality in Nigeria are high and at an unprecedented height. This is a pressing concern for the nation, and various efforts have being geared towards combating the challenge, but all remain inconclusive. Factors responsible for maternal mortality are mainly cultural and systemic. Also, poverty and lack of education influence the seeking behaviour of rural women. It is therefore important to focus on an overall health reform program that will involve maternal education,

access to health care services and women empowerment which will enable them to make informed decisions on issues relating to their reproductive life.

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Prevalence of Malocclusion among Schoolchildren in Makkah, Saudi Arabia

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Abstract

BACKGROUND: The prevalence of malocclusion varies from one region to another and among different age groups and ethnicities. According to the World Health Organization, malocclusion is the third most common abnormal dental condition.

AIM: This study aims to establish the prevalence of malocclusion among schoolchildren in the Holy City of Makkah, Saudi Arabia.

METHODS: A cross-sectional study was conducted among 400 Saudi schoolchildren, 12-15 years of age, of both genders, randomly selected from 15 schools in different regions of Makkah. Molar and canine relationships were examined, in addition to traits such as crowding, spacing, overjet, overbite, cross-bite, scissor bite, and maxillary diastema.

RESULTS: The most prevalent molar relationship was Class I (52.3%), followed by Class II (25%), and Class III (20.5%). Crowding was the most prevalent malocclusion trait (74%), and scissor bite was the least common (2.5%). A statistically significant difference was found between males and females in most of the recorded criteria ($P < 0.005$).

CONCLUSION: Among schoolchildren in Makkah, Class I molar relationship was the most prevalent type of occlusion, and the most prevalent malocclusion trait was crowding.

Introduction

Malocclusion occurs when the dental arches are misaligned, incorrectly related to each other, or when there is an irregularity in teeth position beyond the normal limits [1]. It is usually a developmental condition rather than a pathological one [2]. There are many etiological factors associated with malocclusion, including an abnormal number of teeth, jaw size and form, habits such as nail biting or thumb sucking, and premature loss of or prolonged retention of deciduous teeth [3].

Dental caries and periodontal diseases may result from malocclusion [4], and due to an unpleasant dentofacial appearance, psychosocial problems may develop [5]. Malocclusion may also have unfavourable effects on oral functions, including mastication, swallowing, and speech [6]. Furthermore, if malocclusion remains untreated, it may increase the incidence of temporomandibular joint disorders [7].

Previous studies have shown that the prevalence of malocclusion varies widely among several populations around the world; these variations may be due to differences in age, ethnicity, or size of the studied populations [8].

A study in the northern border region of Saudi Arabia was conducted on 500 Saudi adolescents (male and female) showed a considerable prevalence of malocclusion [9]. Another study in the southern region of Saudi Arabia including only males but with a large cross-section of different ethnicities also showed a high prevalence of malocclusion, and the most common malocclusion trait was crowding [2]. In the central region, specifically in Riyadh, two studies have

reported the predominance of Class I molar and canine relationships among Saudi children (12-16 years of age), and crowding was again the most common malocclusion trait [10], [11]. Another study was conducted in the eastern region of Saudi Arabia, on 330 female patients. They were categorised into an adolescent group (12-17 years) and an adult group (18-35 years). The study revealed that adolescents had more spacing problems and discrepancies involving overjet and overbite than did adults [12].

When reviewing the available literature for similar studies conducted in regions outside of Saudi Arabia, we found one study that showed 70% of adolescent Kuwaitis had moderate-to-severe malocclusion [13]. Another study in Turkey showed that only 3.5% of 1,507 patients had normal occlusion [14].

Because malocclusion can affect a patient's function and appearance, it is better if it is identified and treated at early stages to avoid further complications [15] and to increase the probability of a favourable prognosis [16]. Unfortunately, few studies have examined the prevalence of malocclusion in the western region of Saudi Arabia [17], [18]. Further studies in the western region would form a complete database to aid in planning appropriate orthodontic treatment services among all regions of Saudi Arabia and help to assess the resources needed to overcome the increasing number of malocclusion cases.

Thus, this study aimed to assess the prevalence of malocclusion among schoolchildren in the Holy City of Makkah, which is in the western region of Saudi Arabia.

Methods

Subjects

The study was conducted in the Holy City of Makkah, Saudi Arabia, and included 400 Saudi schoolchildren (200 females and 200 males who were born and lived in Holy City of Makkah, Saudi Arabia) aged 12-15 years. The sample was selected from ten intermediate schools, which were randomly chosen from different regions of the city (two schools from each region): the east, west, north, south, and centre of the city. Students with missing teeth due to previous extraction, previous orthodontic treatment, maxillofacial surgeries, or syndromes were excluded from the study.

Ethics

Ethical approval was obtained from the institutional review board of Umm Al Qura University Faculty of Dentistry and written informed consent was provided by the participants' parents before the examination and after explaining the purpose and style of the study.

Training, Calibration, and Standardization

To standardise examinations, dentists independently examined 10 patients at baseline and discussed outcomes. Following this initial review, the dentists attended a lecture given by an orthodontist on objective diagnostic criteria. Finally, 2 weeks after the baseline examination, the dentists were calibrated by independently examining another 10 patients and comparing the results (Kappa = 0.88).

Clinical examinations and data collection

Clinical examinations were carried out in each school by the examiners using a disposable mouth mirror, ruler (numbering started with zero), and light source. Severely decayed teeth, periodontally affected teeth should be mentioned; the following traits were recorded:

- The molar relationship between right and left sides recorded according to Angle's Classification:

Class I: Mesiobuccal cusp of the maxillary first molar occludes with the mesiobuccal groove of the mandibular first molar in maximum intercuspation.

Class II: Mesiobuccal cusp of the maxillary first molar occludes mesial to the mesiobuccal groove of the mandibular first molar in maximum intercuspation.

Class III: Mesiobuccal cusp of the maxillary first molar occludes distal to the mesiobuccal groove of the mandibular first molar in maximum intercuspation [19].

- The canine relationship between right and left sides, according to Angle's Classification:

Class I: Maxillary canine cusp tip occludes with the embrasure between the mandibular canine and first premolar.

Class II: Maxillary canine cusp tip occludes mesial to the embrasure between the mandibular canine and first premolar.

Class III: Maxillary canine cusp tip occludes distal to the embrasure between the mandibular canine and first premolar [19].

- Crowding/spacing: This is the malalignment of teeth. Crowding was measured as a negative value, while spacing was measured as a positive value.

- Overjet: This is the horizontal distance from the labial surface of the mandibular incisors to the labial surface of the maxillary incisors. It was considered positive when the upper incisors overlapped with the lower, zero when they were edge

to edge, and negative when the lower incisors overlapped with the upper incisors [20]. If the overjet was between 2 and 5 mm, it was considered normal based on examiner clinical experience; greater than 5 mm was considered increased, and less than 2 mm was considered decreased.

- Overbite: This is the vertical measurement of anterior maxillary teeth covering the mandibular anterior teeth in the normal occlusal position of the jaws [20]. If the overbite was one third, it was recorded as normal and more than two-thirds were considered a deep bite. If an overbite is zero, it would be edge-to-edge; in the case of no contact between the maxillary and the mandibular anterior teeth, it would be considered an open bite.

- Anterior cross-bite: This is defined as a palatal positioning of the anterior maxillary teeth to the mandibular anterior teeth [21].

- Posterior cross-bite: This is a transversal relationship between the upper and lower jaws, where the upper buccal cusps occlude the fossa of the lower teeth [22].

- Scissor bite: A scissor bite occurs “if the lingual cusp of the upper tooth occludes buccally to the buccal cusp of the corresponding lower tooth” [23].

- Maxillary diastema: This is defined as space/gap between the maxillary central incisors, which is normally present in the primary and mixed dentition and closed after the complete proper eruption of permanent maxillary canines [24].

Statistical analyses

Primary outcomes included molar and canine relationships. Secondary measures included spacing or crowding, overbite, overjet, scissor bite, and maxillary midline diastema. We calculated descriptive statistics for the demographics and occlusion status for all participants. We reported means and standard deviations for continuous measures and numbers and percentages for categorical measures. Further, the Chi-square test (level of significance = 0.05) was used to compare any differences between male and female students about occlusion classification. Stata statistical software version 14 (StataCorp) was used to analyse the data.

Results

Four hundred Saudi intermediate schoolchildren met the selected criteria (200 males and 200 females); the average age was 13.5 ± 0.9 years.

The most prevalent molar relationship was Class I (52.3%), which was higher in females (P < 0.05), followed by Class II (25%), and Class III (20.5%). Of the study subjects, 2.3% had severely destructed first molars due to dental caries. Therefore, their Angel’s Classification could not be determined (NA) (Figures 1 and 2).

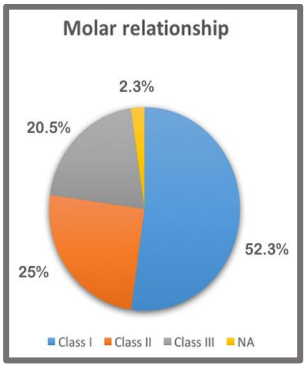

Figure 1: The Prevalence of Molar Relationship (NA = Not applicable: missing tooth)

The prevalence of canine relationship was 56.5%, 27.8%, and 12.8% for Classes I, II, and III, respectively, with a higher prevalence of Class I in females (P < .05) (Table 1).

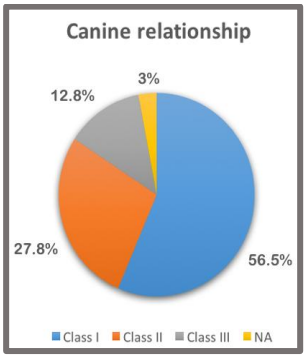

Figure 2: The Prevalence of Canine Relationship (NA = Not applicable: missing tooth)

Crowding was the most prevalent malocclusion trait among the study sample (74%), but it was observed in males more than in females (P < 0.05). The next most prevalent trait was two-third overbite (33.5%), which was relatively comparable between the genders.

Table 1: Prevalence of Molar and Canine Relationships, by gender

	Gender				P-Value
	Male		Female		
	No.	%	No.	%	
Molar Relationship					
Class I	81	40.5	128	64	< 0.001
Class II	65	32.5	35	17.5	
Class III	48	24	34	17	
NA (Not applicable: missing tooth)	6	3	3	1.5	
Canine Relationship					
Class I	96	48	130	65	0.002
Class II	67	33.5	44	22	
Class III	31	15.5	20	10	
NA (Not applicable: missing tooth)	6	3	6	3	

Of all study subjects, 71.3% had a normal

overjet measurement (2-5 mm); 22.3% had a cross-bite; spacing was recorded in 21.5% of them, all the three of the previously mentioned traits were more prevalent in females (P < 0.05).

Maxillary diastema was found in 22% of the cases. The least common trait was Scissor bite (2.5%), and only 3.8% of the subjects had a deep bite. The prevalence of open bite and the prevalence of an edge-to-edge incisal relationship were 3.3% and 4.5%, respectively. There was a statistically significant difference between males and females in all of the measured malocclusion traits (P < 0.05) except for overjet and maxillary midline diastema, both of which revealed no statistically significant difference (P > 0.05) (Table 2).

Table 2: Prevalence of Different Malocclusion

	Gender				Total		P-Value
	Male		Female				
	No.	%	No.	%	No.	%	
Is there spacing or crowding?							
Normal	1	0.5	17	8.5	18	4.5	< 0.001
Crowding	161	80.5	135	67.5	296	74	
Spacing	38	19	48	24	86	21.5	
Is there an overbite?							
Normal	105	52.5	115	57.5	220	55	0.021
Two-Thirds	74	37	60	30	134	33.5	
Deep Bite	3	1.5	12	6	15	3.8	
Open Bite	5	2.5	8	4	13	3.3	
Edge to Edge	13	6.5	5	2.5	18	4.5	
Is there a cross-bite?							
No	165	82.5	146	73	311	77.8	0.022
Yes	35	17.5	54	27	89	22.3	
Overjet Category							
Normal 2-5 mm	133	66.5	152	76	285	71.3	0.077
Reduced <2 mm	47	23.5	30	15	77	19.3	
Increased >5 mm	20	10	18	9	38	9.5	
Is there a scissor bite?							
No	200	100	190	95	390	97.5	0.001
Yes	0	0	10	5	10	2.5	
Is there a maxillary midline diastema?							
No	151	75.5	161	80.5	312	78	0.227
Yes	49	24.5	39	19.5	88	22	

*P-values for comparing male vs female using chi-square test.

Discussion

The goal of this study was to determine the prevalence of malocclusion among school-aged children in the Holy City of Makkah. Four-hundred Saudi schoolchildren were examined, and their occlusion status was recorded. Their ages ranged from 12 to 15 years; this is the preferred age range for orthodontic intervention if malocclusion is found because it is the stage of late-mixed or early-permanent dentition. The subjects were randomly selected from five regions to represent Makkah's population: the north, south, east, west, and centre of the city.

Several studies have been published regarding the prevalence of malocclusion in different populations. However, due to the wide variation in ethnicities and endogenous traits, diverse methods, and differences in sample size, it is difficult to compare the findings.

The present study revealed that the Class I molar relationship was the most prevalent type of occlusion (52.3%), followed by Class II (25%), and Class III (20.5%), which is in agreement with findings by Meer et al., in the southern region of Saudi Arabia [2]; both reported a high prevalence of crowding and a lower prevalence of spacing. The high prevalence of molar Class I, followed by Class II, and Class III found in the present study is similar to that found in studies conducted in Kuwait, Turkey, Jordan, and the northern border region of Saudi Arabia [8], [9], [13], [14]. However, in Riyadh, Asiry reported that Class I was the most prevalent, followed by Class III, then Class II; the same molar-relationship sequence was found in Dammam [10], [12]. The Class I canine relationship was more frequently observed in this study (56.5%) than Class II (27.8%) and Class III (12.8%). Dissimilar findings were reported in Kuwait by Behbehani, who found that the Class II canine relationship was more common than other types [13].

Crowding was the most prevalent malocclusion trait in the current study; it was recorded in 74% of the examined subjects, while in the northern border region of Saudi Arabia, Riyadh, and Aseer, it was much less prevalent (47.2%, 45.4%, and 43.8%, respectively) [2], [9], [10]. However, in Dammam City, crowding was evaluated for both arches: a prevalence of 73.4% was found for the maxillary arch and 67.8% for the mandibular arch [12]. Spacing problems were found in 21.5% of subjects in the present study; this finding is comparable to the findings in Riyadh (20.4%), Asser (16.7%), the northern border region of Saudi Arabia (27.2%), Turkey (12.5%), Jordan (26.7%), and Colombia (25.9%) [2], [8], [9], [11], [14], [15].

We found that 55% of the study subjects had a normal overbite, which is comparable to the findings in the northern border region of Saudi Arabia (64.4%) and Turkey (53.5%) [9], [14]. A deep bite was found in 3.8%, which is higher than the 1.7% found in Kuwait [13] and less than the 8.8% found in Riyadh [11]. An open bite was found in 3.3%, which is comparable to the northern border region of Saudi Arabia (4.6%) and less than that in Colombia (9%) [9], [24]. The prevalence of edge-to-edge was 4.5%, which is less than that observed in Turkey (12.1%) [14].

We found that 71.3% had a normal overjet (2-5 mm), which is comparable to the findings in Riyadh (75.4%). A reduced overjet (< 2 mm) was found in 19.3%, which is more than that found in the northern border region of Saudi Arabia (11.4%). An increased overjet (> 5 mm) was found in 9.5%, which is low in comparison to the findings in Jordan (24.7%) and Turkey (41.7%) [8], [9], [11], [14].

The prevalence of cross-bite was 22.3%, which is slightly high if compared to the findings in Asser (12.6%), Riyadh (17.3%), and the northern border region of Saudi Arabia (14.2%) [2], [9], [10]. Scissor bite was less prevalent than cross-bite, as its

percentage was 2.5% in the present study. However, in Riyadh, there were no incidences of Scissor bite (0%) [10].

In this study, maxillary midline diastema was found in 22% of the study subjects, which is higher than that found in Jordan (6.9%), Turkey (4.5%), and Colombia (7%) [8], [14], [25]. A statistically significant difference ($P < 0.005$) was found between genders in this study among most of the malocclusion traits, which is in agreement with Celikoglu et al., who reported statistically significant differences between males and females with regard to the prevalence of crowding, cross-bite, overjet, and overbite categories [14]. However, Asiry reported different results in Riyadh, as there was no statistically significant difference between the genders among any of the malocclusion traits [10]. The disagreement in these findings could be due to differences in sample size, gender distribution, or registration method, or it could be due to different features of Makkah's population about other regions of Saudi Arabia or other countries.

Further studies are needed in different regions of Saudi Arabia to determine the cause of malocclusion. Studies should examine larger sample sizes and employ additional examination methods such as radiographs and study casts.

In conclusion, this cross-sectional study showed the most prevalent type of occlusion among the Holy City of Makkah to be the Class I molar relationship and revealed normal overjet findings in the majority of cases. Crowding was the most prevalent malocclusion trait, followed by a two-third overbite. A significant difference was detected between males and females in the prevalence of all parameters except for overjet and maxillary midline diastema. These descriptive findings will aid us in understanding the overall picture of malocclusion in these regions and in providing a database that includes the occlusion status among the Saudi population to facilitate the development of appropriate preventive and therapeutic programs. Additionally, they will further increase society's awareness of malocclusion, its aetiology, complications, and when it is necessary to see an orthodontist. However, this research has some limitations, as it was a cross-sectional study that used only basic examination instruments. Thus, further causality studies that include a larger population and utilise additional examination methods are recommended.

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Dental Science

Effect of Commercially Available Denture Adhesives on Microhardness of a Flexible Denture Base Material

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Abstract

BACKGROUND: Various clinical cases of thermopress denture base materials necessitate the use of denture adhesives to achieve proper retention and stability of the removable prosthesis. Therefore; the microhardness of these flexible materials as surface property and its' alterations due to the application of various denture adhesives are still crucial issues to be discussed.

AIM: This study aimed to investigate the impact of two commercially available denture adhesives (DAs) on microhardness of a flexible denture base material.

METHODS: A total of 30 duplicate disc specimens (DS) were fabricated from a thermoplastic injection moulded resin (TR). The obtained 30-disc specimens (DS) were stored in distilled water for seven days, and then their microhardness was measured using Knoop Hardness Test (KHN) under a 10 g load for 10 seconds. The denture adhesives were prepared, and 15 DS were immersed in Corega Super Cream, while the other 15 DS were soaked in Fitty Dent Cream. All DS were stored in distilled water at 37°C. After 30 days of immersion in DAs, microhardness of DS was again measured. T-test for paired observation was used to investigate any alterations in microhardness between the baseline and after 30 days of immersion in the DAs. Statistical analysis was performed with SPSS 20®, Graph Pad Prism® and Microsoft Excel 2016 with a significant level set at $P \leq 0.05$.

RESULTS: Student`s t-test had revealed a significant difference between both groups after application of denture adhesive as a P value < 0.05. The obtained results showed that DA material type, flexible denture base material and their surface interaction provoke a statistically significant outcome on the mean microhardness.

CONCLUSIONS: DAs were found to affect the microhardness of thermoplastic injection moulded resin (TR); which may jeopardise the durability and serviceability of complete denture and patients' acceptance and comfortability.

Introduction

Actually; removable dentures are remaining essential prostheses for many clinical conditions of oral rehabilitation, especially in cases that need restoration of the edentulous ridges; which are located posterior to the remaining anterior teeth [1]. Since its fortunate introduction in 1937, the polymethyl methacrylate (PMMA) persists as the most popular of all polymeric denture base materials used in removable prosthetic base [2]. Commonly, that acrylic resin consists of the powder form of polymethyl methacrylate (PMMA), and liquid form of methyl methacrylate (MMA) proportioned mixture that is processed by a heat cured polymerisation technique [3], [4].

Moreover, PMMA has been the resin material of choice for fabrication of nonmetallic denture base prosthesis owing to many advantages including acceptable biocompatibility, the feasibility of manufacturing and manipulation, good aesthetics, favourable physicochemical properties as well as ease of construction, rebase, relining and repair. However, the inherent disadvantage of PMMA resin causes an allergic hypersensitivity reaction to some laboratory technicians and patients. This tissue reaction is provoked by the continuous leaching out of

the residual MMA monomer; therefore, also compromising the mechanical properties and surface hardness of the cured resin [5], [6]. Furthermore, PMMA denture base resin materials are characterized by low impact strength, weak strength properties, reasonable hardness and low fatigue resistance [7].

Currently, some thermoplastic resins like nylons and polyamides successfully became common alternatives to the PMMA resin materials due to the improvement of certain characteristics. The modified thermoplastic polymer characterised by improved physicomechanical properties [8]. Thermoplastic resins show a smart, flexible nature which produces comfortable stress breaking a design to the removable partial and complete dentures [9]. Also, polyamide thermoplastic denture base materials offer a proper reflection of the oral tissue colour due to their high transparency (i.e. better esthetics). Also, flexible thermoplastic denture base materials present essential advantages to patients in terms of comfort (i.e. lightweight) [10].

Consequently, thermoplastic resins seem suitable denture base materials especially for patients with hypersensitivity to acrylic monomers (as there are almost no free monomers in this material) and for patients who are allergic to nickel. Ridges are having bilateral undercuts and patients having microstomia are also indicated for thermoplastic resins [11]. Recently, variable thermoplastic denture base materials are commercially available like flexible, flexplate, Proflex and Bio-dentaplastas [12].

Whenever used properly, denture adhesives are considered as safe biomaterials that improve patient comfort, retention and stability of removable prosthesis and psychological security. Indeed, although the denture adhesives greatly enhance the removable denture performance and patient confidence, their use should not be aiming to compensate for the prosthetic denture deficiencies [13]. So, the patient should use the denture adhesive only on dentist advice and on the other hand, the dentist should give the patient full instructions about the proper use and precautions of denture adhesives [14].

Ideal requirements of a commercially available denture adhesive (gel powder, or cream form) should be: biocompatible, easily applied and adhere to the fitting surface of the denture, odorless and tasteless, being adhesive for long period (12 to 16 hours), retentive and stable during functions, comfortable and not favorable for microbial proliferation [15].

Surface microhardness may give an idea about material density; as dense materials usually have high resistance to superficial wear. Therefore, evaluating the microhardness of the thermopress acrylic resins indicates the material's ability to maintain the fine details recorded by the impression [16]. Few studies concerning thermoplastic acrylic resin microhardness were conducted [17].

Flexible resin polymers were initially introduced for the construction of provisional and immediate prosthesis [18], [19]. Furthermore, the inherent flexibility of these polymeric materials protects this prosthesis from impact and fatigue fractures [20], [21], [22].

Therefore, the purpose of the presented in vitro study was to evaluate and to record any alteration in the mean microhardness value of a thermoplastic flexible base resin polymer when the denture adhesives are utilised.

This study aimed to investigate the impact of two commercially available denture adhesives (DAs) on microhardness of a flexible denture base material.

Material and Methods

Material

The studied two types of commercially available denture adhesives were: Super Corega Cream (Carboxymethyl cellulose-based, Stafford-Miller, Dungarvan Co. Waterford, Ireland) and Fittydent Cream (Sodium Carboxymethyl cellulose and polyvinyl acetate-based, Fittydent Int. GMBH, Pinkafeld, Austria). The investigated thermoplastic injection moulded resin flexible (TR) denture base material was TCS (Thermopress flexible partial and complete denture base resin, Signal Hill, California, USA).

Fabrication of Disc Specimens (DS)

A total of 30 identical disc specimens (10 mm in diameter and 5 mm in thickness) were fabricated from thermoplastic injection moulded resin (TR). Disc wax patterns were first prepared (Cavex set up regular modelling wax, Holland) in a metal split mould held in a metallic frame. After their solidification, the wax patterns were spread and inserted in a metallic flasks containing dental stone type III (Selenor Verde, Industria Zingardi SrL, Italy) to obtain molds for the processing of the tested thermoplastic materials by the injection molding technique, using the microinjection machine (Biostrong 400, Sabilex, Flexifoil S.A, Argentina).

After wax elimination, the thermopress material was manipulated according to the manufacturer's instructions to fill the moulds. After injection, the flasks were cooled down for 15 min. at room temperature. Then, the flasks were opened, and the discs were finished with very fine sandpaper and polished with polishing paste (Abrasor-Star Glaze, Universal high-lustre polishing paste, Bredent, Germany). The prepared disc specimens were

visually inspected and checked for the clearance of voids or porosity. Moreover, the disc samples containing those defects were discarded. All the specimens were air dried and numbered.

All the test specimens were stored in distilled water at 37°C for 7 days. The 30-disc specimens were then divided into 2 principal groups (each group contains 15-disc specimens): group I: DS immersed in Corega Super Cream DA and subgroup II: DS immersed in Fitty Dent Cream DA. Then, each group was again considered as two subgroups: subgroup A before application of the Corega Super Cream DA, subgroup B after application of the Corega Super Cream DA, subgroup C before application of the Fitty Dent Cream DA and subgroup D after application of the Fitty Dent Cream.

Denture Adhesives Preparation

The prepared DAs (Corega Super Cream and Fitty Dent Cream) were obtained by weighing one gram of the DA and homogeneously mixed with 10 ml distilled water in a closed glass container. The disc specimens were individually immersed in the glass containers containing the prepared diluted DAs. The immersion time was 16 hours/day. The containers were covered and stored in an incubator at 37°C. Next, the specimens were removed out of the prepared DA, rinsed under running water and dried gently with air. After that, each sample was individually stored in distilled water for 8 hours at room temperature. The prepared diluted DA for each disc specimen was replaced and prepared daily, and the procedure was repeated for successive 30 days [23].

Microhardness Test (g/mm^2)

Microhardness was measured with Knoop microhardness tester (Microhardness HV-1000, China) that was calibrated with a load of 10 g for 10 seconds, by implementing Blue Hill Instron computer software program.

The Knoop hardness (KH) indenter was applying a load of 10g smoothly without impact, for 10 seconds and at four different points of each DS. An indentation was made on the block samples using diamond Knoop indenter which; is a pyramid in shape, giving a diamond or rhomboid indentation having a long and short diagonal. The ratio between the long diagonal to the short diagonal is 7:1.

When the indentation was made, the indenter was removed. Stresses were distributed in such a manner that the elastic recovery of the indentation occurs along the short diameter. The physical quality of the indenter and the accuracy of the applied load must be controlled to get the correct results. After the load was removed, the indentation was focused with the magnifying eyepiece and the two impression diagonals were measured, usually to the nearest 0.1 µm with a filar micrometre, and averaged (Figure 1). The KH mean of the four indentations for each DS was calculated in the four subgroups.

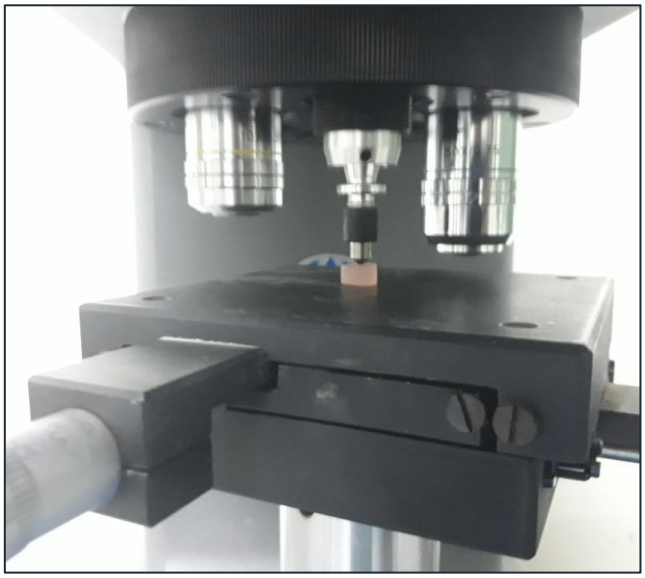

Figure 1: Knoop microhardness testing for TCS thermopress denture base material disc specimens

Statistics

Statistical analysis was performed with SPSS 20®, Graph Pad Prism® and Microsoft Excel 2016 with a significant level set at $P \leq 0.05$. The microhardness data were presented as means (M) and standard deviation (SD ±) values. Furthermore; comparison between both 2 main groups before and after application of denture adhesive was performed using Student`s t-test.

Results

The performed statistical analysis had revealed a significant difference in microhardness of the studied thermopress denture base material before and after exposure to the two denture adhesives. Evidently, for all evaluated disc specimens, the Knoop microhardness test values revealed an obvious reduction in the surface microhardness after the immersion in both types of denture adhesives (Table 1 and 2).

Table 1: Microhardness values in (g/mm2) for TCS thermopress denture base material disc specimens after application of two denture adhesives

	Group I [Corega Super DA]		Group II [Fitty Dent DA]		P-Value
	M	SD ±	M	SD ±	
Before DA	17.20	0.62	17.33	0.50	0.52*
After DA	15.41	0.57	12.11	1.46	0.0001**

*M; Mean, SD; Standard Deviation, P; Probability Level; *Insignificantly difference; ** Significantly difference.*

In that comparative in vitro study, the mean and standard deviation for thermopress microhardness before denture adhesive application was (17.20 ± 0.62) and (17.33 ± 0.5) for subgroup A (Corega Super Cream DA) and subgroup C (Fitty Dent Cream DA) respectively. Also, the mean and standard deviation after denture adhesive application were (15.41 ± 0.57) and (12.11 ± 1.46) for subgroup B (Corega Super Cream DA) and subgroup D (Fitty Dent Cream DA) respectively; (Table: 1, Figure 2 and Figure 3).

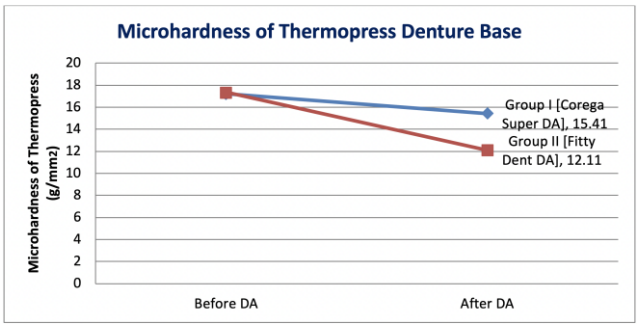

Figure 2: Line chart of reduced microhardness values for thermopress disc specimens after application of two denture adhesives for one month

Student`s t-test was used to a comparison between the two denture adhesive groups before and after application of denture adhesive. That statistical test revealed an insignificant difference between A and C subgroups before application of denture adhesives; P value was > 0.05. However; Student`s t-test revealed a significant difference between B and D subgroups after application of denture adhesives; P value was < 0.05; (Table 1).

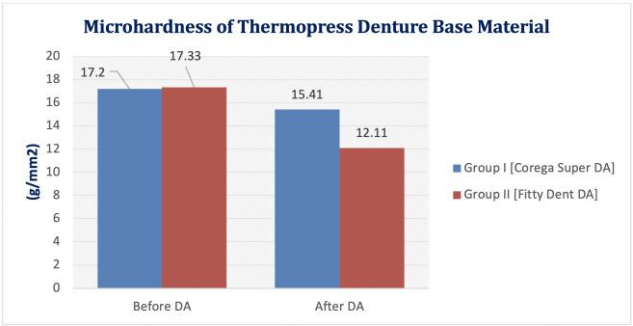

Figure 3: Bar chart of reduced microhardness values of TCS thermopress denture base material after application of two denture adhesives for one month

Moreover; paired t-test was performed to compare the mean microhardness values before and after application of denture adhesive within the same group (i.e. between subgroup A and B and between subgroup C and D); which revealed significant difference between before and after application of denture adhesive; P value was < 0.05 for both groups; (Table 2, Figure 2 and Figure 3). Furthermore; for both DA groups (group I and II), the percentage difference in microhardness before and after application of denture adhesives was calculated according to the following formula:

$$\frac{(\textbf{Values Preoperative}) - (\textbf{Values Postoperative})}{(\textbf{Values Preoperative})} \textbf{ X 100}$$

That percentage difference of reduced microhardness after application of DA in both groups was (-10.41%) and (-30.12 %) for the group I (Corega Super Cream DA) and II (Fitty Dent Cream DA) respectively; (Table 2, and Figure 4).

Table 2: Percentage difference of reduced microhardness values in (g/mm^2) of TCS thermopress denture base material disc specimens after application of two denture adhesives

	Before DA		After DA		P-value	Percentage % difference
	M	SD ±	M	SD ±		
Group I [Corega Super DA]	17.20	0.62	15.41	0.57	0.0003**	- 10.41
Group II [Fitty Dent DA]	17.33	0.50	12.11	1.46	0.0001**	-30.12

M; Mean, SD; Standard Deviation, P; Probability Level, %; Percentage; ** Significantly difference.

Discussion

Indeed; hardness tests are correlated to the surface resistance to indentation and are considered to assess the mechanical properties of dental polymers like tensile strength [17], [24]. After a long term of immersion of conventional denture base acrylics in disinfection solutions or water, Vickers microhardness test was used to investigate alterations in surface properties [25], [26], [27]. However; in that in vitro study, Knoop microhardness method had been implemented as an attempt to consider the elastic recovery and viscoelasticity of the studied flexible polyamide based denture materials [17], [18].

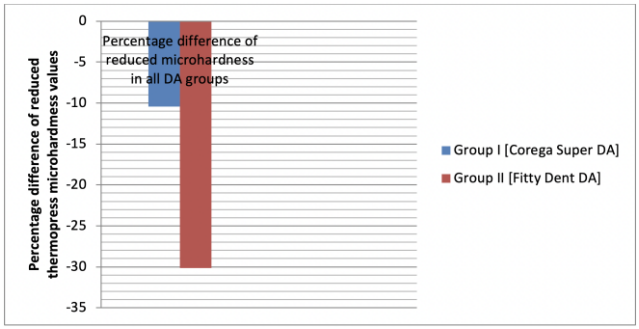

Figure 4: Bar chart for percentage difference of reduced microhardness for thermopress disc specimens after application of two denture adhesives for one month

Furthermore; surface properties of flexible denture base materials are of peculiar importance because they affect their durability, serviceability and longevity during function [28], [29]. Therefore; polymer surface roughness and microhardness had been investigated using different in vitro methods. However;

all those attempted techniques provided valuable information regarding the physicomechanical properties for the tested dental materials, none of the in vitro testing could expose the investigated dental biomaterials in conditions simulating that of the oral environment (i.e. in vivo); such as pH and temperature fluctuations [30].

Generally; polymeric dental materials were characterised by water sorption phenomenon; which decreased their surface microhardness. That reduction was attributed to filler matrix debonding caused by excessive hydration [17], [30]. Also, the absence of cross-linked bifunctional resin in the acrylic-based denture materials could enhance the softening effect of acid solvents [31].

Furthermore; the presence of chemical constituents of DAs had adversely affected the mechanical properties of polyamide based resins. DAs components might produce a plasticising effect which; had attenuated the inter-chain polymer forces and had facilitated the polymer deformation with stress application [32]. Also, the specimens were daily stored in distilled water to simulate the patient saliva which; might have contributed to the reduction in the Knoop microhardness. This was in agreement with Xediek Consani et al., [33] who said that saliva affected similar to water that produced plasticising phenomenon, and thereby reduced the resin microhardness [33], [34].

Compared to heat cured acrylic polymethyl methacrylates, flexible polyamide-based denture base resins showed lower surface microhardness values. Moreover, thermopress flexible resins demonstrated a lesser amount of cross-linking agents, which might interpret the effect of the cross-linking on its surface microhardness. Therefore, those finding showed that thermopress polyamide based resin was much more flexible denture base materials than the conventional heat cure acrylic polymethyl methacrylate [19], [35], [36].

Denture adhesives were usually supplied in the form of powder, paste or cream. The mode of action of denture adhesive was as follow: They absorb too much water, swell, increase many times of its original volumes and consequently, the anions were formed to interact with the cations present in the proteins of the oral mucosa. Furthermore; the viscosity of the denture adhesive was increasing by the resulting thick salivary film, thereby improving the removable denture retention [36].

Recently; new denture adhesive materials were providing strong bio-adhesive as well as cohesive bonding forces. That promising denture adhesive bond was due to the free carboxyl groups produced from the hydration of denture adhesives (such as; sodium carboxyl-methyl cellulose, hydroxymethyl cellulose, methyl cellulose, or polymethyl vinyl ether-maleic anhydride, etc.). Those free carboxyl groups would form electrovalent bonds that produced stickiness to oral mucosa and bio-adhesion. Also, the increased viscosity of the denture adhesive creams caused their lateral spread to exclude saliva and air thereby increasing the removable denture retention [36].

In that study, the decrease in microhardness values of TCS flexible denture base material after immersion for one month, in two different denture adhesives might be due to the enhancement of the degradation process of the polyamide base polymer. The accelerated polymer biodegradability might be because of the presence of addition polymerisation free radicals as well as partially cross-linked polyamide chains containing a large number of residual monomers [37], [38]. This evidence possesses an adverse effect certain physicomechanical properties of the thermopress resin including surface hardness due to diffusion of the residual monomers from the polymer matrix with simultaneous water sorption into the resin microstructure. The consequence of this detrimental scenario is a plasticising phenomenon which; decreases the inter-chain bonding forces and therefore; significantly attenuated the polyamide microhardness facilitates the rapid deformation of polymer chains under load [39], [40].

Within the parameters of this in vitro study design and tested denture base materials, the following conclusions might be drawn:

- Knoop microhardness test was relevant for the assessment of surface microhardness of thermopress denture base material before and after exposure to the two denture adhesives.

- Flexible dentures may be highly indicated as provisional prostheses or space maintainers in certain patient’s requiring replacement of missing teeth in the esthetic area and having some limitations; like allergy to heat cured acrylic resin or metal alloys, restricted jaw opening or severe soft or/and hard tissue undercuts.

- Commercially available denture adhesives (DAs) may have an impact on microhardness of flexible denture base materials.

- Corega Super Cream DA and Fitty Dent Cream DA have decreased the surface microhardness of TCS thermopress denture base material.

- Also; the microhardness of the thermopress denture base materials might be decreased with the use of one type of denture adhesive (Corega Super Cream DA) less than the other (Fitty Dent Cream DA).

- The percentage difference of reduced microhardness after one month of application of DA in both groups was critical and necessitates further evaluation of TCS microhardness after longer periods of DA exposure.

- Transverse strength of the studied

thermopress denture base material needs to be also assessed.

In spite of the innumerable advantages and multiple indications of the flexible thermoplastic polyamide base nylon resin, further in vitro and clinical studies are recommended as the flexible nonmetallic thermopress denture base materials are considered a crucial issue to be addressed.

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Dental Science

Evaluation of The Bio-Stimulatory Effect of Platelet Rich Fibrin Augmented by Diode LASER Compared to Platelet Rich Fibrin Alone on Dental Implant Replacing Posterior Mandibular Teeth. Randomised Clinical Trial: Split Mouth Study

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Keywords: Dental Implant; Platelet Rich Fibrin; Diode LASER; Digital Radiography; Implant stability

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Abstract

BACKGROUND: Restoring masticatory function and replacing missing teeth with minimal pain and discomfort are the most important issues for the patient and clinician. Nowadays dental implants became the most popular line of treatment to replace missing teeth; offering a comfortable long lasting prosthesis. Osseo-integration reflects the long term success of a dental implant. Many bio-modulators are used aiming to improve the osseointegration and healing around dental implants such as Low-Level Laser treatment (LLLT) and Platelet Rich Fibrin (PRF). PRF has been proven to improve bone repair process around the dental implant. LLLT is considered a noninvasive, safe technique that stimulates osteogenesis and alleviates post-operative pain.

AIM: Evaluation of the bio-stimulatory effect of LLLT on a dental implant with PRF compared to PRF alone clinically and radiographically.

METHODS: A randomised clinical trial with the split-mouth design was conducted on nine patients with bilaterally missing lower posterior tooth. All patients received one dental implant on each side with PRF. LASER application was performed to one side twice weekly for one month starting on the day of insertion. Post-operative pain was assessed daily through the first week using numerical rating pain scale (NRS) as the primary outcome. Relative peri-implant bone density was measured using direct digital intraoral radiography immediately after insertion, one, four and nine months postoperatively. Implants stability were measured using radio frequency assessment immediately after insertion, four and nine months post-operative as secondary outcomes.

RESULTS: The NRS for pain was significantly decreased by the end of the first-week postoperatively in the intervention and control group with a mean of (2.22 ± 1.56) (2.11 ± 1.83) respectively. However, there was no statistically significant difference between the test groups at P-Value (0.892). The relative bone density values were decreased by the end of the ninth month of follow-up in the intervention and control group with a mean of (134.42 ± 16.13) (128.77 ± 33.54) respectively. No statistically significant difference was observed between the two test groups at P-value (0.863). The radiofrequency values for implant stability showed no statically significant difference after nine months of follow up when compared to the initial stability values at the day of insertion in the intervention and control group. The mean radiofrequency values were (67.24 ± 1.79) and (66.9 ± 2.57) respectively, and no statistically significant difference was observed between the two test groups at P-value (0.793)

CONCLUSION: There are no statistically significant differences in post-operative pain values, implant stability and bone density between the implant sites treated with PRF augmented by Diode laser compared to implant sites treated by PRF alone.

Introduction

Osseo-integration of the dental implant is currently considered a key parameter for measuring long- term success of the dental implant. The osseointegration assessment could be achieved either clinical by measuring the implant stability, histological evaluating the quality of bone and bone formation or Radiographic through measuring the peri-implant bone density. Among the different techniques used in clinical assessment, Radiofrequency analysis with OSSTEL® device is a reliable and simple technique reflecting implant stability [1]. Digital intraoral radiographic assessment of relative bone density around the dental implant is another applicable and widely used method reflecting the osseointegration [2].

Studies have been conducted to evaluate different techniques, materials and protocols to

improve osseointegration achieving long-term success of the dental implant. Among these trials, surface treatment to dental implant material, developing enhanced implant designs, the use of different implant placement surgical techniques and the use of Bio-modulators to accelerate healing of tissues around dental implants. PRF as a Bio-modulator is a rich source of growth factors that have been successfully used to enhance bone and soft tissue healing. The Diode LASER or Low-level LASER has been used to accelerate healing through induction of formative cells and angio-neogenesis [3], [4], [5].

The implant Osseo-integration is better achieved through the use of bio-modulators such as Platelet Rich Fibrin (PRF) and Low-Level Laser Treatment (LLLT). Each of these treatments PRF and LLLT, when used alone, is proven to have positive effects on bone and soft tissue healing around the dental implant as reported by many studies [2], [3], [4], [5]. However, these studies focused on the effect of either PRF or LLLT alone, the effect of the combination PRF and LLLT both clinically and radiographically has not been studied yet.

The use of LLLT for therapy was adopted in the late 1960s. This technique was applied to stimulate and improve healing, as well as reducing pain because of its stimulatory effect on different cell types. LLLT in soft tissue has been used clinically to speed up the healing of wounds and to control pain [6].

On the other hand, the use of PRF which is rich in platelet and leukocyte cytokines and growth factors are known for its angiographic, hemostatic and osseous conductive properties, has been proven to improve bone healing around dental implant [7], [8], [9].

Subjects and Methods

Trial design

This is randomised clinical trial, split-mouth design. Nine patients are suffering from bilaterally missing mandibular posterior teeth in two parallel groups, with allocation ratio 1:1.

Participants

Eligibility criteria

Inclusion criteria: Missing teeth in the posterior mandibular region bilaterally, Absence of any pathological condition in the posterior region (recipient site) at the time of intervention.

Exclusion criteria: Systemic disease (metabolic bone disease, uncontrolled diabetes myelitis, autoimmune diseases, patients treated with corticosteroids at the last three months, infectious diseases, salivary gland diseases, malignancy) or any disease which may affect the peri-implant healing process. Poor oral hygiene, patients with severe chronic periodontitis as well as aggressive periodontitis in the adjacent teeth to the edentulous area pregnant female. Regular smokers, previous head or neck radiation therapy, Patient with an allergy to any material or medication that will be used in the study, severe psychological problems (Para-functional habits and Bruxism), a patient with the thin wiry ridge (2 mm in width).

Study setting

The patients were recruited in consecutive order from the pool of patients of the central clinic, Faculty of Oral and Dental Medicine, Cairo University, until the target population was reached.

Intervention

Pretreatment measures: the following steps were done for all enrolled patients: detailed recording history including systemic condition, duration as well as diseases and drug history. Undergoing full clinical examination (extra-oral and intra-oral), Giving oral hygiene instructions (verbally and written), Tacking baseline radiographs and photographs.

You are obtaining signed informed consent after explaining the steps of the study and discussing the treatment plan.

Steps for intervention preparation

PRF preparation protocol: the PRF preparation was done according to Choukroun's Protocol [10].

A) For each patient, venous blood was drawn by the same operator from an antecubital vein with pain-free blood test needles (25 Gauge).

B) About 50 ml (0.05 IU) whole venous blood collected in a sterile vacutainer tube without anticoagulant and the vacutainer tubes were then placed in a centrifugal machine at 3,000 revolutions per minute (rpm) for 10 min**.**

C) The resultant gelatinous PRF was placed directly on the buccal bone surface after placement of the implant.

Surgical Procedures

1. All surgical procedures were performed according to Misch's Protocol [11].

2. At the edentulous site, a full thickness flap lingual to ridge crest was performed.

3. Standard drills of sequential diameters were used, and the implant was inserted.

4. Implant placement: Insertion of the suitable implant according to the available bone in each site.

5. PRF placement on the buccal bone surface.

Laser application

Application of LLLT was performed according to Mandić [12]. Patients were briefly informed about the biological effect of Diode Laser and PRF before the operation. The irradiation was performed with a gallium-aluminium-arsenide (GaAlAs) diode low-level laser with continuous emission of 830 nm wavelength. The laser beam power of 100 mW and laser spot size was 0.28 cm^2, resulting in a calculated energy density of 92.1 J/cm^2 and energy of 0.25 J per point. The irradiation time was 30 second per point; the points of irradiation were buccally and lingually with equal time of irradiation; the total delivered energy was 15 J per irradiation session. The application of Diode Laser was performed in 8 visits twice weekly starting from the day of insertion and end 1 month after insertion.

Post-operative pain assessment

It was done daily for 1 week starting from day of implant insertion using NRS for each implant site, for implant stability

Radio Frequency Assessment

Resonance frequency analysis (RFA) was performed using the Osstell™ Mentor instrument. Measurements were recorded immediately after implant insertion and then after four months of insertion then nine months of insertion. A standardised abutment of fixed length was inserted and hand-tightened into each implant. The transducer probe was held so that the probe tip was aimed at the small magnet on top of the Smartpeg™ at a distance of 2-3 mm Figure 1. according to García-Morales et al., 2012.

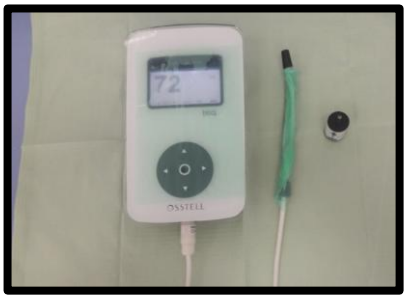

Figure 1: Showing Osstell™ device used in Resonance frequency analysis of implants

Radiographic examination for peri-implant relative bone assessment was done day of the operation, 1 month, 4 months and 9 months using digital intra-oral PSP radiographic assessment (Digora size 2 sensor, Digora Optime UV scanning sensor unit, and X-ray unit Soredex) with exposure parameters 70 kVp, 7 mA and 0.08 Sec) using radiographic stent for Standardized accurate radiographic image, relative bone density around implant was evaluated on DR software by taking three parallel lines at each side (mesial, distal and apical) with 1mm distance between each line and other then the mean of relative bone density at the three lines in each side was calculated to determine the density at each side (mesial, distal and apical) according to Zaky et al., 2016 examination [16].

Results

Demographic data

This study was carried out on nine female patients (split-mouth design) with mean age 45 SD ± 12.5.

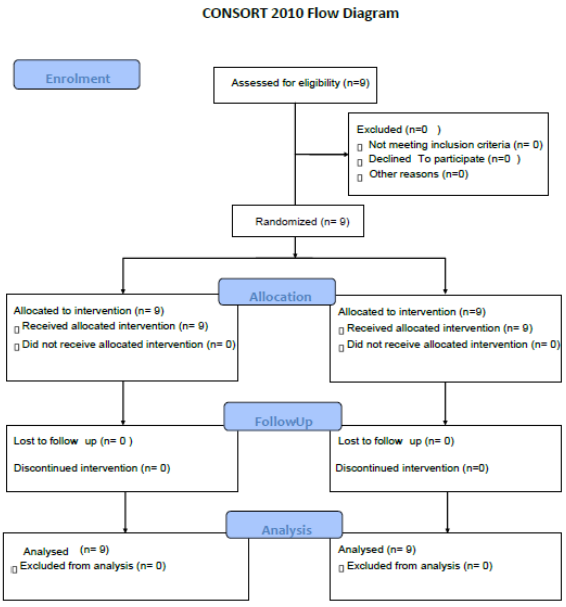

Figure 2: Patients' flow chart

Post-operative pain in each group in different follow up periods:

1) Control group: There was a statistically significant difference between day 1 and day 7 with a mean difference -4.33 ± 1.252, P = 0.0032 using paired T-test.

2) Intervention group

There was a statistically significant difference

in postoperative pain values between day 1 and day 7 with a mean difference -4.66 ± 1.178, P = 0.0011 using paired T-test.

Implant stability in each group in different follow up periods

Control group

The implant stability values comparison showed no statistically significant difference in implant stability through all test periods.

Table 1: implant stability values of the intervention group

Comparatives	Definition	Mean	Mean differences	SD. Deviation	P value	Comment
Pair 1	implant stability at insertion-implant stability at 4m	Insertion 67.13	-1.45556-	4.59622	0.370	Non-significant
Pair 2	implant stability at 4m-implant stability at 9 m	4 months 69.16	1.71111	2.12099	0.042	Significant
Pair 3	implant stability at insertion-implant stability at 9 m	9 months 67	0.25556	5.16602	0.886	Non-significant

Intervention group

The implant stability values comparison showed no statistically significant difference in implant stability between insertion time and 4 months after insertion.

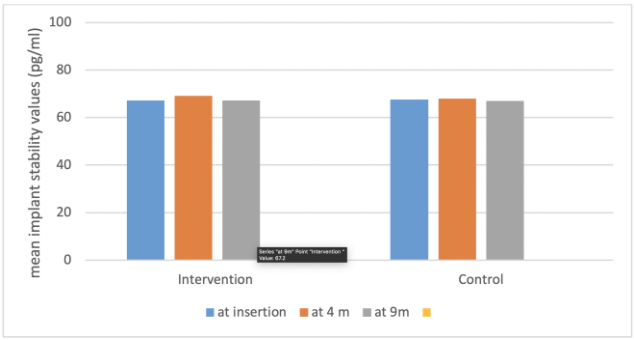

Figure 3: Showing mean implant stability in different follow-up periods

Bone density in each group in different follow up periods

Control group

There were no statistically significant differences between relative bone density values through all periods.

Table 2: Relative bone density of the control group in different follow-up periods

Comparatives	Definition	Mean	Mean differences	SD	P value	comment
Pair 1	bone density at insertion-bone density at 1 m	Insertion 130.46	-5.57	26.62	0.547	Non-significant
Pair 2	bone density at 1 m-bone density at 4 m	1 month 135.98	-3.17	20.15	0.649	Non-significant
Pair 3	bone density at 4 m-bone density at 9 m	4 months 139.24	10.44	28.40	0.302	Non-significant
Pair 4	bone density at insertion-bone density at 4 m	9 months 128.77	-8.75	25.04	0.325	Non-significant
Pair 5	bone density at insertion-bone density at 9 m		1.68	32.26	0.879	Non-significant

*Paired t-test.

Intervention group

There were no statistically significant differences between relative bone density values through all periods.

Table 3: Relative Bone density of the intervention group in different follow-up periods

Compa-ratives	Definition	Mean	Mean differences	Std. Deviation	P value	Comment
Pair 1	Bone density at insertion-Bone density at 1 m	Insertion 136.35	-10.62	33.33065	0.367	Non-significant
Pair 2	Bone density at 1 m-Bone density at 4 m	1 month 145.38	2.52	21.52073	0.734	Non-significant
Pair 3	Bone density at 4 m-Bone density at 9 m	4 months 142.86	17.25	28.93117	0.111	Non-significant
Pair 4	Bone density at insertion-Bone density at 4 m	9 months 134.42	-8.10	39.79447	0.558	Non-significant
Pair 5	Bone density at insertion-Bone density at 9 m		9.155	32.63181	0.424	Non-significant

*Paired t-test.

Comparison between the two test groups

A- Post-operative pain between the two study groups in different follows up periods.

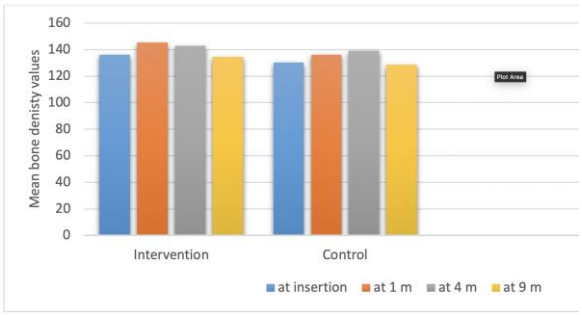

Figure 4: Showing mean density values in different follow-up periods

There were no statistically significant differences in post-operative pain values between both tests groups at all study periods using student t-test P value ≤ 0.05.

Table 4: Comparison between postoperative pain of the two study groups in the different follow-up periods

Comparatives	Intervention	Control	Mean difference	P value	Comments
pain at day 1	6.8	6.4	0.44	0.774	Non-significant
pain at day 2	6.2	4.8	1.33	0.370	Non-significant
pain at day 3	5.2	4.5	0.66	0.554	Non-significant
pain at day 4	4.0	4.2	-0.22	-0.879	Non-significant
pain at day 5	3.2	3.6	-0.44	-0.728	Non-significant
pain at day 6	2.3	2.6	-0.33	-0.728	Non-significant
pain at day 7	2.2	2.1	0.11	0.892	Non-significant

Implant stability between the two study groups in different follow up periods

There were no statistically significant differences in implant stability values between both groups at all study periods using student T-test, Table 5.

Table 5: Comparison between implant stability of the two study groups in the different follow-up periods

Comparatives	Intervention (mean)	Control (mean)	Mean difference	P value	Comment
Implant stability at insertion	67.1	67.5	-0.044	-0.982	Non-significant
Implant stability at 4m	69.1	67.8	1.055	0.281	Non-significant
Implant stability at 9 m	67.2	66.9	0.277	0.793	Non-significant

Comparison between Relative Bone density values of the two study groups in different follow up periods

There was no statistically significant difference in relative bone density values between both test groups at all study periods using Student T-test Table 6.

Table 6: Relative bone density differences between the two study groups in the different follow up periods

Comparatives	Intervention (mean)	Control (mean)	Mean difference	P value	Comment
bone density at 1 m	145.3	135.9	9.98889	0.556	Non-significant
bone density at 4m	142.8	139.5	4.28889	0.760	Non-significant
bone density at 9 m	134.4	128.7	-2.52222	0.863	Non-significant

Discussion

Bio-stimulation is an emerging concept in the field of dentistry that has been proven to accelerate the biological process of healing and regeneration. The purpose of the current study is to evaluate the combined effect of two bio-stimulants, Platelet Rich fibrin and Diode Laser compared to PRF alone. A randomised clinical trial was carried out on nine patients with missing bilateral posterior teeth in a split-mouth study. All nine patients received one dental implant on each side with PRF. On one side LLLT was applied to start from the day of insertion twice weekly for one month postoperatively.

Many of the published studies have assessed the effects of either LLLT or PRF on bone healing around dental implants; however and up to our knowledge this is the first study evaluating the effect of the two techniques together on healing and osseointegration around dental implants both clinically and radiographically using digital radiography [7], [9], [22]**.**

Although LLLT had a strong body of evidence supporting its role in the neo-bone formation and increased implant stability, the absence of a unified protocol of LLLT application around dental implant demands more researches on this point to identify a successful protocol [17], [19], [21].

Post-operative pain was assessed as a primary outcome using numerical rating score daily for seven days. The secondary outcomes assessed were implanted stability and relative peri-implant stability. The results of the present study revealed no significant difference between the two treatments.

Although the results of this study have shown significant pain reduction in each of the test group alone, the comparison revealed no significant differences between the two test groups. In agreement Marenzi et al., in 2015 [4] investigated the influence of PRF on post-extraction healing. Where twenty-six patients were enrolled in their study and a split-mouth design was adopted. Only one side post-extraction sockets received PRF and the other side used as a control. The results were a significant pain reduction in PRF sides.

On the other hand Ozgul et al., 2015 [25] assessed postoperative pain reduction in the presence of PRF compared to non-PRF protocol after third molar extraction in a multicenter randomised clinical trial on fifty-six patient. The study stated no statistically significant differences regarding pain among the groups.

In accordance He et al., in 2014 [3] conducted a systematic review of evidence for LLLT pain reduction capacity post extraction. They pooled the data of 193 patients in a meta-analysis. Despite the authors' comment on the poor quality of the evidence and high risk of bias, their conclusion was a significant pain reduction after laser irradiation post-surgical. Also, Landucci et al., 2016 [24] evaluated postoperative pain in twenty two post extraction patients. The patients were randomly distributed into two groups, one subjected to a single session of laser radiation and the other didn't receive radiation. They reported the same results.

Concerning the implant stability and relative bone density, the present study results have shown no significant differences in each test group starting from the day of insertion tell the end study time also no significant difference was found between the two test groups. In agreement, this was in contradiction to the results reported by Jang et al., in 2010 [2]. The study investigated the ability of PRF to fill peri-implant bone defects in rabbit models. They concluded that peri-implant defect could be successfully repaired by the application of Choukroun PRF. This evidence supported the ability of PRF to induce neo-bone formation. However, the study was an animal study, and the neo-bone formation was histologically evaluated.

A significant difference was found in pain in each group from the 1st day to the 7th day postoperatively in this study. Pain may be reduced as a result of the usage of PRF, finding in agreement of Ozgul et al., 2015 [25] how assessed postoperative pain reduction in the presence of PRF after third molar extraction, and the study of Marenzi et al., in 2015 [4] which investigated the influence of PRF on post-extraction healing. Where twenty-six patients were enrolled in their study and a split-mouth design was adopted. Only one side post-extraction sockets

received PRF and the other side used as a control. The results were a significant pain reduction in PRF sides.

The evaluation of PRF validity in neo-bone formation was evaluated by Jang et al., in 2010 [2] study which detected the ability of PRF to fill peri-implant bone defects in rabbit models. They concluded that peri-implant defect could be successfully repaired by the application of Choukroun PRF. This evidence supported the ability of PRF to induce neo-bone formation.

Zaky et al., in 2016 [16] conducted a prospective randomised controlled trial on 16 patient evaluated the bone healing in maxillary cystic defect reflected by digital radiographic assessment of bone density after single post-operative LLLT session and follow up period for 90 days. The results were a significant increase in bone density in the laser group. This contradiction could be attributed to the nature of the maxillary bone and the different pathological condition. [20], [26], [27].

Castro et al., in 2017 [5] conducted a systematic review of evidence on the ability of L-PRF to induce neo-bone formation in periodontal defects. The results stated that “Favorable effects on hard and soft tissue healing and postoperative discomfort reduction were often reported when L-PRF was used favoured the potential ability of PRF to induce neo-bone formation in periodontal defects”. However, the different material used and different protocol, as well as nature of the disease, may justify this contradiction

In conclusion, there are no statistically significant differences in post-operative pain values, implant stability and bone density between the implant sites treated with PRF augmented by Diode laser compared to implant sites treated by PRF alone.

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Dental Science - Review

Bruxism Unconscious Oral Habit in Everyday Life

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Keywords: Bruxism; Habit; Everyday life; Occlusal splints; TMD; TMJ; Masticatory muscles

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Abstract

BACKGROUND: Bruxism is defined as an unconscious oral habit of rhythmical, unfunctional clenching, grinding and making chewy sounds with the teeth while making movements that are not part of the masticatory function and that lead to occlusal trauma.

AIM: The purpose of this article is to show the habit bruxism, in everyday life, reviewing literature data.

METHODS: Data was researched by using information on the internet on Researchgate, Pubmed, ScienceDirect, by analysing written articles and books and student books. From 200 articles that were analysed, 45 articles and two textbooks were involved in writing of this review article.

RESULTS: Results derived from the analyzed literature, classify the main consequences of bruxism, from fatigue, pain, wasting of the incisal edges and occlusal surfaces of the teeth to loss of teeth, dental implants, headaches, periodontal lesions and TMD (dysfunctions of the masticatory muscles and temporomandibular joint (TMJ)) in severe cases. All these problems negatively affect the quality of everyday life of the patient.

CONCLUSION: Bruxism as a parafunctional habit is present in everyday life needing a multidisciplinary approach for prevention of the teeth, bone and prosthetic restorations. The prevalence of bruxism is growing related to stress, drugs, changes in lifestyle, bad nutrition and sleep problems. The therapist should follow signs and symptoms to ensure the best treatment plan of the patient.

Introduction

Bruxism is defined as an unconscious oral habit of dysfunctional rhythmic pressing, clenching and grinding of the teeth when performing movements that are not part of the masticatory function that leads to occlusal trauma read in the dictionary of dental prosthetic terms. As an oral parafunctional activity it is not related to normal physiological functions, such as speech, breathing, chewing and swallowing.

Bruxism is a complex occlusal parafunction which can hardly be placed in several different categories of parafunction [1]. It can be classified as a sleep disorder, according to the international classification of sleep disorders, when night grinding of teeth occurs in combination with at least one of the following signs: damage to teeth, sounds associated with bruxism and pain of the masticatory muscles. The episodes of bruxism, its duration and intensity in which they appear are different, individual to each patient. The appearance of bruxism can be seen from 6-20% of the population in each age starting from the eruption of deciduous teeth.

This parafunction is discovered when the patient goes for the first time to the dentist. One of the most prominent clinical signs is abnormal wearing of the teeth, caused by clenching and teeth grinding. However, this is not a decisive sign of bruxism because the wearing of teeth can occur when eating acidic foods or improper tooth brushing (erosion and /or dental abrasion). In this way, the therapist should always consider the bruxism antagonist who is also worn, damaged or reduced [2].

Bruxism is common in our population manifested by pinching and grinding of the teeth, as a parafunctional habit is characterised by different intensity and periodic repetition, with tendon to

decrease with age, while generally observed it has a common representation [3].

Psychosocial factors like stress or personal characteristics and pathophysiological factors (e.g., illness, trauma, genetics, smoking, intake of caffeine, medications and illicit drugs), sleep disorders (sleep apnea and snoring) and involving dopaminergic system are often present in the aetiology of bruxism. There is not only one factor responsible for the occurrence of bruxism. It is also evident that there is no generalised treatment effective to eliminate or reduce its occurrence [4].

The aetiology of bruxism is not completely resolved [5]. The anatomy, morphology and dental occlusion are linked to bruxism.

The mobility of teeth, pain, hypertrophic facial muscles and reduced capacity to open the patient's mouth after waking in the morning are changes that are observed. Frequent headaches, especially in the temporomandibular region in everyday life are often noticed [6]. Other clinical signs in patients with bruxism are fractures of the teeth or dental restorations (fillings, or damage to prosthetic restorations as crowns, bridges or dentures) [7].

The purpose of this article is based on own dental experience funded by data from the literature, to show the unconscious habit and its appearance in everyday life, diagnosis of bruxism, etiological factors, clinical manifestations, and treatment using occlusal inserters (splints) [7].

Material and Methods

Bibliographic review of the literature for the habit bruxism was carried out for this article. Its classification, etiological factors, clinical manifestations and therapeutic modalities were obtained with research on the internet. Specialised websites were used such as Researchgate, Pubmed, Science Direct, and through the application of domestic and foreign literature, analysing the written paper's books and textbooks. Keywords such as bruxism, habit, damage to teeth, teeth grinding, muscle pain, temporomandibular dysfunctions, TMD, TMJ, occlusal inserter were applied in the search database. There were 200 articles read and analysed and many internet pages. In the preparation of this paper, 45 texts and two textbooks were incorporated and are cited in the article.

The criteria for selection of papers included the prevalence of the disease, clinical manifestations, diagnosis and the consequences. These papers were used with permission from their authors.

Results

The earliest references to bruxism are described in the Bible, in which dental pain is associates as "the first sentence of the Lord" [8]. In the illustrated medical dictionary by Dorland, the word bruxism comes from the Greek word *brahmin*, which means grinding of the teeth [9].

Bruxism is described by authors as an orofacial motor activity during sleep, characterised by repetitive or sustained contractions of the mandibular elevator muscles, which can cause muscle rigour, about 150-340 kg. This force directed, with exactly obtained voltage during active periods results in fractures and damage of teeth, periodontal problems, pain, muscle fatigue and headaches [10].

Bruxism can be defined as a parafunctional activity on the masticatory system which includes clenching and grinding of the teeth at an unconscious level where the neuromuscular protective mechanisms are absent. This can cause injuries to the masticatory system and TMJ dysfunction. Episodes of occurrence of the disease are highly variable as in a patient and between different patients. The duration of the night grinding can be 5 to 38 minutes as a part of the parafunctional activities. When measuring the strength of the contact between the teeth, it can be three times higher than the normal functional activity of the masticatory system. Other authors proved that during the disease a collapse of the structures of the orofacial system could occur [11].

Bruxism is one of the most relevant, complex and destructive dental disorders [12].

Classified by the degree of difficulty bruxism can be moderate, severe and extreme (mild, moderate, severe) [9].

According to the neuromuscular activity during the bruxism, there can be a division into three types of bruxism: toned, periodic and combined [1].

In the direction, the movements are made bruxism has horizontal and vertical form. During the sleep, bruxism may be horizontal or vertical, but conscious daily bruxism known as bruxomania, cannot be performed with a horizontal movement [1].

It can also be classified as centric and eccentric [9]. Centric bruxism consists of continuously pressing the teeth for some time with the destruction of their supporting structures but also conditions that include the masseters and temporomandibular joint. In eccentric bruxism, there are isotonic muscle contraction and damage on the incisal edges of the teeth particularly in the anterior arch of teeth [12]. However, not all cases of wearing the incisal edges are the result of a parafunctional activity. It may be associated with other habits like biting nails, biting objects among other types of habits [9].

Acute, subacute or chronic is another classification of bruxism. Occlusal disharmony interferes with bruxism when the patient shows signs of/or muscle symptoms, as researched by authors. This occurs in centric relation and/or functional lateral or protrusive phase [13], [14], [15], [16].

Daily and nightly bruxism, is the most common division of bruxism having different characteristics and causes [8], [10]. Bruxism which occurred during the day is called daily bruxism (DB), while bruxism occurring during sleep is called nocturnal bruxism (NB). Daily bruxism is known as bruxomania [1]. These two types of bruxism have different clinical entities that occur in different degrees of consciousness and have different etiologic factors. These two types of bruxism differ and need to be diagnosed and require a different treatment plan [17]. DB and NB are classified as primary when no clear medical causes, systemic or psychiatric disorders occur. Although in most of the literature bruxism is mentioned as a nighttime disorder, there are cases where its appearance was observed daily. Therefore bruxism in accordance to its appearing can be divided as daily, nightly and combined [1].

Primary bruxism occurs without a clear reason. Secondary bruxism is associated with clinical disorders, neurological or psychiatric disorders associated with iatrogenic factors or other types of sleep disturbance [9], [17].

Bruxism can be classified by the complications arising from its destructiveness [1]. The forces that occur in patients with bruxism can cause a devaluation of the successfully achieved prosthetic construction. If prevention measures are not undertaken the existing bruxism may cause very severe complications, such as loss of dental implants [1]. Complications can range from excess damage of the restorations and surrounding dentition, lack of osseointegration, to loosening or fracture of implant restoration. The recommendation made by dental prosthetic specialists includes taking preventive measures and treatment protocols for the patients by wearing occlusal splints [7].

Many authors confirm that the aetiology of the bruxism is multifactorial [1], [6], [18], [19].

The psychosomatic health must be seen as a whole in this condition. Patients with bruxism are seen as bruxers. Some of them are aggressive and they use the stomatognathic system for discharge of their aggressiveness [20], [21], [22], [23], [24], [25], [26]. The neuromuscular mechanism is explained as an interaction between the factors for the presence of early occlusal contacts and the psychological stress in the patient [1].

Aetiology is also associated with local, systemic [23] and neurological factors [11]. Local factors including traumatic occlusion, early contact, excess restorations, dental cysts, the atypical eruption of milk and permanent teeth contribute to the emergence of bruxism. Malocclusions, incorrect restorations, periodontal calculus, tooth mobility, deformity of the lips, gingival hyperplasia and other factors related to the occlusal physiology favour the occurrence of bruxism [27], [28]. The other system factors include nutritional deficiency, parasitosis, Down syndrome, gastrointestinal disorders, allergic reactions, uncontrolled enzymatic digestion, brain damage, adverse effects of drugs, mental retardation and central paralysis [29].

Nutritional factors such as consumption of beverages, coffee, tea, chocolate drinks, non-alcoholic drinks, and smoking habits may be involved, since stimulating the central nervous system, increases anxiety and stress. Because of this, they represent triggering factors for the emergence of bruxism [9], [29].

Several studies focus on explaining the relationship between allergies and intestinal parasites with bruxism. There is an intimate relationship between IgE levels, eosinophilia and bruxism. In allergies, as well as parasitic intestinal infections, levels of IgE and eosinophilia are high, followed by the emergence of oral manifestations [27].

Bruxism is detected in patients with neurological disorders who receive neuroleptic and anticonvulsant therapy. It is discovered in patients with brain abnormalities that are taking levodopa and using stimulants like amphetamines and antidepressants, which are risk factors [11], [29], [30]. The main reason for the parafunction-bruxism is considered to be the disruption of sleep, which is explained by the theory of excitation [31]. In aetiology, despite local and systemic factors, there are factors such as professional practising competitive sports [9].

The diagnosis and clinical evaluation of bruxism are complex because both bruxers and normal individuals may show parafunctional nocturnal activity [9].

Early diagnosis of bruxism is of great importance both for its treatment and for its prevention. Kapusevska in trials of patients at the Clinic for Dental Prosthetics for objective diagnosis of bruxism uses bruxoanalizor applied to determine the horizontal type of bruxism while for determining the vertical type of bruxism bruxoquantifier is applied [32], [41].

The diagnosis must focus on identifying the signs and symptoms reported by the patient or the dentist during clinical examination [14].

Parafunctional forces directly affect the enamel on the teeth that can be observed with their abnormal wearing. It is used as the most common evidence of bruxism. This wearing may be limited to one tooth or the entire dentition [33], [34]. The radiographic analysis may show loss of laminate changes of the periodontalniot space that can either

disappear or be with increased resorption of the tooth root, a fracture or changes in the dental pulp, sometimes the appearance of the pulpal stones [9].

The main lesions caused by the present bruxism can be summarised as changes in teeth, periodontium, masticatory muscles, TMJ, headaches, behavioural and psychological effects. Other signs are also symptoms of the parafunctional hypermobility in the absence of periodontal disease, pulpitis, tooth pain (with normal pulp, partial crown fracture and migration of teeth) [7]. Muscle symptoms include fatigue, increased tension in masseters, especially in elevator muscles (m. Masseter and m. Temporalis). The most common symptom is muscle fatigue that represents resistance over a sustained effort, without clear signs or symptoms of pain or discomfort [9], [36].

Problems with the movement of the body can develop because of bruxism. In addition to that, it can affect the masticatory muscles and back muscles of the cervical spine, which can cause muscle pain and chronic future permanent changes [20].

The harmful habit bruxism causes relevant changes in the structure of the stomatognathic system, causing friction, inflammation, necrosis of the pulp and mobility of teeth. There may be the appearance of muscle pain and tenderness on palpation of TMJ, pain, cracking and other sounds from the joint as a lack of coordination of the lateral pterygoid muscles. In certain patients, capitulum mandibulae may change, and loss of the vertical dimension and mandibular displacement on the maximum intercuspidal position (MIP) may develop [37].

It is of significant importance for a differential diagnosis to be obtained depending on the aetiology and clinical signs observed during the clinical examination of the patient and including symptoms derived from the history. Treatment and therapy vary from patient to patient [13].

The treatment for bruxism requires a multidisciplinary approach, including psychology, psychotherapy and speech therapy [10].

Planning the therapy must be with meticulous attention to details. The purpose is to reduce physical and mental stress, treating signs and symptoms [3].

Early treatment involves reducing the psychological stress through the use of relaxation methods like exercises, massages and physiotherapy [38]. This treatment reduces symptoms but does not remove the cause. The habit may be reactivated when the tolerance of the patient to occlusal change decreases [10].

Occlusal therapy may include occlusal adjustment of the situation in the mouth. Although occlusal position works with minimal impact on the disease process of occlusal adjustment is an irreversible therapeutic method to minimise the damage caused by clenching and grinding of the teeth. But it is not a treatment for the disease [9]. Applying interocclusal inserter (splints) [7] reduces the symptoms of bruxism. Their application may not stop the disease, but will not allow its progression, because it allows the exact condyle position in the fossa mandibular [38], [39].

The occlusal inserter may vary in material, rigidity, resiliency and extent of occlusal coverage. In this way, according to the therapeutic indications splints can lead to various intermaxillary relations [4], [6], [28]. Depending on the complexity of the case it is usually recommended to be used at night, with weekly suggested controls. The author's processed papers for implementation of various types of occlusal inserter, which are analysed from different perspectives. Occlusal inserter can be made of different materials. There is hard and soft occlusal inserter. Soft can be used to prevent further attrition caused by bruxism and various etiologic agents [40]. Occlusal inserter can be made of high quality and modern way and with alternative methods. From Kapusevska’s trials of patients treated with occlusal inserter made with the material, the eclipse was given the conclusion that they are superior to conventional occlusal inserter [41]. Furthermore, occlusal inserter (splints) may be separated from the treatment required for horizontal and vertical bruxism. For the treatment of patients with horizontal bruxism and TMD Kapusevska et al., recommend applying repozitional occlusal inserter, while in patients with bruxism and vertical musculofascial pain occlusal stabilisation inserter should be used [42].

Despite the aetiology of bruxism, the occlusal therapy can be suitable, because it promotes functional comfort, prevention of further damage to components of the masticatory system. Pharmacological treatment with drugs such as dopamine agonists, anxiolytics, buspirone, nonbenzodiazepamic hypnotics, anticonvulsants and botulinum toxin are appropriate when bruxism is very pronounced [5], [23], [24], [25].

Discussion

Early diagnosis of bruxism is necessary to avoid damage to the TMJ and other oral or facial structures such as teeth and masticatory muscles. Bruxism is present as an unconscious habit every day. Having that in mind, lesions caused by bruxism can easily affect the quality of life of patients mainly due to the high association of pain and discomfort [43].

Bruxism diagnosis is usually made clinically and is based on the clinical history of the patient and the presence of typical signs, including tooth mobility,

damage to teeth, masseteric hypertrophy, indenting the tongue, hypersensitive teeth, and pain in the masticatory muscles. It is possible for cracking or locking of the TMJ to appear [43].

However, it is important to understand that bruxism as an isolated condition cannot cause damage to the teeth. Another possible reason for the damage is acid erosion, which can occur in people who drink acidic juices as concentrated fruit juice or in people who have frequent vomiting or regurgitation of stomach acid. People also demonstrate normal levels of damage to the teeth, associated with the normal function. The presence of damage to teeth only indicates that it occurred at some point in the past and shows that the loss of tooth substance does not progress. People who grind their teeth and minimally apply parafunctional pressure on them also show no damage to the teeth [44].

Bruxism diagnosis usually involves the exclusion of dental, temporomandibular disease and rhythmic movements of the jaw resulting from disorders associated with seizures. This usually includes dental examination and electroencephalography possible, in case you need to diagnose the disorder attack. Damage to teeth may be perceived during a routine dental examination.

When a patient has daily bruxism, he usually denies that he is pressing and clenching his teeth because they're unaware of the habit [45]. Generally, in these patients sleep is relatively good. Usually, the sleeping partner is the person who sleeps less awakening from the grinding sound of the bruxers.

Few authors have studied the effectiveness of sleep in patients with bruxism finding different values for REM sleep [46]. That is why Bader finds its prolongation and Butros et al., [47] its reduction.

Conclusion

With the analysis of such abundant area on the field of bruxism, it is aware that bruxism represents a high frequency of appearance in all age groups and is an important oral health issue. This parafunctional activity represents a significant change in the stomatognathic cavity and thus on the entire body. Because of its presence, a multidisciplinary approach is indicated for the reduction of injuries in osteo-dental structures.

It is noted that recently the prevalence of bruxism is growing and is associated with many symptoms such as stress, drugs, anxiety and sleep disorders.

An opportunity is created using the main scientific databases for better understanding the main principles for treatment of the oral habit-bruxism as a significant health issue.

By learning what is bruxism dental professionals will understand the better diagnosis, risk factors, prevention and treatment methods used. Prevention should be considered as a basic measure in the treatment of bruxism, avoiding the risk of development of various oral diseases with their complications and improving patient's lives.

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Dental Science - Review

The Effect of Local Pharmacological Agents in Acceleration of Orthodontic Tooth Movement: A Systematic Review

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Abstract

AIM: Acceleration of orthodontic tooth movement has gained a massive interest to decrease the total treatment time. Local pharmacological agents might be used for that purpose as a practical, effective and inexpensive alternative. A systematic review was achieved to evaluate the evidence in that topic.

METHODS: A search was conducted on electronic databases including PubMed, Lilacs, Web of Science (Thompson Reuters), EMBASE (OvidSP), and Cochrane Database of Systematic Reviews (Wiley) in addition to hand searching of relevant journals till June 2018. Only studies written in English were utilised. Publications were selected, assessed systematically and graded by two observers according to Bondemark grading system.

RESULTS: Only two human studies were found investigating the effect of Relaxin and Prostaglandins in the rate of orthodontic tooth movement. No obvious side effects were reported. Relaxin showed no increase in the rate of tooth movement while prostaglandin showed a marked increase in the rate of orthodontic tooth movement.

CONCLUSION: There is below moderate evidence showing no effect of relaxin on orthodontic tooth movement, while inconclusive evidence was found regarding Prostaglandin in the acceleration of orthodontic tooth movement. More prospective well-conducted clinical trials are needed to reach a proper conclusion regarding the local pharmacological agents which can be safely used to accelerate orthodontic tooth movement.

Introduction

Orthodontic tooth movement has been defined as the production of a biological reaction to an interruption in the physiological equilibrium related to the dentofacial complex by an externally applied force [1].

The lengthy duration of orthodontic treatment is considered a major disadvantage. This may lead to loss of patient compliance. This may be considered a problem especially for patients who require extraction of teeth as the treatment takes a relatively longer period than patients who don't require extraction of teeth [2]. This may also lead to increasing the risk of caries [3] and periodontal breakdown [4]. Therefore, attention was paid to find methods to accelerate orthodontic tooth movement [5]. These methods were surgical, mechanical or physical. Examples of these methods are low-level laser therapy, corticotomy, electrical current, pulsed electromagnetic fields, and dentoalveolar or periodontal distraction. Evidence showed that corticotomy is effective and safe to accelerate orthodontic tooth movement [5]. Unluckily, very few patients can accept this surgical intervention to accelerate tooth movement due to its aggressive and invasive nature.

Another direction was focused on pharmacological approaches either locally or systemic administration to accelerate orthodontic tooth movement [6], [7].

Most of the previous systematic reviews concentrated on the physiological and surgical interventions with little concentration on the

pharmacological interventions [5], [8], [9], [10].

This systematic review aims to investigate - in a systemic methodology and critical analysis - the available scientific literature discussing locally administrated pharmacological agents used in the acceleration of orthodontic tooth movement in humans.

Material and Methods

This section was written following the PRISMA 2009 checklist [11].

Protocol and Registration

There was neither a detailed protocol nor a systematic review registration done.

Information Sources and Search strategy

A search was conducted on electronic databases including PubMed, Lilacs, Web of Science (Thompson Reuters), EMBASE (OvidSP), and Cochrane Database of Systematic Reviews (Wiley) in addition to hand searching of relevant journals till June 2018. Only studies written in English were utilised.

The terms used in the search were shown in Table 1.

Table 1: Terms used in the search strategy of the systematic review

PICO item	Synonyms
P	Orthodontic patient OR Orthodontic therapy OR Orthodontic treatment Or Orthodox* Or Tooth Movement (Mesh) OR teeth
I	Pharmacological OR Drug Or Local Factor OR Pharmacol* OR vitamin D OR Prostaglandin OR Cholecalciferol
C	Control OR Regular Orthodontic treatment
O	Accelerate tooth movement OR Fast treatment OR Treatment time OR Accelerate* movement OR Rapid tooth movement OR Quick treatment

Eligibility Criteria

The PICOS format (P = Population, I = Intervention, C = Comparison, O = Outcome, S = Study design) was constructed in order to state a clinical question with particular inclusion criteria.

P - Patients at any age undergoing orthodontic tooth movement

I - Local pharmacological interventions to accelerate tooth movement

C - Conventional orthodontic therapy without local pharmacological intervention.

O - Rate of tooth movement

S - Randomized controlled studies and non-randomized controlled studies

Inclusion criteria: - Local intervention; - Injectable; - Clinical trials; - Trial aiming to accelerate tooth movement.

Exclusion Criteria: - Animal study; - Systemic drug; - Histological study; - Trial comparing drugs decelerating tooth movement; - Subcutaneous, Intramuscular, Intravenous administration.

Review question

Are the local pharmacological interventions able to accelerate tooth movement compared to conventional orthodontic treatment without local pharmacological intervention?

Study selection

Two independent reviewers examined the article titles and abstracts. Full-text articles were retrieved when the articles were either potentially eligible or when the eligibility criteria couldn't be determined. Full-text articles were assessed following the inclusion and exclusion criteria. Reviewers' results were compared. Discussion of the data was done to resolve any disagreement.

Data Items

From the studies that met our inclusion criteria, specific data items were extracted including (drug, frequency, dose, site, duration, total, dose, control, appliance, outcome, Risk Ratio, Mean Deference and side effects).

Data collection

The data items were extracted independently by 2 reviewers. The results were compared for accuracy and reassessment of the extracted data was done in case of any discrepancy until resolving the disagreement.

Bias Assessment

A quality assessment was performed based on the method described by Bondemark et al. [12, [13]. Following this method, studies were assigned to the grading of A, B, & C. A was considered high-quality evidence, B was a moderate value of evidence and C was considered the low value of evidence. In case of disagreement between the two reviewers or inadequately described criteria, the study was discussed thoroughly until reaching a consensus (Table 2).

Table 2 : Bondemark grading system

Grade A	Grade B	Grade C
All criteria should be met: A randomised clinical study or a prospective study Diagnostic reliability tests and reproducibility tests described Defined diagnosis and endpoints Blinded outcome assessment	All criteria should be met: Cohort study or retrospective case series with defined control or reference group Diagnostic reliability tests and reproducibility tests described Defined diagnosis and endpoints	One or more of the conditions below: The high rate of attrition (1/3 or more of subjects lost during the study) Poorly defined patient material Unclear diagnosis and endpoints

Summary measures and synthesis of the results

The final level of evidence was determined based on Bondemark et al., [12] The protocol divided evidence level to 1 (strong), 2 (moderate), 3 (limited) and 4 (inconclusive) (Table3).

Table 3: Evidence level

Level	Evidence	Definition
1	Strong	Minimum of 2 studies level A
2	Moderate	At least 1 study level A and two studies level B
3	Limited	Minimum of 2 studies level B
4	Inconclusive	Less than 2 studies level B

Approach to Data synthesis

A meta-analysis was considered if the available collected data was adequate.

Results

Study selection

A flowchart showed the selection process in each stage of the systematic review. (Figure 1) Five hundred seventy-eight articles were excluded by title and abstract while 4 articles were selected for full review. Two of these articles weren't written in English [14], [15].

Two articles were included in our review utilising relaxin [16] and prostaglandin [17] as local pharmacological interventions aiming to accelerate tooth movement.

Study characteristics

Methods. One study was a randomised controlled study while the other study was a prospective study which was divided into 3 phases. Subjects. Total of 65 patients was involved in both studies. Intervention. Relaxin hormone and prostaglandin were used in selected studies.

Quality assessment

One study was graded A (High value of evidence) [16], while the other was graded B (moderate level of evidence) [17].

Results of Individual Studies

The primary outcome assessed in both studies was the effect of the local agent in the acceleration of orthodontic tooth movement. Secondary outcome included side effects resulted from using prostaglandin and effect of Relaxin on relapse (Table 4). The effect of prostaglandin was investigated both macroscopically and using radiographic images. There was no side effect observed on the gingiva or bones. Relaxin showed no effect on short-term stability.

Table 4: Results of individual data

Title	Author	Year	Design of the study	Number	Groups	Split-mouth
A randomized, placebo-controlled clinical trial on the effects of recombinant human relaxin on tooth movement and short-term stability	Susan P. McGorray, a Calogero Dolce,b Susan Kramer,c Dennis Stewart,d and Timothy T. Wheelere	2012	RCT	40	2	no
Clinical application of prostaglandin E1 (PGE1) upon orthodontic tooth movement.	Yamasaki, K Shibata, Y.Imai, STani, Y.Shibasaki, Y Fukuhara, T	1984	3 phases	25	3	yes

Title	Drug		Sample	Frequency	Dose	Site	Duration	Total dose	Control	Appliance and force
A randomized, placebo-controlled clinical trial on the effects of recombinant human relaxin on tooth movement and short-term stability	Human relaxin		40	/7 days	25µg/site/0.1 ml	2 sites buccal and lingual of the target teeth	56	400µg	Vehicle 1.6 ml	Invisalign vacuum aligners
Clinical application of prostaglandin E_1 (PGE_1) upon orthodontic tooth movement.	PGE_1	Phase I	9	3 to 5 times	10 µg PGE_1	Injection of the submucosal area of the buccal side of the first premolar	Up to 26 days		Lidocaine	Double spring soldered on lingual arch(100 gm)
		Phase II	8	3 to 4 times	10 µg PGE_1	Injection of the submucosal area distal to the canine	Up to 21 days		Lidocaine	Sectional contraction loop (150 gm)
		Phase III	8	5 to 13 times	10 µg PGE_1	Injection of the submucosal area distal to the canine	Up to 5 months		Lidocaine	Compressed open-coil springs or ringlets (150 grams)

	Outcome	Frequency days	Duration (days)	Dose	Mean difference (intervention/control) (ratio)	Side effects
A randomized, placebo-controlled clinical trial on the effects of recombinant human relaxin on tooth movement and short-term stability	Rate of tooth movement, rate of relapse	/7	56	25µg/site/0.1 ml	1	No
Clinical application of prostaglandin e_1 (pge_1) upon orthodontic tooth movement.	Otm and side effect was examined macroscopically	3 to 5 times in 26 days 3 to 4 times in 14 days 5 to 13 times	15-28 10-21 45-144	10 µg pge_1	2.14±0.33 Not mentioned 1.6 ±0.09	No

Risk of bias

The study investigating Prostaglandin effect was found to be of high risk of bias as it was not a

randomised controlled trial, while some concerns regarding the allocation concealment were detected in the other study investigating Relaxin effect.

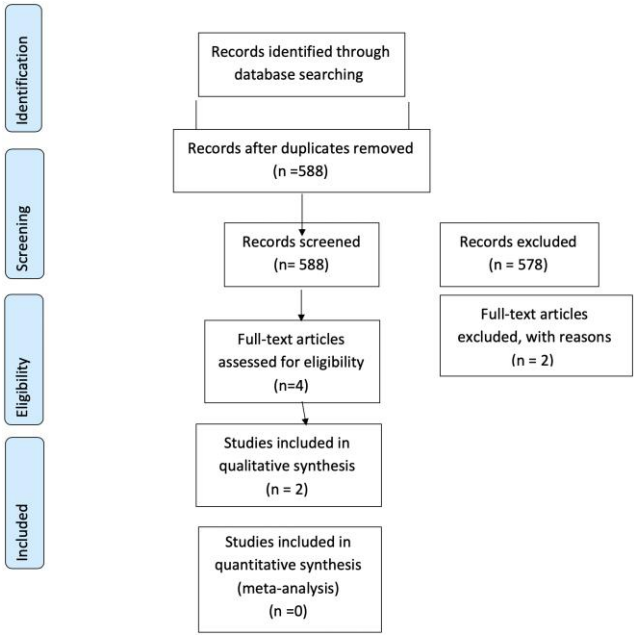

Figure 1: Prisma flow chart

Synthesis of Data

A meta-analysis was not possible due to the presence of insufficient studies.

Discussion

This study aimed to investigate the ability of the local pharmacological agent to accelerate orthodontic tooth movement. Surgical interventions have shown potential in the acceleration of orthodontic movement [8]. Its side effects had always been a barrier in the generalisation of these interventions. Local pharmacological interventions would be an excellent replacement if proven their efficiency, especially if not accompanied by any side effects. In spite of the huge focus on the acceleration of orthodontic tooth movement, there were only 2 human studies investigating the effect of local pharmacological agents on the acceleration of OTM. There were much more animal studies [18], [19], [20], [21] on the same topic. The obvious reason was the risk of side effects that accompany the tested interventions [22].

From the current systematic review, Relaxin [16] and Prostaglandin [17] were tested on humans. Prostaglandin showed a marked increase in OTM. The study was divided into 3 phases in which all phases showed acceleration of OTM in intervention sides, yet the study was considered having a high risk of bias. The study wasn't a randomised controlled study, and the sample size wasn't enough for each phase.

Evidence level was below moderate regarding Relaxin and inconclusive regarding prostaglandin according to Bondemark grading system [12]. Only 1 study was found which was graded A for Relaxin. While for Prostaglandin, only one study which was graded B was found.

The RCT investigating Relaxin showed that there was no significant difference and was considered of low risk of bias. Yet they used aligners which might not have delivered a consistent force-that couldn't be measured-necessary for proper comparison. Both studies were based on submucosal injection in the areas adjacent to OTM.

This study showed the need for further studies investigating the use of local pharmacological agents in the acceleration of orthodontic tooth movement.

Strength and limitations

Previous studies investigated different approaches with no concentration on the pharmacological approaches [23]. This study, however, focused on the local pharmacological agents used in orthodontic treatment to accelerate tooth movement. There were not enough studies to conduct a meta-analysis. There were only a few heterogynous human studies. The quality of evidence was poor in that topic indicating the need for further studies to reach a proper conclusion.

Conclusion

There is below moderate evidence showing no effect of relaxin on orthodontic tooth movement, while inconclusive evidence was found regarding Prostaglandin in the acceleration of orthodontic tooth movement. More prospective well-conducted clinical trials are needed to reach a proper conclusion regarding the local pharmacological agents which can be safely used to accelerate orthodontic tooth movement.

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NPC POPLINE: A Tool for Population and Reproductive Health Evidence-Based Decisions in Egypt

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Abstract

BACKGROUND: Population and Reproductive Health Research (PRHR) should have a crucial role in the policy process in Egypt, providing the evidence for problem identification, priority setting, laying out the alternatives, monitoring and evaluation of implemented evidence-based decisions. Minimally, the practice of evidence-based population and reproductive health requires the access and visibility of such information.

AIM: In response to the current situation, the Egyptian National Population Council in collaboration with the Information and Decision Support Centre of the Egyptian Cabinet developed the first online bilingual PRHR database entitled "NPC POPLINE" aiming at providing a tool for evidence-based decisions in the field of population and reproductive health in Egypt

METHODS: NPC POPLINE is operated by the electronic Library Information System using MARC21 format. Data was collected from all research centres and institutions conducting PRHR in Egypt; the Egyptian Universities Library Consortium and the international POPLINE database by using structured data collection forms.

RESULTS: NPC POPLINE combines a unique coverage in terms of language (English and Arabic); subject (population and reproductive health) and publication type (peer-reviewed research and grey literature), in addition to the marked search flexibility and the availability of different formats to display the search results.

CONCLUSION: NPC POPLINE goes beyond the definition of an advanced search engine; it can be used to perform bibliometric studies to evaluate the quantitative and qualitative aspects of PRHR conducted in Egypt. Further studies should be initiated to assess the alignment of the database content to the national and international priorities regarding population and reproductive health.

Introduction

Health research generates knowledge that is necessary for countries to achieve better health, equity and development [1]. Through research, countries can improve their health systems, discover new ways to prevent and treat diseases, understand people health needs and consequently improve individuals' and populations' health [2], [3], [4]. In this context, Population and Reproductive Health Research (PRHR) are considered a multifaceted domain that is closely linked to a wide scope of population health services [5].

However, research is a complex process, generation of knowledge is only one output of the research process; for knowledge to be useful, it should be shared with other researchers and communicated to all users and stakeholders as health professionals, policymakers, patients …etc [6]. So, the two processes should work side by side, the production of knowledge in addition to sharing and dissemination of the old through an accessible and free resource [7], [8], [9]. Furthermore, research should have a key role in the policy process, providing the evidence for problem identification and prioritisation, laying out the alternatives for addressing policy issues, and feeding back the appropriateness of the implemented evidence-based decisions [10].

According to the United Nations estimates, Egypt is considered one of the most populous countries in the world [11]. With a 2016 estimated population of 92 million, accounting for almost 1.2% of the global population and occupying the 15th rank. Moreover, Egypt's population is generally rising at an extremely progressive rate, which is causing a

growing concern within the government, and an increase in expenditure on reproductive health policies [12]. The demand for evidence-based population and reproductive health policies and programs seems likely to grow as never before. Minimally, the practice of evidence-based population health requires the availability and access to such information [13].

In response to this critical situation, the Egyptian National Population Council (NPC) which is the official entity responsible for planning and setting strategies and policies related to population and reproductive health issues [14], [15], developed the first online PRHR database as a part of the electronic library of the information centre under the auspice of the NPC to achieve all the above mentioned purposes and beyond under the name of "NPC POPLINE".

NPC POPLINE was developed to provide a tool for decision makers to access the PRHR conducted in Egypt through one unified portal considering the marked variation in the capacity of research users to access the different types of existing research evidence across the country. NPC POPLINE also supports and catalyses PRHR transfer from producers to potential users namely, decision-makers, policy-makers, planners, service providers and researchers to enhance the utilisation of knowledge generated from research in decision-making and policy development at the national level.

This article describes the development of NPC POPLINE as well as the scope, geographic coverage, services and types of publications targeted by the database.

Methods

Database Design and Technology Platform

NPC POPLINE is an online electronic bibliographic database that has two user interfaces (English and Arabic) targeting PRHR conducted in Egypt from the year 2005 till 2017. NPC POPLINE is a free online resource maintained by the NPC information centre in collaboration with the Information and Decision Support Centre (IDSC) of the Egyptian Cabinet.

NPC POPLINE is managed and operated by the electronic Library Information System (eLIS) developed by the IDSC. eLIS is a web-based software program that uses Machine Readable Cataloging 21 (MARC21) format to build the electronic bibliographical database. eLIS provides many facilities to assist performing archiving and indexing activities as acquisition, serial control, cataloguing, information services as well as flexible search and filter engines.

Data Collection Source

Data Collection work was divided according to the source of data collection into field and office work. The fieldwork included manual data collection from all research centres and institutions in Egypt conducting research related to population or reproductive health domains. The NPC list of all research centres and institutions conducting PRHR in Egypt was updated at an experts' meeting during the preparation of the study. The list included 35 research centres and institution all over Egypt. On the other hand, office work was confined to electronic data collection from; the online website for the Egyptian Universities Library Consortium (EULC) to retrieve all theses addressing our target and the online international POPLINE database to retrieve all indexed work from Egypt.

EULC is the national consortium providing a wide range of academic services to all the university libraries in Egypt, supervised and monitored by the Egyptian Supreme Council of Universities. It includes an online database indexing all theses approved by the Egyptian Supreme Council of Universities [16]. The international POPLINE database is the world's most comprehensive resource for population, family planning and reproductive health literature. Its main mission is to share knowledge that has been produced, synthesised, or encapsulated in this crucial field, to help users in low- and middle-income countries as well as supporting agencies and organisations to gain access to this type of literature [17].

A two days training program on manual and electronic data collection was conducted at the NPC (40 trainees), followed by recruitment of the 2 data collection teams from NPC based on the results of pre and post-training tests. The first team (20 researchers) was assigned for the fieldwork and the second (5 researchers) was responsible for the office work.

Data Collection Tool

A bibliographic data collection form was developed in English and Arabic languages to include the following items:

- Title of the research;
- Names and affiliations of the authors;
- Publication year;
- Publication type;
- Publication source;
- Major publication subject;
- Minor publication subject;
- Abstract (the researchers were trained on how to write an abstract if it is not available in the original document).

Data Entry

Data entry was conducted by five full-time staff who works for the NPC information centre after receiving a five-day training program at the IDSC of the Egyptian Cabinet on the data entry as well as the utilisation of different facilities of the NPC POPLINE.

Data Quality Control

Each bibliographic data collection form was given a record number and reviewed by an independent data quality control team (3 trained researchers from outside the NPC) for completeness, consistency and proofreading. Duplicated records were omitted.

Launching of NPC POPLINE

Before the online launching of NPC POPLINE, testing of the database was carried out for 3 months. Additionally, backup and data retrieval operations were tested and secured.

Ethical Considerations

All the administrative approvals were obtained from the targeted research centres and institutions before conducting the fieldwork. Regarding the online data sources, a link to the original data source was provided for each record.

Results

Geographic Coverage

NPC POPLINE covers all research conducted in Egypt related to population or reproductive health during the period 2005-2017. However, no restrictions were made regarding the nationality of the authors.

Table 1: NPC POPLINE Subject Coverage

1.	Adolescent reproductive health
2.	Family planning methods
3.	Family planning programs
4.	Gender
5.	Communication programs
6.	AIDS/HIV
7.	Maternal and child health
8.	Population dynamics:
a.	Fertility
b.	Mortality
c.	Migration
d.	Marriage and family
e.	Geographic distribution
f.	Population growth and size
9.	Population strategy and Laws
10.	Population, health and Environment
11.	Population characteristics:
a.	Vital characteristics
b.	Labor force
c.	Age composition
d.	Social characteristics
12.	Population and economic resources and poverty
13.	Population and non economic factors:
a.	Population Problem
b.	Human Development
c.	Nutritional status
d.	Unemployment
e.	Education
14.	Sexually transmitted diseases

Subject Coverage

The international POPLINE database subject index was used after adding specific subjects to match the Egyptian priorities to finally end up with 14 main subjects as shown in Table 1. Each publication is classified according to the major subject (only one subject) and minor subject(s) (more than one subject can be selected).

Language Coverage

NPC POPLINE has two user interfaces: English (Figure 1) and Arabic (Figure 2).

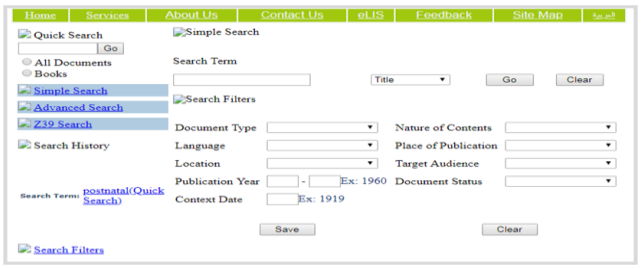

Figure 1: English interface of a simple search of NPC POPLINE

The database covers mainly all PRHR released in English or Arabic languages. Other languages are also covered as long as English or Arabic abstracts are available.

Figure 2: Arabic interface of a simple search of NPC POPLINE

Publication type

The database exclusively covers all types of publications including peer-reviewed articles; scientific conferences abstracts; research reports; books; booklets and all other technical, programmatic publications. Additionally, all theses (Master and Doctoral degrees) targeting PRHR and accepted by the Egyptian Supreme Council of Higher Education are included.

Search Engine Flexibility

NPC POPLINE is characterised by remarkable search flexibility as well as easy retrieval and storage of data. NPC POPLINE is a user-friendly database. It permits 3 different types of search: quick search, simple search and advanced search. Simple search provides searching by the affiliation of authors;

authors; ISSN; ISBN; publisher and keywords as illustrated in Figure 3. Moreover, the "filter" function that is incorporated in the search engine, limits the search by the type of publication; language; publication source; research source; publication year and the target audience as shown in Figure 1.

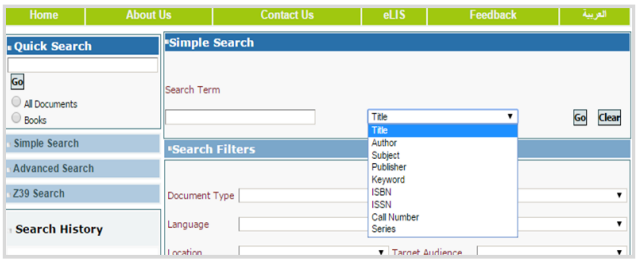

Figure 3: Dropbox list for simple search options of NPC POPLINE

Figure 4 displays the advanced search that offers a wide range of options, for example: using tags to search in various fields of the bibliographic record; using Boolean operators (AND, OR, NOT); using the "*" wildcard, a special character that will match any other(s), including "no character" to broaden a query as well as using the "filter" function that is incorporated in the search engine.

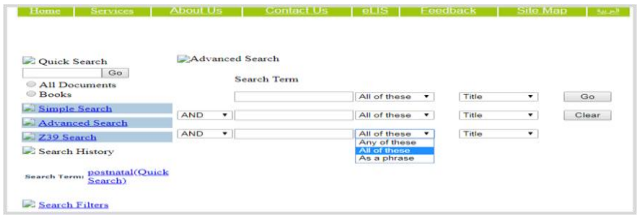

Figure 4: Advanced search interface of NPC POPLINE

Search results display

As illustrated in Figure 5, search results are displayed in different formats depending on the user requests, either full details or short records can be displayed. These results can be printed or exported to an external file for further processing or emailed.

Figure 5: The NPC POPLINE results page for the search term "natal."

Discussion

Over the last three decades, the NPC has worked hard to support the research capacity of individuals and institutions, in addition to providing technical assistance and policy analysis to support evidence-based decisions at the national level regarding population and reproductive health issues [14], [15]. In this context, NPC POPLINE was developed as a free online tool that compiles all forms of PRHR that were conducted in Egypt from the year 2005 to 2017. The NPC POPLINE seeks to fill the gaps regarding 2 critical drawbacks of PRHR conducted in Egypt, namely poor access to grey literature which remains for many years stuck in closed lockers inside different institutions and invisibility of PRHR produced in the Arabic language. The current work goes in accordance with the recommendations of the Canadian Institutes of Health Research, Institute of Population and Public Health (CIHR-IPPH) to support population and public health research generation, diffusion, transfer and uptake by key stakeholders through innovative use of different technologies and accessible language for various audience [13], [18].

According to the CIHR-IPPH, evidence-based decisions requires that the right people should have access to the right knowledge at the right time in the right usable format, by another meaning "just-in-time" use of evidence [13]. Despite that the question of how to define evidence is simple, the answer is still indefinite and complex. The definition of evidence in the scientific literature tends to line up with researchers' definitions (i.e., "evidence" means peer-reviewed research indexed in international bibliographic databases as Pubmed and Scopus). On the other hand, decision-makers define "evidence" more broadly to seek a range of evidence, only one of which is peer-reviewed research [19]. Results of studies from both developing and developed countries reveal that decision-makers use a wide array of evidence, including peer-reviewed research, grey literature including theses, governmental and nongovernmental reports and policy documents in addition to community views and complaints [20], [21].

Furthermore, studies show that when evidence is accessed and used correctly, research can be used as a tool to guide decision-makers rather than being used only in an instrumental fashion to defense an already made decision [22], [23]. Adding to the complexity, most of the research in the developing countries go to low-impact journals or local journals or remain unpublished such as scientific reports and theses. In addition, many of these journals do not have online access or are indexed in international bibliographic databases [6], [24]. From this perspective, NPC POPLINE covers all types of publications related to PRHR including peer-reviewed articles; theses; scientific conferences abstracts;

research reports; books; booklets and all other technical, programmatic publications to align with the current policy needs and facilitate making decisions related to advocacy, policy, planning and management, allocation of resources, and different programs development and strengthening.

One of the most important values added by this research database compared to other research databases as the international POPLINE database which cities worldwide literature in the field of PRHR [17], is the indexing of PRHR produced in the Arabic language. There is a widespread consensus that most international bibliographic databases ignore non-English research, which may be more important than mere invisibility of the existing scientific information; it can result in gaps and biases in our knowledge hindering appropriate evidence-based decisions [25], [26]. To the researcher's knowledge, NPC POPLINE is the first bilingual research database including both English and Arabic PRHR. It provides a unique model to other countries that face language barriers which impede compilation and application of scientific knowledge. Even though English is currently the language that dominates worldwide scientific research, yet many researchers and potential users of scientific information including decision-makers, communicate daily in their native languages, which without doubt hinders the transfer of knowledge and its translation into action [25], [27].

The unique feature of the NPC POPLINE is its combination of language (English and Arabic), geographic (Egypt), subject (population and reproductive health), publication type (peer-reviewed research and grey literature) coverage. The overlap between NPC POPLINE and other bibliographic databases is minimal as no other research database has the same mandate as the NPC POPLINE. Major bibliographic research databases as MEDLINE, EMBASE and international POPLINE databases don't index publications in the Arabic language [28]. Additionally, their selection criteria and indexing policies do not allow these to be indexed in such databases, being classified as a type of grey literature which is considered difficult to track and acquire [24], [28].

NPC POPLINE goes beyond the definition of an advanced search engine for PRHR citations; it can also be used to perform bibliometric studies to evaluate the quantitative and qualitative aspects of research outputs in the scope of the population and reproductive health. It is also worth mentioning that to date, researchers and decision makers in developing countries tend to exist in different worlds. Accordingly, research often has limited impact on or relevance to the policy process [8], [24], [29]. NPC POPLINE can be utilised to identify the discrepancies between researchers' priorities versus the national population and reproductive health policy priorities. Similarly, the current database can ensure that the research priorities are aligned to the international sustainable development goals launched by the United Nations in 2002 [30]. Not to mention that NPC POPLINE aims at grouping homogenous and diverse academic and research institutes together through providing a central resource for knowledge transfer in the field of PRHR to achieve the optimum saving in expenses, avoid duplication and resources sharing through facilitating accessing all the potential information resources.

Limitations

The sustainability of NPC POPLINE in a developing country with scarce resources like Egypt poses a challenge in terms of upgrading of the software program, technical maintenance of the server and updating of the content of the database regularly. Additionally, to increase the recognition and visibility of the database to the potential users, a facility should be added to NPC POPLINE to allow it to be searchable through different commercial search engines like Google Scholar.

In conclusion, NPC POPLINE is a shift from invisible, inaccessible and limited-sharing of PRHR to a free, bilingual, online and authorised central resource-sharing including all forms of PRHR conducted in Egypt. However, to attain the maximum expected benefit of NPC POPLINE as a tool for evidence-based decision-making in the field of population and reproductive health, further studies should be initiated for quantitative and qualitative evaluation of the content of the database and to assess its alignment to national and international population and reproductive health priorities.

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Letter to the Editor

Primary Regressive, But Metastasizing Melanoma!?

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Abstract

BACKGROUND: Cases of regressive melanomas represent a diagnostic and therapeutic challenge because time intervals between the presence of the primary tumour formation, the metastasis and the involution of the primary tumour may intertwine or occur at different times. The regression of cutaneous melanomas does not necessarily guarantee prevention from the development of locoregional or distant metastases. There are cases in which the prognosis of patients with the development of subsequent metastasis within regressive melanomas may be better depending on the number and location of metastases.

CASE REPORT: We are presenting a 42-year-old patient with two timed removals of enlarged inguinal lymph nodes within one year, as the subsequent histological examination identified histopathological data for metastasis of melanoma. BRAF testing was positive for BRAF mutation. Within the anamnesis, it was further clear that the patient had an irritated melanocytic lesion in the lateral right thigh area, which over the time disappeared and shortly after that, the enlargement of locoregional lymph nodes has been noted.

CONCLUSION: In the presented case prognosis and therapeutic options for treatment of patients with regression melanomas and subsequent development of lymph node metastasis have been discussed. Currently, there is no consentaneous opinion on the applicability of the early adjuvant therapy with targeted therapies or immunotherapy in patients with regressive and non-regressive type melanomas. We suggest and share the idea that early adjuvant therapy may be beneficial generally in patients with stage III melanomas.

Dear Editor,

We present a 42-year-old man who was hospitalised 1 year ago in a surgical department for enlargement of right inguinal lymph nodes. The lymph nodes were surgically resected. The histological evaluation found metastatic melanoma. BRAF testing revealed a wild type genotype. After a thorough examination and PET Scan, the primary tumour was not identified. Twelve months later, PET/CT of the whole body was performed due to an enlarged lymph node in the inguinal area (clinically/palpatory and ultrasound), and the scans confirmed a single enlarged, metabolically active, right/inguinal lymph node. Surgical removal of the enlarged lymph node from the proximal part of the right thigh was performed, below the inguinal fold (Figure 1a). The histological and immunohistochemical examination of the removed material identified a metastatic melanoma with infiltration through the lymph node capsule and perinodal soft tissues. The patient was visiting the Department of Dermatologic Surgery to clarify the diagnosis and to elucidate the further therapeutic regimen. During the clinical examination, a subungual pigmentation of the left foot's thumb was observed (Figure 1c), which was subsequently identified as a post-traumatic subungual hematoma. Concomitantly, the presence of a melanocytic lesion with peripheral involution, suspicious for regression melanoma was found in the right femoral region (Figure 1b and 1d). The patient also reported the

presence (6 years earlier) of a mole in the right femoral area, which was traumatically irritated and treated with "silver water", resulting in a complete disappearance. Immediately afterwards, the patient observed repeated swellings localized in the right inguinal area, and in the first 2-3 years they were reversible, but subsequently, the tumor formation increased and became painful for which it was surgically removed and identified as lymph node metastasis from melanoma. A re-excision of the regression lesion is contemplated with a view to discussing the benefits of adjuvant therapy.

Figure 1: a) Postoperative view after second surgical excision: surgical wound after inguinal lymph dissection to the right; b) and d) Clinical examination: melanocytic lesion with peripheral involution, clinically suspected for regression melanoma, located in the right femoral area; c) Subungual pigmentation of the left leg's thumb, identified as a post-traumatic subungual hematoma

Cases of regressive melanomas represent a diagnostic and therapeutic challenge [1]. The greatest difficulty in data interpretation and subsequent diagnosis is caused by cases of patients with metastatic melanoma with complete regression of the primary lesion [2]. The time intervals between the presence or emergence of the primary tumour formation, the metastasis and the involution of the primary tumour may intertwine or occur at different times. This often confuses clinicians and is the reason for subsequent inadequate diagnostic and therapeutic steps. Interesting is the fact that 1) the regression of melanomas do not necessarily guarantee the prevention/protection from the development of locoregional or distant metastases [3] and that [2] the prognosis in some patients with metastasis within regressive melanoma may be better depending on the number and location of metastases (as in our case). In this regard, as important factors of major importance should be considered, on the one hand, primary tumour localisation, and, on the other, the location of metastasis development.

As areas of involvement from the primary tumour, the head and neck are probably associated with worse prognosis due to the more complicated and complex lymphatic drainage system (as well as the thoracic area), which in turn, in the case of metastatic involvement, is further problems due to the need for lymph dissection. An example of a possible predictive significance/relevance link between location and the subsequent prognosis is the recently described in the literature metastasis of the internal organs (such as gastrointestinal) in patients with regression type melanomas compared with metastases in the inguinal area (as seen in our patient) [4]. In the case of inguinal metastatic involvement, better overall prognosis and survival should be expected compared to disseminated intestinal involvement, for example. We describe a young patient with primary regression of melanoma and subsequent development of locoregional (inguinal) metastases. A re-excision of the regression lesion, located in the right thigh area, is planned, with the possible benefit of subsequent therapy remaining open.

New literature data suggest that early adjuvant therapy with targeted therapies or immunotherapy could provide greater benefits [5]. By definition, however, early adjuvant therapy is applied after resection of a primary melanoma, which opens the question of its applicability in the case of regression melanomas that are masked in the form of other skin diseases such as vitiligo [5], [6]. We propose an idea that early adjuvant therapy may be a key factor and also beneficial in patients with stage III melanomas in the case of completely eradicated metastatic tumours.

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