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Basic Science

Physical Exercise Increased Brain-Derived Neurotrophic Factor in Elderly Population with Depression

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Keywords: Exercise; Brain-Derived Neurotrophic Factor; Depression

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Abstract

BACKGROUND: Depression is very common in the elderly population. Physical exercise is one of the non-pharmacological procedures that promise to be a solution to improve the severity of depression. Brain-Derived Neurotrophic Factor (BDNF) plays a role in maintaining the survival of neuronal cells and in the regulation of synapse plasticity, affecting serotonin production in the hippocampus and thus the depressive symptoms.

AIM: This study aimed to assess the role of physical exercise in affecting BDNF levels in elderly with depression.

METHODS: Thirty-five elderly women (age ≥ 50 years) with depressive episodes based on Diagnostic and Statistical Manual of Mental Disorders (DSM)-V criteria were enrolled as treatment group, and 35 elderly women without depressive episodes were enrolled as control group, and underwent physical exercise in the form of treadmill with a speed of 6 km/h for 15 minutes. Physical exercise was carried out once a day for 28 days. As much as 1 ml of blood from the study, subjects were obtained from the cubital vein before the exercise commenced. Brain-Derived Neurotrophic Factor (BDNF) serum level was assessed by Enzyme-Linked Immunosorbent Assay (ELISA). Data were presented in the form of mean ± SD. An independent T-test was used to test levels after exercise in the depression group compared to the non-depression group.

RESULTS: Pre-exercise BDNF levels in the depression group were lower than the group of elderly without depression. Physical exercise increased BDNF production in both elderly groups with and without depression. In the depression group, the increasing percentage of BDNF level was higher compared to non-depressive elderly.

CONCLUSION: The increasing percentage of BDNF level was found to be higher in depressive elderly performing physical exercise. Physical exercise may be beneficial in supporting the therapy of elderly with depression.

Introduction

Depression is one major health problem experienced by more than 300 million people worldwide. This disorder is characterised by the presence of typical symptoms in the form of sadness or anhedonia which are persistent and followed by somatic, cognitive, and symptomatic disorders in the form of decreased appetite, sleep disturbances, feeling unenergized, decreased concentration, guilt and suicidal thoughts or attempts [1]. Depression is very common in the elderly population. The elderly population generally has retired, which sometimes initiates a slight psychic disorder in the form of feeling no longer useful and post-power syndrome. These psychological changes generally initiate depression in the elderly population [2].

In addition to psychological problems, depressive disorders that generally occur in the elderly can be caused by physiological changes in the body. Ageing will be followed by a decrease in bodily functions, including a decrease in brain function and neurotransmitters. Neurobiological depression is caused by a decrease in the serotonin neurotransmitter in the hippocampus. The decrease in serotonin is caused by a decrease in neuronal cells. Brain-Derived Neurotrophic Factor (BDNF) is a growth factor that plays a role in maintaining the survival of neuronal cells and in the regulation of synapse

plasticity. Decreasing BDNF levels will reduce the survival of neuronal cells, which will reduce serotonin production in the hippocampus, leading to the occurrence of depressive symptoms [3].

Physical exercise is one of the non-pharmacological procedures that promise to be a solution to improve the severity of symptoms in people with depression. Previous studies showed the role of physical exercise in increasing BDNF levels. Physical exercise in the form of jogging for about 30 minutes every day is believed to be able to improve cardiovascular function and improve cognitive function through regulation of BDNF production [4], [5].

This study aimed to assess the role of physical exercise in affecting BDNF levels in elderly with depression.

Material and Methods

Study Population

The study was conducted on the geriatric population at Kambang Iwaki Palembang elderly community. This study included 70 subjects, where 35 older women (age ≥ 50 years) with depressive episodes based on Diagnostic and Statistical Manual of Mental Disorders (DSM)-V criteria in the past 1 year, enrolled as the treatment group. As many as 35 older women (age ≥ 50 years) without any psychotic symptoms or depressive episodes were enrolled as a control group. Assessment of the degree of depression was done by referring to the Hamilton Rating Scale for Depression (HRSD). Subjects were categorised as depressed when HRSD score > 7. Baseline assessment of the level of physical activity was carried out by The Habitual Physical Activity (HPA) score. Subjects who were determined to experience depression were first treated with an antidepressant of Selective Serotonin Reuptake Inhibitors (SSRI) for 3 months before the study was conducted. They were subjected to routine blood tests in the form of blood glucose levels, cholesterol levels, uric acid levels and vital sign examinations to determine the overall health status of the subjects before the exercise. Also, subjects were tested for cognitive function, with the Mini-Mental State Examination (MMSE) test.

The subjects had received informed consent regarding the study. This study was by the Declaration of Helsinki and had received ethical approval from the ethics committee for medical and health research, Bioethics and Humanities Unit, Faculty of Medicine, Sriwijaya University (No.153/kptfkunsri-rsmh/2018).

Physical Exercise

Physical exercise was carried out in a measurable manner, where each study subject conducted a treadmill with a speed of 6 km/h for 15 minutes. Physical exercise was carried out once a day for 28 days.

Blood Collection

Before the commencement of the physical exercise, as much as 1 ml of blood from the study, subjects were obtained from the cubital vein. Furthermore, the blood of the research subjects was stored in the centrifuge tube. The samples that had been stored in the centrifuge tube were then centrifuged at a speed of 5000 rpm for 10 minutes at 25^{o}C. Then, the supernatant was separated from the pellet and put into a 1.5 ml centrifuge tube. Samples were stored at -20°C.

Measurement of BDNF

BDNF serum level was assessed by Enzyme-Linked Immunosorbent Assay (ELISA) (detection limit of 62.5 pg/ml). ELISA kit (Cloud-Clone Corp, Texas, USA) consisted of 96-well microplate pre-coated with antibodies for BDNF. A total of 10 ul samples were inserted into the microplate, then incubated for 30 minutes, at 37°C. Next, horseradish peroxidase (HRP)-conjugate (Sigma-Aldrich, St. Louis, Missouri, USA) was added as much as 50 ul on each microplate, then pre-incubated at 37°C for 30 minutes. As much as 50 ul of chromogen A and B were added to each microplate. The stop solution was added and read at a wavelength of 450 nm so that the optical density value was obtained.

Data Analysis

Data were presented in the form of mean ± SD (standard deviation). Variables of age, HRSD, MMSE, (Body Mass Index) BMI, HPA, systolic and diastolic blood pressure, respiratory rate, hemoglobin, blood glucose, cholesterol level, uric acid level were analyzed by independent T-test to determine the differences in overall health status of the subjects and to put aside the possibility of chronic diseases involvement in physical exercise results. BDNF serum was analysed by dependent T-test to assess the levels before and after exercise. An independent T-test was used to test levels after exercise in the depression group compared to the non-depression group. Significance value was set at $p < 0.05$. The analysis was carried out with SPSS 24.0 (SPSS Inc., Chicago, USA).

Results

As seen in Table 1, the differences in baseline characteristics between the depression and non-depression groups were not statistically significant. The difference between the depression and non-depression groups was observed only in the HRSD score, which exhibited that the depression group was indeed depressed, while the control was less likely to experience depression. Statistics of independent T-test on age, MMSE, BMI, HPA, systolic and diastolic blood pressure, respiratory rate, haemoglobin, blood glucose, cholesterol level, uric acid level showed no difference between depression and non-depression groups. These results, of course, exhibited that both groups were comparable in overall health status and only differed in depressive psychological status.

Table 1: Baseline Characteristics of Depression and Non-Depression Group

Variables	Depression N = 35 (Mean ± SD)	Non-Depression N = 35 (Mean ± SD)	P*
Age (years)	58.2 ± 5.78	58.9 ± 6.13	0.567
HRSD	9.7 ± 2.1	2.7 ± 1.1	0.002
MMSE	29.3 ± 2.6	29.7 ± 2.3	0.756
BMI (kg/m^2)	25.7 ± 3.4	24.8 ± 3.9	0,713
HPA	8.7 ± 2.2	8.4 ± 2.5	0.811
Systolic Blood Pressure (mmHg)	129.4 ± 10.2	128.7 ± 11.1	0.542
Diastolic Blood Pressure (mmHg)	82.7 ± 6.6	81.1 ± 7.3	0.643
Respiration Rate (x/minute)	19.7 ± 1.1	20.2 ± 1.8	0.482
Hemoglobin (g/dl)	12.9 ± 9.6	12.4 ± 8.4	0.463
Blood Glucose (mg/dl)	100.7 ± 11.3	97.7 ± 8.9	0.354
Cholesterol Level (mg/dl)	169.7 ± 9.8	167.9 ± 10.1	0.451
Uric Acid Level (mg/dl)	3.7 ± 1.1	3.4 ± 1.1	0.431

***** Independent T-test, p = 0.05; SD: Standard Deviation; HRSD: Hamilton Rating Scale for Depression; MMSE: Mini-Mental State Examination; BMI: Body Mass Index; HPA: Habitual Physical Activity.

As shown in Table 2, pre-exercise BDNF levels in the depression group were lower than non-depression This showed that patients with depression possessed lower BDNF levels than non-depression. Physical exercise increased BDNF levels in both depression and non-depression group.

Table 2: BDNF Levels Before and After Exercise in Depression and Non-Depression Group

Variable	Depression N = 35		p	Non-Depression N = 35		p
	Pre-Exercise (Mean ± SD)	Post-Exercise (Mean ± SD)		Pre-Exercise (Mean ± SD)	Post Exercise (Mean ± SD)	
BDNF (pg/mL)	231.7 ± 18.5	313.5 ± 28.7	0.001*	412.4 ± 22.3	478.9 ± 33.2	0.001*

*Dependent T-test, p = 0.05; SD: Standard Deviation; BDNF: Brain-Derived Neurotrophic Factor.

T-test showed that there were significant differences in BDNF levels before and after exercise. In the depression group, BDNF level increased about 35.3% from the initial value (from 231.7±18.5 to 313.5±28.7). In the non-depression group, BDNF level increased about 16.3% from the initial value (from 412.4±22.3 to 478.9±33.2). For the BDNF levels after exercise, the differences between depression group and non-depression were significant, as shown in Table 3.

Table 3: BDNF Levels After Exercise in Depression and Non-Depression Group

Variable	Depression N = 35 (Mean ± SD)	Non-Depression N = 35 (Mean ± SD)	p
BDNF (pg/mL)	313.5 ± 28.7	478.9 ± 33.2	0.001*

*Independent T-test, p = 0.05; SD: Standard Deviation; BDNF: Brain-Derived Neurotrophic Factor.

As shown in Figure 1, depression and non-depression group yielded an increase in post-exercise BDNF level. Depression group showed lower initial BDNF level compared to non-depression that eventually elevated after physical exercise.

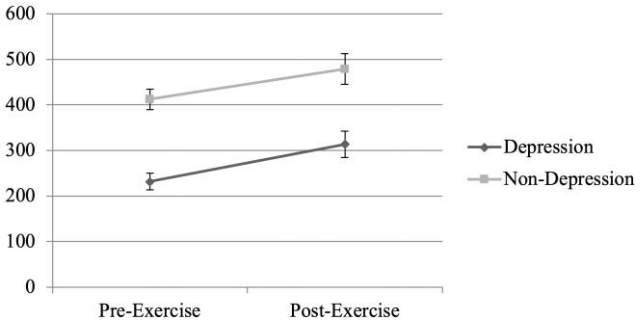

Figure 1: BDNF Levels in Depression and Non-Depression Group

Discussion

In this study, baseline characteristics of the subjects were similar between both study groups and only differed in depressive psychological status. The measures were taken into consideration since overall health status would affect physical activity related to the physical exercise and thus, the outcome of BDNF levels. Overall health status as may be compromised by chronic diseases that may be detected from BMI, blood pressures, respiration, as well as chemistry panel to put aside anaemic, hyper or hypoglycemia, hypercholesterolemia, or hyperuricemia conditions, that may confound results of physical exercise [6], [7]. The MMSE scores that almost reached the highest value (30) also indicated that all subjects were in normal cognitive function [8]. The cognitive evaluation was performed since depressive state, and cognitive function may be intertwined and degree of cognitive impairment is related to many factors in depression such as numbers of previous episodes, duration, and onset as well as treatment factors [9]. Evaluation of these factors and characteristics was aimed to include homogenous patient groups and make a comparison of the main result more precise.

This study showed that older adults with depression possessed BDNF levels far lower than those of the elderly without depression. The results of this study were in line with various previous studies,

which stated that BDNF levels tended to be lower in the elderly with depression. Decreasing BDNF levels will cause a decrease in the ability of neuronal cells to survive, thus affecting the neuroplasticity of neuronal cells [2], [10], [11]. Neuronal cells are producers of neurotransmitters that play a role in communication between neuronal cells. One of the main neurotransmitters produced by neurons is serotonin. Serotonin produced by neuronal cells in the hippocampus holds a major role in mood regulation. Decreased serotonin production will cause an alteration in mood, and patients will tend to undergo anhedonia, or to be sad and feeling guilty. Decreased survival of neuronal cells will reduce their ability to produce serotonin [4], [5], [12].

Physical exercise increased BDNF production in both elderly groups with and without depression. In the depression group, the increasing percentage of BDNF level was higher compared to the non-depression group. Physical exercise will reduce blood glucose levels. This causes the production of ketone bodies from the liver to increase as compensation for decreasing glucose levels in the brain, to maintain the stability of energy sources for the brain [13], [14]. Ketone bodies can increase BDNF expression in neuronal cells by inducing activation of monocarboxylate transporter-2 (Mct2) in the hippocampus [15], [16]. Increased activity of Mct2 transporters will increase the expression of BDNF and tropomyosin receptor kinase B (TrkB) levels, which leads to increased survival of neuronal cells. In addition to the role of ketone bodies, the presence of intermediate compounds such as ketoglutarate is believed to also play a role in increasing BDNF expression. Ketoglutarate will initiate histone demethylation. Histone demethylation is an epigenetic factor that possesses a role in increasing transcription and translation of the BDNF gene in the hippocampus [15], [16], [17], [18], [19].

In conclusion, physical exercise increased BDNF production in both elderly groups with and without depression. The increasing percentage of BDNF level was found to be higher in depressive elderly performing physical exercise. Physical exercise may be beneficial in supporting the therapy of elderly with depression. Further studies may explore physical exercise in improving depressive symptoms as well as BDNF level as performed in an extended period of study.

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Effect of a Small Selective Inhibitor of C-Jun N-Terminal Kinase on the Inducible mRNA Expression of Interleukin-6 and Interleukin-18

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Abstract

BACKGROUND: The expression of many inducible genes, involved in cell growth and differentiation as cytokine genes are regulated by receptor-activated intracellular signalling pathways, including the c-Jun N-terminal kinase (JNK) mitogen-activated protein kinase pathway.

AIM: We examined the involvement of the JNK signalling pathway in the regulation of the inducible interleukin-6 (IL-6) and interleukin-18 (IL-18) gene expression at the transcriptional level.

METHODS: Peripheral blood mononuclear cells (PBMC) from healthy donors were stimulated with lipopolysaccharide (LPS) and C3 binding glycoprotein (C3bgp) with or without SP600125 and cultured for 6 h. After mRNA isolation, a qRT-PCR was performed.

RESULTS: Regarding IL-6 and IL-18 mRNA expression, donors were divided into two groups of high and low responders. SP600125 inhibited significantly IL-6 mRNA transcription in the high responder group and did not influence the transcription level in the low responder group. Concerning IL-18 mRNA, we detect the significant effect of SP600125 on the inducible mRNA in high responder group upon C3bgp stimulation.

CONCLUSION: JNK transduction pathway is involved in the production of IL-6 mRNA, after LPS and C3bgp stimulation. We suggest that the inhibition of JNK may be beneficial only for higher responding patients during the treatment of inflammatory and autoimmune diseases.

Introduction

The expression of many inducible genes, involved in cell growth and differentiation as cytokine genes are regulated by a receptor-activated intracellular signalling pathways including c-Jun N-terminal kinase (JNK) mitogen-activated protein kinase (MAPK) pathway. JNK is a serine-threonine protein kinase that by phosphorylation activates c-Jun, a part of the transcription factor AP-1 [1]. Many target genes regulating the cell cycle, apoptosis and cell survival with AP-1 binding sites are regulated by JNK transduction pathway [2]. In immune cells, JNK regulates the transcription of a lot of inducible genes, including inflammatory cytokine genes [3]. More precisely, JNK is involved in the regulation of TNF-α, IL-12p40, IL-10 and IL-23 as is shown by previous studies of our laboratory [4], [5], [6].

Interleukin-6 (IL-6) is a part of the inflammatory response initiated by recognition of antigens referred to as pathogen-associated molecular pattern (PAMP) molecules to the pattern recognition receptors (PRR) expressed by immune cells. IL-6 is a proinflammatory and immunoregulatory cytokine with hormone-like activity – it is involved in immune regulation, inflammation, and oncogenesis [7]. During acute – phase inflammatory response liver cells secrete CRP, serum amyloid A, complement proteins and fibrinogen in response to IL-6 stimulation [8]. IL-6 also plays a key role in the humoral immune response by stimulation of proliferation of activated B cells and antibody production [7]. IL-6 induces the development of Th17 cells from naive T cells together

with TGF-β. Dysregulation and overproduction of IL-6 are connected to the development of autoimmune diseases such as multiple sclerosis (MS) and rheumatoid arthritis (RA), in which Th17 cells are suspected as the major cause of the diseases [9]. Although many somatic cells and cells of the immune system produce IL-6 the major source of this cytokine are the activated monocytes/macrophages. IL-6 expression and transducing IL-6 receptor system are well understood [10]. Human IL-6 is synthesised as a protein containing up to 212 amino acids, including a 28-amino-acid signal peptide. The core protein is 20 kDa and glycosylation accounts for the size of 21 – 26 kDa of natural IL-6 [8].

Interleukin-18 (IL-18) is also a pro-inflammatory cytokine, but it is included in Th1 polarisation. It's inducible gene expression after recognition of PAMPs by PRR lead to the synthesis of an inactive protein (pro – IL-18). Pro – IL-18 (24 kDa) is converted into biologically active IL-18 (17.2 kDa) by another activation pathway in inflammasome complexes through caspase 1-mediated cleavage [11], [12]. IL-18 is involved in the development of successful Th1 cell-mediated and antitumor immune response through its ability to induce IFN-γ secretion [13]. Unlike IL-6, the mechanisms regulating IL-18 gene expression and processing remains unclear.

In this regard, our study was designed to investigate the involvement of the JNK transduction pathway in the regulation of the expression of IL-6 and IL-18 mRNA and identify the anthrapyrazolone inhibitor as a novel potential regulator of LPS-induced cytokine production in human blood mononuclear cells.

Methods

PBMC isolation

With the approval of the local ethics board, blood samples were taken from 18 healthy donors 30-40-year olds 9 male and 9 females. Informed consent was obtained from each participant. The peripheral venous blood (10 ml) was collected in sterile tubes with ethylenediaminetetraacetic acid (EDTA). Peripheral blood mononuclear cells (PBMC) were isolated by Histopaque-1077 (Sigma-Aldrich-Merck, Darmstadt, Germany) density gradient centrifugation. The interface containing PBMC was harvested and washed twice with cold RPMI-1640 medium.

Cell cultures and stimulation

PBMC (1 x 10^6 cells/ml) cultures were carried out in RPMI-1640 (Sigma-Aldrich-Merck, Darmstadt, Germany) supplemented with: 10% FBS, 100 U/ml penicillin, 100 μg/ml gentamycin and 0.3 mg/ml L-glutamine. The cells were stimulated with: 30 μg /ml C3 binding glycoprotein (C3bgp) isolated as described previously [14] or 1 μg/ml Lipopolysaccharide (LPS) from Escherichia coli serotype 026: B6 (Sigma-Aldrich-Merck, Darmstadt, Germany). The concentrations of the stimuli used were determined, according to Stanilova et al., [15] and Takahashi et al., [16]. As shown by our previous study of inducible cytokine gene expression transcripts, the maximum mRNA quantities were reached after 6 h of stimulation and were independent of stimuli used [5]. Therefore, PBMC cultures were incubated at 37°C for 6 h. After incubation, the cultures were centrifuged at 1800 rpm for 10 min. The cell pellet was separated, and the RNA was isolated.

Inhibition of JNK MAPK pathway

One hour before the stimuli addition, some of PBMC cultures were pre-treated with an inhibitor of JNK kinase. For inhibition of c-jun N-terminal kinase, we used the selective anthrapyrazolone inhibitor SP600125 (Sigma-Aldrich-Merck, Darmstadt, Germany). It competitively inhibits JNK 1, 2 and 3 with > 20-fold selectivity vs the wide range of kinases according to Bennett et al., [1]. SP600125 is dissolved in 100% dimethylsulfoxide (DMSO) (Sigma-Aldrich-Merck, Darmstadt, Germany), and the final concentration of JNK inhibitor in cell cultures is 20 μM. Non-stimulated cell cultures were used as controls. Therefore, our experiment included the following PBMC cultures: non-stimulated, stimulated with LPS, stimulated with LPS and pre-treated with SP600125, stimulated with C3bgp, stimulated with C3bgp and pre-treated with SP600125.

RNA extraction and reverse transcription

Total RNA from cell culture pellet was isolated using innuPREP blood RNA isolation kit AJ Roboscreen (Leipzig; Germany) with the additional step of treatment with DNase I to remove traces of genomic DNA. The total RNA was quantified by spectrophotometrical analysis. Synthesis of cDNA was performed manually according to the manufacturer's instructions with the High-Capacity cDNA Archive kit (Applied Biosystems, Foster City, CA) that uses random primers and MutliScribe TM MuLV reverse transcriptase enzyme. Incubation conditions for reverse transcription were 10 min at 25°C followed by 2 h at 37°C and was performed on a GeneAmp PCR System 9700 (Applied Biosystems).

Quantitative real-time polymerase chain reaction

Quantitative real-time polymerase chain reaction (qRT-PCR) was performed on a 7500 Real-Time PCR System (Applied Biosystems). The qRT-PCR primers and probes were purchased from

Primerdesign, UK and Termoscientific as predesigned inventoried assay reagents. The following validated PCR primers, and TaqMan MGB probes (6FAM-labeled) were used: IL-6 (assay ID: Hs00985639_m1) and for IL-18 mRNA (AX-7948-00-0200). As endogenous controls were used eukaryotic 18S ribosomal RNA (Hs999999_s1) and β2 microglobulin (Hs00187842_m1). An aliquot of 5 µl of the RT reaction was amplified in duplicate in a final volume of 20 µl using a TaqMan Universal PCR Master Mix and Gene Expression Assay mix, containing specific forward and reverse primers and labelled probes for target genes and endogenous controls (Applied Biosystems). The thermocycling conditions were: initial 10 min incubation at 95°C followed by 40 cycles of denaturation for 15 s at 95°C and annealing/extension for 1 min at 60°C. PCR data were collected with Sequence Detection System (SDS) software, version 2.3.

Relative quantitative evaluation of cytokine mRNAs was performed by the comparative ΔΔCt method. The mean ΔCt obtained in nonstimulated controls for each cytokine mRNA was used as a calibrator, after normalisation to the average of endogenous controls 18S rRNA and β2 microglobulin. The results are calculated as an n-fold difference relative to the calibrator ($RQ=2^{-\Delta\Delta Ct}$).

Statistical analysis

The data were presented as a mean±standard error. One-way ANOVA test was used to compare the mean values between all stimuli used, followed by post hoc Fisher LSD test to analyse the effect of JNK inhibition on LPS or C3bgp induced gene expression. Differences were considered significant when the p values were < 0.05.

Results

IL-6 mRNA expression

Results presented in Figure 1, showed that the transcription of IL-6 mRNA was upregulated after stimulation with both LPS and C3bgp. Moreover, the healthy donors were divided into two groups – individuals with high (RQ > 200) (6 healthy donors) and with low (RQ ≤ 200) (12 donors) levels of the inducible IL-6 mRNA. In the high responder group, the level of mRNA expression in stimulated cultures RQ value varied between 219-2387 for LPS and 394-4728 for C3bgp stimulation. The gene expression was significantly affected by a used stimulus in high responder group ($F = 3.68$; $df = 3$; $p = 0.029$) in contrast to low responder group ($F = 1.16$; $df = 3$; $p = 0.35$). In the low responder group, the RQ value is 11-120 for LPS and 13-165 for C3bgp, respectively. We found statistically significant inhibition of IL-6 gene transcription in cells pretreated with SP600125 compared to cultures without JNK inhibitor in high responder group – LPS vs. LPS + SP600125 $p = 0.014$; C3bgp vs. C3bgp + SP600125 $p = 0.014$ (Fisher LSD test). In the same experimental condition, we did not detect the inhibitory effect of SP600125 on the IL-6 gene transcription in the low responder group.

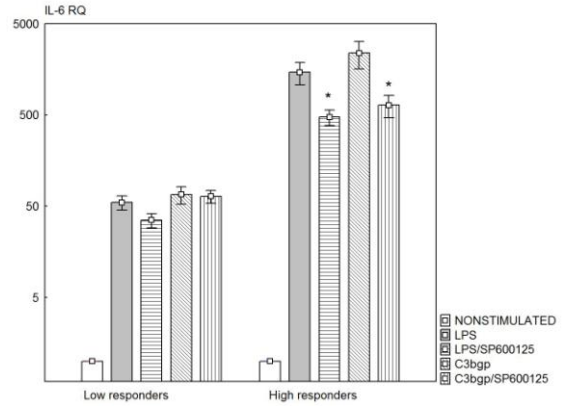

*Figure 1: Levels of IL-6 mRNA transcription in PBMC of 18 healthy donors after stimulation with 1 µg/ml LPS and 30 µg/ml C3bgp, with or without 20 µM JNK inhibitor SP600125. The results are presented on a logarithmic scale. The cells were harvested at 6 h after stimulation. After mRNA isolation, a qRT-PCR was performed, and the results were calculated as an n-fold difference relative to calibrator $RQ = 2^{-\Delta\Delta Ct}$. The results are presented as mean ± standard error; *p < 0.05 for stimulus vs stimulus + SP600125*

IL-18 mRNA expression

The levels of the inducible IL-18 mRNA were significantly lower (more than 100-fold) than those of IL-6 mRNA after stimulation with LPS and C3bgp. Regarding IL-18 mRNA expression individuals were also subdivided into two groups: high responders (8 healthy donors) (RQ between 1.201-1.979 for LPS and 1.324-3.249 for C3bgp) and low responders (10 donors) (RQ varied between 0.720-1.147 for LPS and 0.454-0.882 for C3bgp), Figure 2.

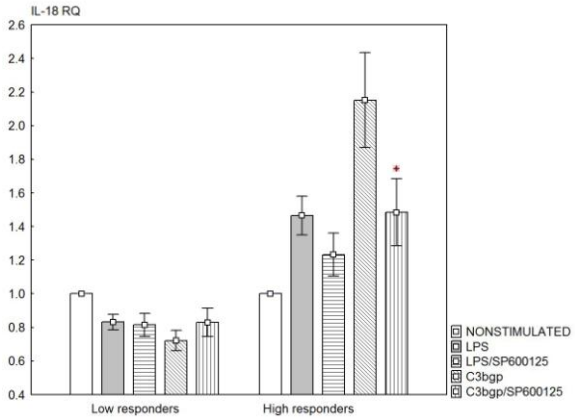

*Figure 2: Levels of IL-18 mRNA transcription in PBMC of 18 healthy donors after stimulation with 1 µg/ml LPS and 30 µg/ml C3bgp, with or without 20 µM JNK inhibitor SP600125. The cells were harvested at 6 h after stimulation. After mRNA isolation, a qRT-PCR was performed, and the results were calculated as an n-fold difference relative to calibrator $RQ = 2^{-\Delta\Delta Ct}$. The results are presented as mean ± standard error; *p < 0.05 for stimulus vs stimulus + SP600125*

The gene expression of IL-18 was also significantly affected by a used stimulus in high responder group ($F = 3.21$; $df = 3$; $p = 0.039$) in contrast to low responder group ($F = 0.89$; $df = 3$; $p = 0.46$). JNK inhibition significantly decreases C3bgp-induced IL-18 gene expression (C3bgp vs. C3bgp + SP600125 $p = 0.035$, Fisher LSD test) in contrast to LPS-induced gene expression (LPS vs. LPS + SP600125; $p = 0.43$) in high responder group. Also, upregulation of IL-18 gene expression was significantly higher after stimulation with C3bgp compared to LPS ($p = 0.04$).

Discussion

To investigate the involvement of JNK MAPK in the inducible IL-6 and IL-18 gene expression, we used a small selective inhibitor of JNK transduction pathway SP600125. The effect of JNK inhibition on the IL-6 and IL-18 mRNA expression we determined in an experimental model of LPS and C3bgp-stimulated human PBMC pretreated with inhibitor. Cell cultures were harvested on 6 h, and steady-state mRNA of IL-6 and IL-18 gene was evaluated by qRT-PCR.

We found that healthy donors were divided into two groups in terms of mRNA expression of both IL-6 and IL-18 genes-individuals with high mRNA expression (high responders) and those with low mRNA expression (low responders) of the target genes. In most cases, the same donors showed high or low IL-6 and IL-18 mRNA expression. The observed level of expression was distinct between two studied groups, showing principal differences of individual response in the regulation of IL-6 and IL-18 expression. Our experiments revealed that value for IL-6 after LPS and C3bgp stimulation varied between individuals more than a 10-fold range. This variability is much greater than would be expected due to technical reasons. It is obvious that mechanisms determining this variability of induced proinflammatory cytokine production should be searched elsewhere, probably in the individual genetic background and regulation of gene expression as well. Other authors also have a similar observation on the inter-individual variability in proinflammatory cytokine response, especially in response to LPS [17], [18]. Indeed, in their study Wurfel et al., discovered 80 genes that were differentially expressed in LPS-low and LPS-high healthy responders in the presence of LPS and 21 genes in the absence of LPS [19].

In this study for the first time, we showed that JNK inhibitor SP600125 selectively mediated its effect in PBMC from healthy individuals – SP600125 downregulated IL-6 gene expression in high responder group and did not affect the expression of this gene in the low responder group. Other researchers did not divide its donors depending on the magnitude of IL-6 expression and reported that inhibition of JNK reduced IL-6 expression in a different type of human cells on the protein level [20], [21] IL-6 expression is tightly regulated transcriptionally by several transcription factors. NF-kB and AP-1 are included between the major transcription factors responsible for IL-6 gene regulation [22]. Therefore, the downregulation of IL-6 gene expression by JNK inhibitor in high responder group is not an unexpected result. On the contrary, the lack of inhibitory effect of SP600125 on low responders indicates that in these individuals, the JNK transduction pathway may not be involved in inducible IL-6 gene expression. Additional experiments with inhibition of ERK and p38 is necessary to clarify whether these MAP kinases are involved in the regulation of IL-6 production in the low responder group.

At present, it is known that IL-18 mediated Th1 type of the immune response, because of its property to induce IFN-γ secretion from activated T cells and NK cells [13]. There is evidence that treatment with IL-18 has significant antitumor action due to enhanced cell-mediated immune response [11]. Also, IL-18 administration in mice inoculated with tumour cell line stimulated IFN-γ production and IL-12 independent Th1 cell-mediated antitumor immune response [23]. Our study indicated that SP600125 downregulated significantly IL-18 mRNA expression in PBMC after C3bgp, but not after LPS stimulation. There are few studies about JNK inhibition and its effect on IL-18 secretion. Similar results regarding the absence of an inhibitory effect of SP600125 after LPS stimulation was reported in primary human keratinocytes activated by β-defensins in the work of Niyonsaba et al., [24]. However, our results support the study of Miyauchi et al., reporting that JNK inhibition by SP600125 in human tubular epithelial cell from type 2 diabetic subjects significantly suppressed TGF-β-induced IL-18 expression [25] or Wang et al., showing that SP600125 inhibited IL-18 mRNA expression in murine peritoneal macrophages after heat shock response [26].

Currently, it is widely accepted that IL-6, together with TGF-β, induced the development of normal Th17 cells, whereas the additive presence of IL-23 and IL-1 lead to the development of pathological Th17 subset [27]. The pathological Th17 cells are recognised as essential cells responsible for the development of chronic inflammatory conditions and autoimmune diseases. The upregulated prolonged synthesis of IL-6, both genetically and epigenetically determined, has a significant role in the development of many diseases, including autoimmune and cancers. Dysregulated IL-6 production and subsequently, the presence of pathologic CD4 + Th17 cells were described in rheumatoid arthritis, multiple sclerosis, myeloma, systemic lupus erythematosus and others [9], [22]. Because of the pathologic role of IL-6 in the therapy of these diseases is used anti-IL-6R

monoclonal antibody tocilizumab that block IL-6 mediated signal transduction. There was an observation that tocilizumab alleviated diseases symptoms, but also several side effects are described, including hypercholesterolemia, acute pyelonephritis, inflammation of the upper respiratory system and parotid glands [28]. During immune response development of the Th1 type of the response suppressed the development of the Th17 cells by the secretion of IFN-γ [29]. Therefore, in the context of an autoimmune response downregulation of IL-6 expression by the SP600125 application may provide a desirable effect in the treatment of these diseases in high responding patients.

The results and conclusions in this article are based on studies conducted within 18 healthy donors and PBMC isolated from them.

In conclusion, the JNK MAPK transduction pathway is selectively involved in the production of IL-6 mRNA, after LPS and C3bgp stimulation, and IL-18 mRNA after C3bgp stimulation from PBMC isolated from higher responding individuals. We suggest that the inhibition of JNK may be beneficial only for higher responding patients during the treatment of inflammatory and autoimmune diseases mediated by the unbalanced Th1/Th17 type of immune response.

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Basic Science

Characteristics of an Outpatient Cohort with HBeAg-Negative Chronic Hepatitis B

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Abstract

BACKGROUND: Patients with hepatitis Be antigen-negative chronic hepatitis B (HBeAg-negative CHB), and patients' inactive carriers (IC) have similar laboratory and serologic characteristics and are not always easy to distinguish.

AIM: To characterise hepatitis Be antigen (HBeAg) negative chronic hepatitis B cohort based on their laboratory and virology evaluations at one point of time.

METHODS: A prospective non-randomized study was conducted on 109 patients with HBeAg negative chronic hepatitis B treated as outpatients at the Clinic for Infectious Diseases and Febrile Conditions. All patients underwent laboratory and serology testing, quantification of HBV DNA and HBs antigen (qHBsAg).

RESULTS: A group of 56 patients were inactive carriers (IC), and 53 patients had HBeAg-negative CHB (AH). The mean values of ALT, HBV DNA and qHBsAg in IC were 29.13 U/L; 727.95 IU/ml and 2753.73 IU/ml respectively. In the AH group, the mean values of ALT, HBV DNA and quantitative HBsAg were 50.45 U/L; 7237363.98 IU/ml and 12556.06 IU/ml respectively. The serum value of ALT was more influenced by qHBsAg than HBV DNA in both IC and AH groups ($R = 0.22$ vs $R = 0.15$) ($p > 0.05$).

CONCLUSION: patients with inactive and active HBeAg-negative CHB have similar laboratory and serology profile. It is necessary to combine analysis of ALT, HBV DNA and qHBsAg for better discrimination between patient's IC and patient with HBeAg-negative CHB.

Introduction

Infection with hepatitis B virus (HBV) remains the leading cause of liver damage and represents one of the major health problems worldwide. Nowadays, it is considered that approximately 30% of the world population has serologic evidence of current or past infection with HBV and 248 to 257 million people are chronic HBV carriers on a global level [1]. Chronic hepatitis B virus infection is associated with serious complications such as cirrhosis, hepatocellular carcinoma (HCC) end-stage liver disease and death [2], [3], [4]. The natural history of chronic hepatitis B (CHB) is characterised by different phases of infection, and patients may evolve from one phase to another or may revert to a previous phase and not necessarily in sequential order. The phases of the natural history of chronic HBV infection have been schematically divided into five phases, taking into account the presence of HBeAg, HBV DNA (hepatitis B virus deoxyribonucleic acid) levels, alanine transaminase (ALT) values and the presence or absence of liver inflammation [5]. The hepatitis B-e

antigen (HBeAg) positive phase is characterized by high serum HBV DNA levels, and HBeAg negative phase is characterised with HBeAg loss and seroconversion with the occurrence of anti HBe antibodies, which is usually associated with the decline of HBV DNA levels, and normalisation of ALT values [5], [6]. In some patients, this process of seroconversion to HBeAg negative phase is associated with the selection of HBV variants that express little or no HBeAg at all and is usually characterised with continuing HBV DNA replication and progression of liver damage [5], [6], [7]. Usually, most of the chronically infected HBV patients experience the inactive phase with normal ALT levels, low viraemia and negative HBeAg after HBeAg seroconversion. However, up to 10-30% of chronic HBV infected adults subjects may suffer from HBeAg-negative hepatitis flare after HBeAg seroconversion, especially in those who experience late HBeAg seroconversion, and are associated with increased life-long risk of liver cirrhosis and HCC [8], [9]. It has been estimated that the median prevalence of HBe antigen-negative chronic hepatitis B infection is around 33% in the Mediterranean, 15% in the Asia Pacific, and 14% in the USA and Northern Europe [10]. Patients with HBeAg-negative CHB represent a heterogeneous group characterised with a different range of viral replication and liver disease severity, seen by fluctuating levels of HBV DNA and transaminases with temporary remissions during the disease [11]. Therefore, it is necessary to make a distinction among those with active hepatic necrotic inflammation and persistent viraemia as they have higher rates of complications (patients with chronic HBeAg negative hepatitis B) in contrast to HBeAg-negative CHB patients who are inactive carriers. Both forms of CHB, patients with HBeAg negative chronic hepatitis and patients' inactive carriers, have similar laboratory and serologic characteristics and are not always easy to distinguish [8]. In an inactive carrier, ALT usually remains normal on serial monitoring with undetectable to low levels (i.e., < 2000 IU/ml) of HBV DNA. However, the same may also occur in a patient with HBeAg-negative CHB. It is known that maintained high levels of HBV DNA are associated with progressive liver disease. Serum DNA levels are a prognostic factor, and contribute to defining the phases of CHB infection, the treatment indication, and allow an assessment of the efficacy of antiviral therapy [11], [12].

Eradication of HBV should be useful both for the patients and the society. There are consensus guidelines that help the clinicians to make decisions about whether or not to treat a patient. The viral load cannot be considered as the only treatment criterion. HBV DNA persists even in persons who have serological recovery from acute HBV infection [13] Areas of uncertainty whether and when to treat patients with HBeAg negative chronic hepatitis still exist, and clinicians, patients, and public health authorities must, therefore, continue to make choices on the basis of the evolving evidence [11], [14], [15]. The identification of patients with chronic HBeAg negative infection (inactive carriers-IC) versus patients with chronic HBeAg-negative chronic hepatitis B (AH) is a complex issue due to the dynamic character of hepatitis B infection. Proper and timely assessment of patients with HBeAg negative chronic hepatitis B is important for early treatment decision and consecutive prevention of disease progression and development of chronic hepatitis B virus-associated complications. In this study, we evaluated the laboratory, serological and virological characteristics of an outpatient cohort of HBeAg negative chronic hepatitis B.

Material and Methods

A prospective non-randomized study was carried out on 109 patients with HBeAg negative CHB, treated at the Clinic for Infectious Diseases and Febrile Conditions in Skopje, the Republic of Macedonia from the period of November 2016 till January 2018. All patients who were HBsAg-positive for at least six months, but HBeAg-negative, anti-HBe-positive, and had detectable HBV DNA in the serum were included in the study. Patients under the age of 18 years, all patients who tested positive for hepatitis A, hepatitis C and HIV were not included in the study. Other excluding criteria were previous or current exposure to antiviral hepatitis B treatment, alcoholic and autoimmune liver diseases, incomplete serum profile and a follow-up period of fewer than six months. Patients with hepatocellular carcinoma (HCC), decompensated liver disease and pregnant patients were excluded from the study.

The following data were obtained for all the patients: age, sex, alcohol consumption, complete blood count, bilirubin levels, transaminase, AFP, serology, quantification of HBV DNA, quantification of hepatitis B surface antigen (qHBsAg), abdominal ultrasound, total protein electrophoresis and presence of clinical signs and symptoms for cirrhosis. Cirrhosis was determined with the presence of ascites, encephalopathy, palmar erythema, telangiectasia, jaundice, hypoalbuminemia and ultrasound finding of cirrhosis. Complete serology profile was performed with ELISA (enzyme-linked immune assay) tests. The normal upper limit of serum transaminase both for alanine aminotransferase (ALT) and aspartate aminotransferase (ALT) was 40 U/L, according to the traditional cut-off values. Quantification of HBV DNA levels in the plasma was performed in-house, by real-time polymerase chain reaction (RT-PCR) on COBAS AmpliPrep COBAS TaqMan HBV test and Abbott m 2000 sp/m 2000 rt with a lower detection limit of 10 IU/mL. The serum level of HBsAg (qHBsAg) was quantified with Architect HBsAg assay (Abbott Laboratories) in-house, according to the

manufacturers' protocol. The detection level of HBsAg varies from 0.05 to 250 IU/ml. HBsAg levels above 250 IU/ml were further diluted in a ratio of 1:500.

We evaluated the serum values of alanine transaminase (ALT) aspartate transaminase (AST), qHBsAg and HBV DNA. A multiple regression analysis was performed to establish the correlation between the serum levels of ALT with qHBsAg and HBV DNA.

Relevant clinical variables were gender, age, platelet count, ALT, AST, HBsAg, hepatitis B e antigen, HBV DNA. The value of ALT and AST are expressed in units per litre (U/L), and those of qHBsAg and HBV DNA were expressed in international units per millilitre (IU/ml).

Adopted Definitions

An inactive carrier was considered when HBeAg nonreactive with normal transaminase levels, HBV-DNA < 2000 IU/ml [11].

Active chronic HBeAg negative hepatitis was considered when HBeAg nonreactive, and if ALT was elevated above the upper normal limit and HBV-DNA was more than 2,000UI/mL [11].

The study was approved by the Ethics Committee of the Medical Faculty in Skopje.

Statistical Analysis

All data were processed using a statistical computer program Statistica 7.1 for Windows and SPSS Statistics 17.0. Series with attributive variables were analysed with percentages of structure. For numerical variables descriptive statistics ((Mean; Std. Deviation; ± 95.00% CI; Minimum; Maximum) was used, where frequencies and percentages were used for the description of the categorical variables. Distribution of the data was tested with Kolmogorov-Smirnov tests; Lilliefors test; Shapiro-Wilks test(p). The differences between the groups were analysed with Pearson Chi-square (p) and Fisher's exact test (p). T-test for independent variables (t/p) and Mann-Whitney U test (Z/p) were used depending on the distribution of the data. Multiple Regression (R/p) was used to determine the correlation between ALT, qHBsAg and HBV DNA. For all analyses P values of <, 0.05 were considered significant.

Results

Out of 109 patients included in the study, 80 (73.39 %) were male, and 29 (26.61%) were female. If in the analysis of the presentation forms at one point of time we considered only the baseline values of ALT and HBV DNA according to definitions used, 56 patients (51.37%) had chronic hepatitis B infection, or as previously defined inactive carriers (IC), and 53 (48.62%) had HBeAg-negative CHB (AH). In the group of inactive carries, the mean age of the patients was 37.50 ± 10.84 years, while in the group of AH was 43.91 ± 11.72 years. For Pearson Chi-square = 0.002 and p > 0.05 (p = 0.97) there was no statistically significant difference between both groups of patients in terms of gender. Patients with AH for t = -2.96 and p < 0.01 (p = 0.004) were significantly older than patient's IC (Table 1)

Table 1: Demographic and descriptive statistics in patients' inactive carriers and patients with HBeAg-negative chronic hepatitis B

Characteristics						n (%)		
		IC				56 (51.37%)		
		AH				53 (48.62%)		
	Sex			Male		80 (73.39 %)		
				Female		29 (26,61%)		
Parameter	Group	N	Mean	Confidence -95,00%	Confidence +95,00%	Minimum	Maximum	Std.dev.
Age	IC	56	37,50	34,60	40,40	19	67	10,84
(years)	AH	53	43,91	40,67	47,14	22	74	11,72
ALT U/L	IC	56	29,13	24,43	33,82	10	89	17,53
AST U/L		56	22,20	20,10	24,29	14	57	7,81
ALT U/L	AH	53	50,45	39,83	61,07	10	173	38,53
AST U/L		53	34,74	29,37	40,10	12	101	19,46

Abbreviations: IC-inactive carriers; AH- HBeAg-negative chronic hepatitis B; ALT-alanine transaminase; AST-aspartate transaminase.

The mean value of ALT and AST in IC and AH patients was 29.13 ± 17.53 U/L; 22.20 ± 7.81 U/L; 50.45 ± 38.53U/L and 37.74 ± 19.46 U/L, respectively (Table 1). When the levels of transaminases were compared, patients with AH had significantly higher ALT values compared to IC for Z = -3.18 and p > 0.01 (p = 0.001), as well as for AST (Z = -4.06 и p < 0.001 (p = 0.000)) (Table 2).

Table 2: Differences in transaminases levels in patients' inactive carriers and patients with HBeAg-negative chronic hepatitis B

Parameter	Rank Sum IC	Rank Sum AH	U	Z adjusted	p-level	N IC	N AH
ALT U/L	2556.50	3438.50	960.50	-3.18	0.001	56	53
AST U/L	2410.50	3584.50	814.50	-4.06	0.000	56	53
alkaline phosphatase U/L	3028.00	2967.00	1432.00	-0.32	0.75	56	53
Gamma GT U/L	2649.50	3345.50	1053.50	-2.61	0.009	56	53

Abbreviations: IC-inactive carriers; AH- HBeAg-negative chronic hepatitis B; ALT-alanine transaminase; AST-aspartate transaminase; Gamma GT-Gamma-glutamyl transferase.

The mean value of HBV DNA in IC and AH group were 727.95 ± 584.24 IU/ml and 7237363.98 ± 46513427.91 IU/ml respectively. The mean value of quantitative HBsAg in IC was 2753.73 IU/ml and in the AH group 12556.06 ± 27188.85 IU/ml (Table 3).

Table 3: Quantitative HBsAg and HBV DNA levels in patients' inactive carriers and patients with HBeAg-negative chronic hepatitis B

	Parameter	N	Average	Confi-dence -95,00%	Confi-dence +95,00%	Min	Max	Std.dev
IC patients	qHBsAg IU/ml	56	2753.73	1494.72	4012.74	0.05	19636.84	4701.29
	HBV DNA IU/ml	56	727.95	571.49	884.41	10	1997	584.24
AH patients	qHBsAg IU/ml	53	12556.06	5062	20050	12.95	155311.00	27188.85
	HBV DNA IU/ml	53	7237363.98	-5583325	20058053	2061	338999252	46513427.91

Abbreviations: IC-inactive carriers; AH- HBeAg-negative chronic hepatitis B; HBV DNA-hepatitis B virus deoxyribonucleic acid; qHBsAg-quantitative hepatitis Bs antigen.

In the group of patients IC, 29 (51.79%) had qHBsAg < 1000 IU/ml, and 27 (48.21%) had qHBsAg > 1000 IU/ml, while in the group of patients with active hepatitis (AH) 6 (11.32%) had qHBsAg < 1000 IU/ml, and 47 (88.68%) had qHBsAg > 1000 IU/ml. When the levels of HBV DNA in AH group were stratified, 27 (50.94%) had HBV DNA > 2000 ≤ 20 000 IU/ml and 26 (49.06%) had HBV DNA > 20000 IU/ml.

Obviously, all patients' inactive carriers had HBV DNA level < 2000 IU/ml (Table 4). For Pearson Chi-square = 20,45 and p < 0.001 (p = 0.000) in the AH group of patients qHBsAg > 1000 IU/ml is significantly more represented.

Table 4: Distribution of quantitative HBsAg and HBV DNA in patients' inactive carriers and patients with HBeAg-negative chronic hepatitis B

	IC					AH			
qHBsAg	No.	Cumulative No.	%	Cumulative %	qHBsAg	No	Cumulative No.	%	Cumulative %
< 1000 IU/ml	29	29	51.79	51.79	< 1000 IU/ml	6	6	11.32	11.32
> 1000 IU/ml	27	56	48.21	100.00	> 1000 IU/ml	47	53	88.68	100.00
Missing	0	56	0,00	100.00	Missing	0	53	0.00	100.00
HBV DNA	No.	Cumulative No.	%	Cumulative %	HBV DNA	No.	Cumulative No.	%	Cumulative %
< 2000 IU/ml	56	56	100.00	100.00	≥ 2000 AH ≤ 20000 IU/ml	27	27	50.94	50.94
					> 20000 IU/ml	26	53	49.06	100.00
Missing	0	56	0.00	100.00	Missing	0	53	0.00	100.00

Abbreviations: IC-inactive carriers; AH- HBeAg-negative chronic hepatitis B; HBV DNA-hepatitis B virus deoxyribonucleic acid; qHBsAg-quantitative hepatitis B s antigen.

Patients with AH for Z = -5.10 and p < 0.001 (p = 0.0000) had statistically significant higher values of qHBsAg compared to patients' inactive carriers. Likewise, for Z = -8.99 and p < 0.001 (p = 0.000) patients with active hepatitis have significantly higher values of quantitative HBV DNA than patient's IC (Table 5).

Table 5: Differences between quantitative HBsAg and HBV DNA in patients' inactive carriers and patients with HBeAg-negative chronic hepatitis B

Parameter	Rank Sum IC	Rank Sum AH	U	Z adjusted	p-level	N IC	N AH
qHBsAg IU/ml	2238.00	3757.00	642.00	-5.10	0.000	56	53
HBV DNA IU/ml	1596.00	4399.00	0.00	-8.99	0.000	56	53

Abbreviations: IC-inactive carriers; AH- HBeAg-negative chronic hepatitis B; HBV DNA-hepatitis B virus deoxyribonucleic acid; qHBsAg-quantitative hepatitis Bs antigen.

Individualized analysis of the serum profile and the measurements of HBV DNA/ALT/qHBs antigen showed that the increase of the level of HBV DNA is followed with a non-significant decrease of ALT, both in IC and in patients with AH, while the increase of the level of quantitative HBsAg is followed with the increase of the level of ALT in both groups of patients. The influence of qHBsAg is significantly stronger than that of HBV DNA.

For each single increase of serum HBsAg, serum ALT increases for 0.0008 IU/ml p > 0.05 (p = 0.11) in IC group of patients, while in AH group of patients, the serum level of ALT increases for 0.0002 IU/ml p > 0.05 (p = 0.33) (Table 6).

Table 6: Multiple regression analysis of ALT/HBV DNA/qHBsAg in patients' inactive carriers and patients with HBeAg- negative chronic hepatitis B

	IC						AH					
	Dependent Variable: ALT; R= 0.22; F(2.53)=1.39 and p<0.26						Dependent Variable: ALT; R= 0.15; F(2.50)=0.59 and p<0.66					
	Beta	Std.Err. of Beta	B	Std.Err. of B	t(53)	p-level	Beta	Std.Err. of Beta	B	Std.Err. of B	t(53)	p-level
Intercept			28.29	3.89	7.27	0.000			47.64	5.93	8.03	0.000
qHBsAg	0.22	0.13	0.0008	0.0005	1.64	0.11	0.17	0.17	0.0002	0.000	0.99	0.33
HBV DNA	-0.07	0.13	-0.002	0.004	-0.49	0.63	-0.04	0.17	-0.000	0.000	-0.22	0.83

Abbreviations: IC-inactive carriers; AH- HBeAg-negative chronic hepatitis B; HBV DNA-hepatitis B virus deoxyribonucleic acid; qHBsAg-quantitative hepatitis Bs antigen.

Discussion

HBeAg negative, antique positive chronic hepatitis B is a capricious disease characterised with a dynamic and complex interaction between the virus, the hepatocytes and the host's immune system [6], [7], [8], [16], [17]. The Republic of Macedonia has an estimated HBsAg prevalence around 1-4% [18] and having in mind that chronic HBV infection is the major pathogen causing chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC) in the world, this imposes serious burden both on the individual and society as well [3], [5], [19]. It is very important to distinguish chronic inactive HBsAg carriers from HBeAg-negative CHB patients because the progression of the liver damage occurs primarily during the active hepatitis phase, and this group of patients has the potential of developing marked viral reactivation and has less chance of response to antiviral medications [20]. In the currently available guidelines, the recommendation is that HBV DNA, ALT, and HBeAg be analysed together and with great care for therapy decision making and the indication of the need for biopsy [11], [14], [15].

The results from the patients included in our study showed that there is no statistical significance between inactive carrier group and patients with HBeAg-negative CHB in terms of gender, although in the literature HBeAg-negative CHB is more expressed in males [21], [22], [23]. At the same time, the patients in our cohort with HBeAg-negative CHB are older than the patient's inactive carriers, which is consistent with the findings described in the literature [5], [23]. Chu et al., [24] followed 1.965 inactive HBV carriers' patients during 11.5 years and found out that 314 patients had reactivation of HBV. The risk for reactivation had a positive correlation with older age (p < 0.0001) and male sex (p < 0.0001). At the same time, the risk for developing cirrhosis also had a statistically significant correlation with advanced age and HBV reactivation (p = 0.004) and (p < 0.0001), respectively. The study showed that male sex (p = 0.037) and advanced age (p = 0.006) were two independent factors for HBV reactivation. The REVEAL-B study carried out by Chen et al., [23], besides HBeAg positivity and high HBV viraemia identifies male gender, older patients,

alcoholism and high BMI (body mass index), as factors associated with HBV disease progression similar to the findings of Fattovich et al., [5].

Alanine transaminase (ALT) levels have traditionally been used for treatment decisions in chronic hepatitis B virus-infected patients. In the study of Ijaz et al., [25] 567 patients with HBeAg negative CHB were investigated, and 228 were classified as chronic inactive carriers, and 339 with chronic active hepatitis B. The serum enzyme levels of ALT, AST showed significant and high AUROC in differentiation between HBeAg negative IC and HBeAg negative patients with chronic hepatitis. The AUROC for ALT and AST was 0.997 and 0.969, respectively. Similar to Ijaz's study, when the levels of transaminases were compared in our cohort, patients with HBeAg-negative CHB had significantly higher ALT and AST values compared to IC patients. The serum level of ALT is a factor to consider in the treatment of CHB patients, and a high ALT level helps to distinguish between the inactive carrier state and asymptomatic HBeAg-negative CHB patients with normal ALT [26]. The findings of low serum levels of transaminases in the patients inactive carriers included in our study is compatible with the observation that low and normal levels are expected in both patients inactive carriers and patient with HBeAg negative chronic hepatitis B, [5], [27], and it is prudent to emphasize the need for serial monitoring of the levels of transaminases over time and that the sole monitoring of ALT is not strong enough criteria for evaluation of hepatic injury as described in the study of Hadziyannis et al., [7].

It is known that maintained high levels of HBV DNA are associated with progressive liver disease. Serum DNA levels are a prognostic factor, and contribute to defining the phases of CHB infection, the treatment indication, and allow an assessment of the efficacy of antiviral therapy. Kumar et al., [26] in a recent, large prospective study have shown clearly that baseline ALT, and DNA level is good predictors of histologically significant fibrosis. The ten years long retrospective study conducted by Madan et al., [28] showed that the mean level of HBV DNA is lowest in patients' inactive carriers, and it is highest in patients with chronic active hepatitis B. Patients inactive carriers had significantly lower serum values of HBV DNA than patients with HBeAg negative chronic hepatitis B. The cut-off values of 3,5 log 10 cp/ml had sensitivity and specificity of 83 and 58% respectively in differentiation of patient's IC from patients with HBeAg negative CHB. The study of Zacharakis et al., [29] investigated 263 patients with chronic hepatitis B, genotype D, patients who were treatment naïve, HBeAg negative, antique positive, and all patients inactive carriers had low or almost undetectable levels of HBV DNA < 2000 IU/ml, and only 2% had HBV DNA reactivation with the level of HBV DNA > 2000 IU/ml. Similar to these studies, from the patients included in our study, patients inactive carriers had significantly lower values of serum HBV DNA compared to patients with HBeAg-negative CHB. An interesting finding in our study was that when the levels of HBV DNA were stratified in patients with HBeAg-negative CHB, 50.94% had HBV DNA > 2000 ≤ 20 000 IU/ml and 49.06% had HBV DNA > 20 000 IU/ml. The levels of HBV DNA have to be monitored on a close and regular basis in order, not to mistaken patients with active hepatitis for inactive carriers, especially in patients with HBeAg negative chronic hepatitis. In an inactive carrier, ALT usually remains normal on serial monitoring with undetectable to low levels (i.e., < 2000 IU/ml) of HBV DNA but the same can occur in a patient with HBeAg-negative CHB [20]. As the understanding of the complex problem that HBeAg negative chronic hepatitis B represents grows, it is understood that HBV DNA is not always a useful indicator for treatment decision. A Chinese study, involving 165 patients, reported that a single HBV DNA measurement misdiagnosis 45% HBeAg-negative CHB as chronic inactive HBsAg carriers. The study further revealed that even HBV DNA measurement on 3 separate occasions also misdiagnoses 30% cases [30]. Moreover, a study of 196 CHB patients revealed that 10.5% HBeAg-negative CHB patients had HBV DNA < 30,000 copies/mL [31].

Evaluation of the level of quantitative hepatitis B surface antigen (qHBsAg) reflects the amount of transcriptional activity of cccDNA (covalently closed circular DNA) and the integrated DNA in the hepatocytes representing one of the main serologic markers in chronic HBV infection; accurately monitoring both disease progression and prognosis as well as response to antiviral therapy [32], [33] At the same time, the correlation between the serum levels of HBsAg and HBV DNA improves and helps in better understanding and following the phases and outcome of CHB during its natural history and treatment as well [34].

In our cohort, the patients with HBeAg-negative CHB had significantly higher values of qHBsAg compared to patients IC. These findings correlate with the studies found in the literature. The study of Zhu et al., [35], included 124 patients with chronic hepatitis B and demonstrated that there is a correlation between HBV DNA and qHBsAg and that the serum level of HBsAg reflects the amount of HBV DNA replication. The serum levels of HBsAg were significantly higher in patients with HBV DNA > 1×10^3 cp/ml compared to patients with HBV DNA level < 1×10^3 cp/ml ($t = 5.983$, $p = 0.000 < 0.05$). Based on the HBV DNA level, the patients were divided into three groups: group A (HBV DNA level between 1×10^3 and 1×10^5 cp/ml, group B (1×10^5 cp/ml till 1×10^7 cp/ml) and group C (> 1×10^7 cp/ml). It was shown that the level of HBsAg increased with the level of HBV DNA and that it was higher in-patient group C Pearson's correlation ($r = 0.657$, $p = 0.000 < 0.05$) showed that there is a positive correlation between serum HBsAg and HBV DNA.

In many centres which do not have molecular biology testing, and in practical terms, ALT levels are used to predict the presence of viral replication and progression of liver damage. Our study showed that the increase of the serum level of HBV DNA is followed with a non – significant decrease of ALT both in IC and in patients with AH, while the increase of the level of quantitative HBsAg is followed with the increase of the level of ALT in both groups of patients. In our study, the influence of qHBsAg on values of ALT was significantly stronger than that of HBV DNA. The research done on patients with HBeAg negative chronic hepatitis have already demonstrated the weak correlation between viral load and transaminases [6]. The study of Kim et al., [36] showed that correlation of qHBsAg with ALT and HBV DNA can better predict the liver (dis)function and that at the same time this correlation can be used to discriminate between patients' inactive carriers and patients with HBeAg negative chronic hepatitis B.

As it has been said, the assessment of viral load is not a sufficient factor for treatment decision, and additional factors such as histological factors (fibrosis/cirrhosis and liver inflammation), patient age, disease evolution over time, family history of HCC, have to be taken into account for treatment decision. The most recent international guides also point out the need for multiple clinical applications, with repeated measurements of transaminases and HBV-DNA for the determination of the phases of the disease and better management of the infected patient [11], [14], [15].

Our study showed that both groups of patients, the inactive carriers and patient with active HBeAg negative chronic hepatitis have similar laboratory and identical serology profile and that it is difficult to determine who to treat based on the level of HBV DNA measured at one point of time. The presence of a positive correlation between the levels of qHBsAg and ALT may suggest the presence of more advanced liver disease and active HBV DNA replication, which can be taken into consideration for treatment decision. An especially interesting group of patients in our study are the patients who have normal ALT values and HBV DNA level above 2000 IU/ml, but less than 20 000 IU/ml, which in our cohort present 50.94% of patients with chronic HBeAg negative hepatitis. The decision of when to start antiviral treatment will be the most difficult in this set of patients.

The primary limitations of our study were the adoption of the same ALT reference value for male and female patients and the absences of the histological staging of the liver disease.

In conclusion, determination of the phases of chronic hepatitis B in patients who are HBeAg negative, anti HBeAg positive is of enormous clinical importance in order to avoid misclassification of patients inactive carriers with patients with HBeAg-negative CHB since laboratory and virology analysis were taken at one point of time can show normal transaminase activity and undetectable to low HBV DNA viraemia due to the typical intermittent profile of HBeAg negative chronic hepatitis B.

In conformity with literature, the results of our study suggest that due to the dynamism of the chronic infection by HBV, the infected patient should be continuously and carefully evaluated with a joint analysis of the clinical, serologic, biochemical, molecular biology, and sometimes histologic parameters in order to make a timely and proper decision when and who to treat. Proper and timely initiation of antiviral therapy will prevent the development of chronic HBV associated complications, and reduce the overall morbidity and mortality of these patients.

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The Correlation between Levels of Transforming Growth Factor-β with Pulmonary Fibrosis in Post Pulmonary Tuberculosis in Medan, North Sumatera – Indonesia

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Keywords: TGF-β; Post-pulmonary tuberculosis; Lung fibrosis

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Abstract

BACKGROUND: Untreated or undertreated, pulmonary tuberculosis could cause severe complications until death. After treatment, residual lesions might occur. The presence of residual lesions is varied, including fibrosis, cavity, bronchiectasis and calcification. Transforming growth factor-β (TGF-β) is a cytokine associated with lung inflammation, which plays a role in lung fibrosis. However, only a few studies have assessed the serum level of TGF-β in post-treatment tuberculosis patients.

AIM: The main objective of this study was to determine the correlation between TGF-β levels and pulmonary fibrosis in patients with pulmonary tuberculosis.

DESIGN: A group of 51 patients that had undergone anti-tuberculosis treatment were observed, consisting of 31 men, 20 women. Of all patients, there were 26 people with a smoking history, including 25 men and 1 woman. All patients had been recovered, confirmed by the clinical state, laboratory and radiology examination. The ELISA test was performed to measure TGF-β level, while the chest X-ray was used to look for the occurrence of pulmonary fibrosis.

RESULTS: The mean level of TGF-β in patients with a lesion (+) was 7628.02 (SD: ± 4928.38) while the mean level of TGF-β in patients with a lesion (-) was 2315.11 (SD: ± 505.83). The statistical test showed a significant relationship between TGF-β level and fibrosis lesion ($p < 0.001$).

CONCLUSION: TGF-β level was significantly higher in post-tuberculosis patients with pulmonary fibrosis.

Introduction

Tuberculosis (TB) is an infectious disease caused by germs from the *Mycobacterium tuberculosis*. Pulmonary TB is a type of TB infections which invade the pulmonary parenchyma, including respiratory bronchiole and alveolus [1]. According to the Global Tuberculosis Report 2016, Indonesia is the second country with the highest incidence of pulmonary TB after India and followed by China [2].

TB patients who are recovering from TB usually have residual lesions and sign and symptoms similar to chronic obstructive pulmonary disease (COPD) such as shortness of breath during activity [3], [4]. In a case series study, it showed that in patients with post pulmonary TB, some of them develop a reduction of lung functions, with the majority of patients had a moderate obstruction and mild restriction [3]. TB sequelae is a condition of which there is permanent deformity after TB treatment. TB sequelae may appear in lung and extra lung. Lung fibrosis is one of the variations in residual lesions that occur in the lung parenchyma [5], [7].

To find out the process of fibrosis formation, recent studies had discussed the molecular level of lung fibrosis of which several inflammatory cytokines were found to be involved in defence mechanisms against bacterial infections, including pro-inflammatory cytokines such as interferon-γ (IFN-γ), and anti-inflammatory cytokines such as Tumor Necrosis Factor Alpha (TNF-α), and Transforming Growth Factor – β (TGF – β) [4], [6], [7]. TGF – β is a cytokine that plays an important role in cell proliferation and differentiation. TGF-β has been recognised as the suppressor in the immune system and stimulator in

the pathogenesis of pulmonary fibrosis. Thus excessive production of TGF-β can cause the excessive deposit of scar tissue and fibrosis [7], [8], [9].

Smoking is one of the aggravating factors in forming lung fibrosis. Cigarette smoke can induce pro-inflammatory and immunosuppressive cytokines that stimulate fibrogenesis in post-pulmonary TB. A study showed the increased level of TNF-α could decrease the lung functions in post-pulmonary TB [4].

The studies of TGF-β level in post-TB patients have not been widely conducted in Indonesia. Figen Devici et al. found that inactive TB patients, inactive TB and healthy people, TGF-β level were 258.1 ± 306.6, 185.1 ± 216.5, 110.3 ± 83.2 [10]. A Study about the correlation between TGF-β level and pulmonary fibrosis in post-TB patients has never been conducted in one of the provinces in Sumatera. These facts interest the authors to conduct a study about the relationship between TGF-β level and pulmonary fibrosis in post-TB-patients.

Material and Methods

Study population

This study was an analytical study with cross-sectional design and conducted in Haji Adam Malik General Hospital, Medan, North Sumatera.

Inclusion Criteria

1. Patients with pulmonary TB who had been recovered, confirmed by negative smear examination in the sixth month of treatment.

2. Patients aged over 17 years.

3. Willing to follow the study after receiving information about this study (written informed consent).

Exclusion criteria

1. Patients diagnosed with asthma; and/or

2. Patients suffer from diseases that can interfere with lung function.

Procedures

After had been contacted via mobile phone, all participants were informed about the content of the study, and if they were willing to participate, they were asked to come to the hospital. Then, they needed to sign the informed consent and were interviewed about the history of TB. TGF-β examination was conducted from a vein blood sample, processed with Enzyme Link Immunosorbent Assay (ELISA) by using TGF-β kit. Pulmonary fibrosis was confirmed by Chest X-Ray.

Statistical Analysis

All data were analysed using SPSS version 23. Then, they were analysed if they were normally distributed by Kolmogoroff-Smirnov. In this data, Independent T-test was used to determine the significant correlation between TGF-β levels and pulmonary fibrosis. P-value < 0.05 considered to be significant.

Results

This study involving 51 subjects who matched inclusion criteria and did not have any exclusion criteria. The study samples were post-TB patients and were grouped based on gender, age, smoking history, Brinkman-Index, level of education, occupational, and fibrosis lesson (Table 1).

Table 1: Characteristics of study subjects based on gender, age, smoking history, Brinkman Index, level of education, occupational, and fibrosis lesson

Variable		N (%)
Gender		
Male		31 (60.80)
Female		20 (39.20)
Total		51 (100)
Age		
15-30 Years		16 (31.4)
31-45 Years		12 (23.5)
46-60 Years		22 (43.1)
61-75 Years		1 (2.0)
Total		51 (100)
Average Age (Year)	40.75 ± 14.3	
Smoking History		
Yes		26 (51.0)
No		25 (49.0)
Total		51 (100)
Brinkman Index		
Mild		10 (38.4)
Moderate		10 (38.4)
Severe		6 (23.2)
Total		26 (100)
Level of Education		
< Senior High School		24 (47.1)
Senior High School		16 (31.4)
Diploma		4 (7.8)
Bachelor		7 (13.7)
Total		51 (100)
Occupational		
Indoor		29 (56.9)
Outdoor		22 (43.1)
Total		51 (100)
Fibrosis Lesion		
Yes		42 (82.4)
No		9 (17.6)
Total		51 (100)

From the table, we showed the majority of participants were men consisted of 31 people (60.80%), while the female 20 (39.20%). Based on age, the highest age group was the age group of 46-60 years, which consisted of 22 people (43.1%), with an average age of 40.75 years. Study subjects who had a smoking history were 26 people (51.0%), with mild and moderate Brinkman Index in 10 people

(38.4%), respectively and severe Brinkman Index in 6 people (23.2%) and subjects who had no smoking history were 25 people (49.0%). The characteristics of the study subjects based on the presence of fibrosis lesions, there were 42 subjects (82.4%) with fibrosis lesions on the chest X-ray and 9 subjects without fibrosis lesions in the chest X-ray (17.6%).

Table 2: TGF-β level of study subject

Variable	Min - Max	Mean	SD	Median
TGF-β (pg/ml)	692 – 19100	6690.45	4913.46	5190.00
Smoking History (+)	692 – 16700	6621.50	4856.77	5370.00
Smoking History (-)	1590 - 19100	6762.16	5070.95	4780.00

Table 2 shows that TGF-β level in the study subjects obtained an average level of 6690.45 pg/ml ± 4913.46 pg/ml, median 5190 pg/ml, with a minimum value of 692 pg/ml and maximum value 19100 pg/ml. The study subjects with smoking history status obtained an average level of TGF-β were 6621.50 pg/ml ± 4856.77 pg/ml SD, median 5370.00 pg/ml, and a minimum value of 692 pg/ml and a maximum of 16700 pg/ml. In the study subjects without smoking history status, the average level of TGF-β was 6762.16 pg/ml ± 5070.95 pg/ml SD, median 4780.00 pg/ml, and a minimum value of 1590 pg/ml and a maximum of 19100 pg/ml.

Table 3: Correlation between TGF-β level and pulmonary fibrosis

Fibrosis Lesion	TGF-β Level				
	Mean	SD	Median	Min-Maks	p-value
(+)	7628.02	4928.38	7185.00	692-19100	0.001*
(-)	2315.11	505.83	2177.00	1620- 3430	

*Significant with the independent t-test correlation test.

The correlation between TGF-β level and the presence or absence of pulmonary fibrosis in a chest X-ray can be seen in Table 4. Based on the independent t-test, the p-value was 0.001. This result indicated that there was a significant correlation between TGF-β and the presence of pulmonary fibrosis in a chest X-ray as indicated by the presence or absence of lesions in post-TB patients ($p < 0.05$). In other words, the value of TGF-β was significantly related to the presence of fibrosis lesions.

Discussion

In this study, the majority of the subject was male (n = 31, 60.80%). This is in line with a study conducted by Adhanta SR et al., (2009), Chung et al., (2011) and Manji et al., (2016) which found that male gender is more common in TB cases [11], [12], [13]. In this study, the majority of participants was in the age group 46-60. According to the Indonesian Ministry of Health for the Prevention of Tuberculosis 2016, the most vulnerable groups of TB infections were young and productive age group [14]. On the other hand, based on 2013 Foundational Health Research in Indonesia, the age group > 45 years old has a higher prevalence among other groups [15].

This study found that 42 subjects (82.4%) had fibrosis lesions on chest X-ray. The fibrosis lesions also presented in the chest X-Ray of patients that had been recovered from TB. The presence of sequelae lesions is varied. Menon et al., (2015) found 40.36% of patients had residual lesions, including: fibrosis (38.7%), cavity (21.4%), bronchiectasis (4.3%) and calcification (3%) [16]. The presence of these fibrosis lesions correlates with TGF-β levels. Based on the Independent T-Test, the p-value was 0.001. This result indicated that there was a significant correlation between TGF-β level and the presence of pulmonary fibrosis in chest X-ray ($p < 0.05$).

TGF-β plays a role in growth regulators and cell differentiation. TGF-β is produced as an inactive form in part of a larger molecule that requires an acid or enzymatic process to activate. TGF-β is present in various tissues and interacts with specific cell membrane receptors [17], [18].

The importance of TGF-β in causing and maintaining fibrosis was seen in high levels of TGF-β in various fibrosis diseases as shown by few studies [19], [20]. This positive feedback process could explain the important role of TGF-β in promoting, strengthening and perpetuating fibrosis. Therefore, a potential therapeutic target for fibrosis might involve disconnecting one of the links with TGF-β induction found in myofibroblasts [19], [20].

TGF-β contributes to the differentiation of myofibroblasts by stimulating the expression of α-sma. TGF-β also activates myofibroblasts and then produce greater amounts of Extracellular Matrix protein, stimulating chemotaxis migration of fibroblasts to the inflammation site, and increasing myofibroblast ability to contract. In addition to increasing α-sma expression, TGF-β induces stress fibre formation and adhesion complex maturation. TGF-β also induces the biochemical release of contraction of stress fibres. In the other study, TGF-β also acts as the main mediator of EMT, providing a further source for myofibroblasts. This explains the TGF-β involvement in the pathogenesis of fibrosis found in various organs and tissues. TGF-β is considered a "major switch" in fibrotic processes, plays a role in parenchymal and interstitial fibrosis, as well as vascular remodelling. Thus the therapeutic target in diseases with fibrogenesis should target the TGF-β pathway. Many attempts have been made to block TGF-β. Pirfenidone, recently approved for the treatment of IPF, weakens the production and work of TGF-β, albeit with an unclear mechanism [21], [22], [23], [24].

In conclusion, there is a correlation between TGF-β level with the presence of pulmonary fibrosis in chest X-ray in post-pulmonary tuberculosis patients.

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Control of the Transdermal Delivery Process of Active Substances of the Phytocomplex during Phonophoresis in Model Experiments

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Keywords: Phonophoresis; Transdermal delivery; Plant extract; Flavonoids

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Abstract

BACKGROUND: The scientific substantiation for the selection of therapeutically significant dosage of phytocomplex in the dosage form for phonophoresis, control over the delivery of active substances into the body, and what affects this process require the study of the kinetics of phytocomplex flavonoids delivery during phonophoresis.

AIM: The aim was to study the possibilities of controlling the process of transdermal delivery of phytocomplex active substances (flavonoids) during phonophoresis in vitro model experiments.

METHODS: Working compositions with different concentrations of phytocomplex for phonophoresis were used. The content of flavonoids in the compositions was determined using the spectrophotometric method and was calculated equivalent to quercetin, the flavonoid prevailing in the phytocomplex. The study of the kinetics of flavonoids delivery from working compositions was carried out using Franz diffusion cells and Carbosyl-P membranes. The authors determined the main parameters of the process and established the dependence of the delivery rate of flavonoids on their initial concentration in the working composition. The authors studied the effect of dimethyl sulfoxide and the base-forming substances of the working composition on the kinetics of phytocomplex flavonoid delivery during phonophoresis.

RESULTS: The authors recorded an increase in the rate of delivery of the active substances from working compositions containing dimethyl sulfoxide into the model medium by almost 1.5-2 times during the first ten minutes of the experiment (approximate duration of the phonophoresis procedure). The authors proposed technological techniques for improvement of the phonophoresis method for the phytocomplex. The possibilities of control over the process of transdermal delivery of the phytocomplex active ingredients during phonophoresis in vitro model experiments were shown.

CONCLUSION: The obtained results provide information for further pharmacological studies of the nature and mechanism of the effect of phytocomplex flavonoids during phonophoresis in the rehabilitation of patients with osteoarthrosis.

Introduction

One of the promising trends in modern physiotherapy is the creation of new, more effective pharmaco-physiotherapeutic methods for the rehabilitation of patients, including the use of herbal drug products [1], [2], [3], [4], [5], [6]. When developing new methods, ensuring control over the delivery of active substances into the body and the ability to influence and control this process is crucial [7], [8], [9], [10], [11], [12]. Duration of physiotherapeutic procedures is limited; therefore, it is necessary to quickly and fully utilise the resources of the drug for its rational use and maximum therapeutic efficacy.

This paper shows the possibility of regulating the process of transdermal delivery of active substances by the example of phonophoresis of phytocomplex in the rehabilitation of patients with osteoarthrosis. Previous studies have shown the need for improved technology and new, more effective phytocomplex dosage forms for phonophoresis [13], [14]. The study of the transdermal delivery of the phytocomplex active substances is also needed for the possible industrial production of a new drug based on the phytocomplex in the form of an ointment or gel suitable for phonophoresis. This allows for wider use of a new pharmaco-physiotherapeutic method.

The proposed phytocomplex is a dry extract from grass and roots of marsh cinquefoil, grass or alfalfa, and multiple fruits, or cones, of common hop

(TOR 9375-021-00003938-11 “Marsh cinquefoil, alfalfa, and common hop dry extract (phytocomplex)”) [14]. It contains flavonoids, polysaccharides, coumestans, tannins, essential oils, phenol carboxylic acids, macro- and microelements, and vitamins [15]. The main active ingredients of the phytocomplex are flavonoids, which have anti-inflammatory, analgesic, antioxidant and other effects. This stipulates the possible use of the phytocomplex in medicine for inflammatory and degenerative diseases of the musculoskeletal system, including osteoarthritis.

Currently, there are no systematic works in the literature devoted to the penetration of flavonoids from the medications through the skin during phonophoresis.

The study aimed to study the possibilities of controlling the process of transdermal delivery of the active ingredients (flavonoids) of the phytocomplex during phonophoresis in *vitro* model experiments.

Material and Methods

Phytocomplex working compositions with concentrations of 5% (W1), 10% (W2) and 15% (W3) were used for phonophoresis in the study. The following substances were used as base-forming substances for the preparation of 10% working composition: Carbopol 974P (Carbomer 974P, BufferGel) (C974P, Noveon Inc.) (W2) [16], [17]; ointment composition with 48.5 g of vaseline (FS.2.2.003.15, State Standard 3582-84), 15.0 g of anhydrous lanolin (TOR 9154-015-00333865-05), 1.5 g of distilled monoglycerides (TOR 9145-357-00336444-2005), and up to 100.0 g of purified water (FS.2.2.0020.15, State Standard 6709-72) (W4); ultrasound gel – Ecoultragel (Pirrone & Co. S.p.A., Italy) (W5). Analysis of the rheological characteristics of the working compositions was carried out using a Rheotest rotary viscometer (RN 4.1 modification, RHEOTEST Messgerate Medingen GmbH, Germany).

Previously it was established that the main active ingredients of the phytocomplex are flavonoids, and the working compositions are resistant to exposure to ultrasound with an intensity of 0.1-1.0 W/cm^2.

The content of flavonoids in working compositions was determined using the spectrophotometric method [15] and calculated equivalent to quercetin (Q 0125, Sigma), the flavonoid prevailing in the phytocomplex. To do this, an exact weight of the working composition (about 1 g) was placed in a flask with a capacity of 150 ml, 30 ml of 70% ethanol was added, the flask was connected to a reflux condenser and heated in a boiling water bath for 30 minutes. Then the flask was cooled to room temperature, and its contents were filtered through a filter paper into a 100 ml volumetric flask. The extraction was repeated twice, with 70% ethanol for 30 minutes. The extracts were filtered into the same volumetric flask, the filter was washed with 70% ethanol, and the filtrate was brought to the mark with a solution of 70% ethanol (solution A). In a volumetric flask with a capacity of 25 ml, 2 ml of solution A was added and brought to the volume of the solution to the mark with 70% ethanol (solution B). Studies were performed using a Titrtek MCC 1340 spectrophotometer (Finland) at a wavelength of 370 nm. The absorption spectra of the working compositions and quercetin were previously investigated. It was established that the base-forming substances do not shift the maximum optical density of quercetin, based on the intensity of which photometry was carried out.

The content of flavonoids was 0.35 ± 0.01% in W1, 0.70 ± 0.02% in W2, 1.04 ± 0.03% in W3, 0.72 ± 0.03% in W4, and 0.69 ± 0.02% in W5.

The study of the kinetics of the transdermal delivery of flavonoids from working compositions during phonophoresis performed by diffusion through a semipermeable membrane in Franz diffusion cells (# 4G-01-00-09-05, SES GmbH-Analysesysteme, Germany) in a V6-SFCS system using Carbosyl-P membranes (TOR 66-2-512-92). During phonophoresis, penetration of flavonoids through the skin follows the Fick's law, according to which the flux of the particles *(I)* diffusing through the plane perpendicular to the direction of diffusion is directly proportional to concentration gradient *(dc/DX)*:

$$I = -D(dc/dx), \quad (1)$$

where *D* is the diffusion coefficient.

The second Fick's law is usually used for analysing the majority of diffusion experiments:

$$dc/dt = D(d^2c/dx^2) \quad (2)$$

From equation (2) it follows that the change in the concentration over time *(dc/dt)* at a distance *x* from the initial plane is proportional to the rate of changing the concentration gradient towards *x* at moment *t*. For the practical use, equation (2) should be integrated under appropriate boundary conditions [18], [19].

Sodium hydroxide solution, 0.1 n. (quercetin is very poorly soluble in water) And 0.9% sodium chloride solution (physiological solution) were used as model media. Phonophoresis was carried out using the labile contact technique in a continuous mode with ultrasound intensity of 0.6 W/cm^2 using the UZT-1.07F device (Maloyaroslavets instrument factory, Russia). Samples were taken at certain time intervals with a complete replacement of the model medium (this system can be considered as a flow system at a first approximation) and with taking 4 ml samples with their subsequent return and addition of the original medium

to maintain required volume if necessary (closed system).

Statistical processing of the results was carried out using SPSS Statistics v17 Multilingual-EQUiNOX (SPSS Inc.) software.

Results

We studied the influence of the base-forming substances in the working composition on the delivery of flavonoids into 0.1 n. Sodium hydroxide solution in a flow system during phonophoresis. It was found that, during the first ten minutes of the experiment (the approximate duration of the phonophoresis procedure), more than 5% of flavonoids diffused into the model medium from the working composition W2, about 4% from the composition W4 and less than 3% from the composition W5. According to the results of the organoleptic control, the drying-up testing, the pH value determination and the thermal stability, the compositions with the best properties were W2 and W4. Analysis of the rheological characteristics of the working compositions demonstrated that the working composition W2 is optimal for the use in ultrasound therapy, it can be used with the prolonged application to the skin, it is sufficiently stable over time, which facilitates the preservation of the good quality of the drug during storage. Therefore, we used a basis with Carbopol to prepare the working compositions W1, W2, and W3 for further research.

The study of the kinetics of the flavonoids delivery from working compositions with different phytocomplex concentrations into 0.1 n. Sodium hydroxide solution in a closed system during phonophoresis showed that within four hours of the experiment approximately 43% of flavonoids diffused into the model medium from the working composition W1, about 26% – from composition W2, and about 20% – from composition W3 (Figure 1).

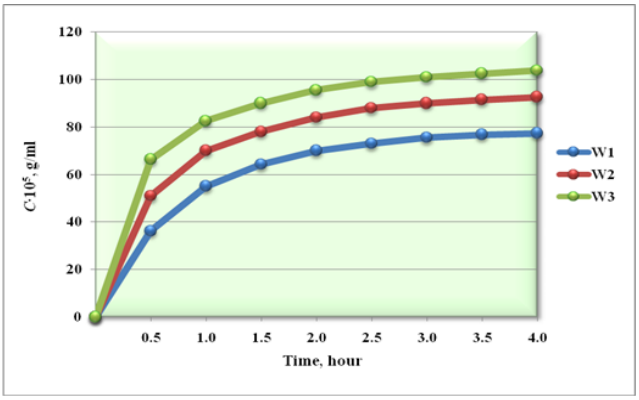

Figure 1: Kinetics of flavonoids delivery from the phytocomplex working compositions into 0.1 n. sodium hydroxide solution through Carbosil-P membranes when the equilibrium was reached in a closed system at 23°C during phonophoresis (C is the concentration of flavonoids in the model medium)

The equilibrium was reached in 3.5-4 hours. "Time lag" was 2.5-3 minutes. The flavonoids delivery rate from working compositions with different phytocomplex concentrations was directly proportional to the initial active substances concentrations in the compositions at the beginning of the experiment and sharply decreased by the time the equilibrium was reached.

It is known that the time lag has a significant effect on the diffusion process of substances into the model medium through a membrane. The diffusion coefficient (D) is calculated using the formula:

$$D = l^2 / 6\Theta,$$

where l is the film thickness, Θ is the time lag.

For comparison, we determined the time lag for the flavonoid's delivery from the composition W2 into 0.1 n. Sodium hydroxide solution without exposure to ultrasound – it was 3.5 minutes. Increasing the temperature of the applied working composition to 42°C reduced the time lag to three minutes. For rational use of the phytocomplex and increased therapeutic efficacy of the method, it is advisable to apply warm (40-42°C) working composition to the skin surface in the affected joint area, leave it for three minutes and then expose to ultrasound.

Periodic replacement of the model medium (flow system) allowed us to obtain a broader picture of the kinetics of the flavonoids delivery from working compositions with phytocomplex during phonophoresis — the maximum rate of flavonoids delivery from the composition W1 into 0.1 n. Sodium hydroxide solution was achieved after 30 minutes of the experiment, from compositions W2 and W3 – after 20 minutes (Table 1).

Table 1: Kinetics of flavonoids delivery from phytocomplex working compositions into 0.1 n. sodium hydroxide solution through Carbosil-P membranes inflow system at 42°C during phonophoresis

Time	Rate (V) and proportion (P) of flavonoids delivery					
	5% phytocomplex working composition (W1)		10% phytocomplex working composition (W2)		15% phytocomplex working composition (W3)	
	$V \cdot 10^5$, g/ml·h	P, %	$V \cdot 10^5$, g/ml·h	P, %	$V \cdot 10^5$, g/ml·h	P, %
10 min	60.9 ± 0.2	5.6	114.7 ± 0.3	5.3	147.9 ± 0.4	4.7
20 min	81.8 ± 0.3	13.2	141.1 ± 0.6	11.8	178.2 ± 0.5	10.5
30 min	94.0 ± 0.3	21.9	136.1 ± 0.4	18.1	154.8 ± 0.6	15.4
40 min	71.1 ± 0.2	28.5	130.9 ± 0.5	24.2	145.1 ± 0.5	20.1
1 h	55.3 ± 0.2	38.7	106.2 ± 0.5	34.0	125.6 ± 0.5	28.1
2 h	39.9 ± 0.2	60.9	78.8 ± 0.3	55.9	113.9 ± 0.4	50.0
4 h	18.1 ± 0.1	81.0	38.0 ± 0.2	77.0	62.3 ± 0.4	74.0
6 h	9.2 ± 0.1	91.3	19.2 ± 0.1	87.7	28.7 ± 0.2	85.0
8 h	4.4 ± 0.1	96.2	9.3 ± 0.1	92.9	14.1 ± 0.1	90.5

To increase the delivery rate of flavonoids, during the first ten minutes of the experiment, dimethyl sulfoxide (DMSO) was added into the working composition W2 with 10% and 15% DMSO concentrations were chosen also due to anti-inflammatory, analgesic and antimicrobial effects. A significant effect of DMSO on the delivery of flavonoids during the first several minutes of the experiment was recorded. For example, after ten

minutes of the experiment, the rate of flavonoids delivery from the working composition W2 with 10% of DMSO increased by more than 1.5 times, and 15% DMSO concentration resulted in an almost twofold increase (Table 2). The time lag was 2-2.5 minutes.

Table 2: Kinetics of delivery of flavonoids from working compositions containing DMSO, in 0.1 n. sodium hydroxide solution through membranes Carbosil-P inflow system at 42°C with phonophoresis

Time	Rate (*V*) and proportion (*P*) of flavonoids delivery					
	W2 + 10% of DMSO		W2 + 15% of DMSO		W2 + 10% of DMSO*	
	$V \cdot 10^5$, g/ml·h	*P*, %	$V \cdot 10^5$, g/ml·h	*P*, %	$V \cdot 10^5$, g/ml·h	*P*, %
10 min	173.3 ± 0.5	8.0	273.0 ± 0.7	10.5	216.0 ± 0.5	10.0
20 min	222.0 ± 0.6	18.3	264.8 ± 0.6	24.9	110.2 ± 0.4	15.1
30 min	190.1 ± 0.5	27.1	233.3 ± 0.5	35.7	67.0 ± 0.3	18.2
40 min	159.8 ± 0.4	34.5	183.6 ± 0.5	44.2	45.4 ± 0.2	20.3
1 h	145.8 ± 0.4	48.0	126.4 ± 0.4	55.9	40.0 ± 0.2	24.0
2 h	78.5 ± 0.3	69.8	66.6 ± 0.2	74.4	36.4 ± 0.2	34.1
4 h	31.1 ± 0.2	87.1	28.4 ± 0.2	90.2	26.8 ± 0.2	49.0
6 h	12.8 ± 0.1	94.2	9.5 ± 0.1	95.5	16.9 ± 0.1	58.4
8 h	4.1 ± 0.1	96.5	-	-	5.2 ± 0.1	61.3

*The working composition was exposed to ultrasound (beginning of the experiment) three minutes after it was applied warm (42°C) on the Carbosyl-P membrane. Ultrasound exposure time was ten minutes.

The results for the flavonoids delivery from working compositions based on W2 with 10% and 15% of DMSO into a 0.9% sodium chloride solution also indicated an increase in the phytocomplex active ingredients delivery rate.

An analysis of the rheological properties of working compositions with DMSO showed that composition W2 with 10% of DMSO had optimal plastic viscosity and yield value for ultrasound therapy.

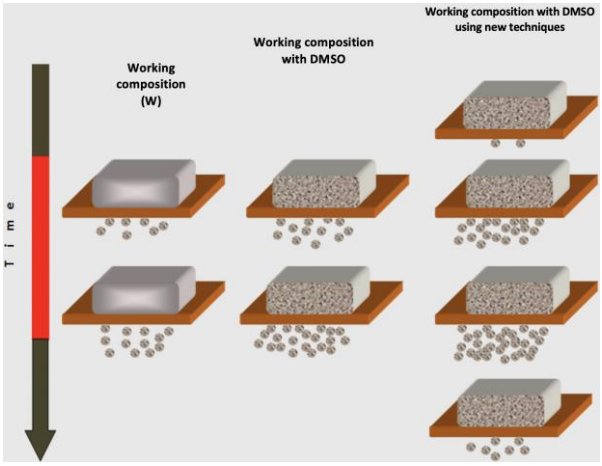

Figure 2: Control of the transdermal flavonoids delivery process from the phytocomplex working compositions during phonophoresis (red colour indicates the time of the phonophoresis procedure)

In order to increase pharmacokinetics measuring parameters of the process and make the use of the phytocomplex more rational, the following techniques were proposed: application of a thin layer of warm (40-42°C) working composition locally to the skin surface in the affected joint area followed by two or three minutes of wait time before the ultrasound exposure using contact labile method with an intensity of 0.6-0.8 W/cm^2 in a continuous mode lasting ten minutes, with working composition subsequently left on the skin for up to 6-8 hours (as an ointment) (Table 2, Figure 2). It should be noted that with the use of this technology, the flavonoids delivery rate from the working composition W2 with 10% of DMSO immediately after exposure to ultrasound is similar to that of composition W2 with 15% of DMSO, and the proportion of active substances delivery at the end of the experiment is almost eight times higher compared to traditional phonophoresis procedure.

Discussion

We presented pharmacokinetics measuring parameters of the transdermal flavonoids delivery process from the phytocomplex working compositions during phonophoresis in *vitro* model experiments: the maximum concentration of flavonoids in model media, the time of achievement of maximum concentration in model media, the rate and degree of release of flavonoids from the dosage form. The optimal dosage of the phytocomplex in working compositions was established – 10%. The main parameters of the process were determined; the dependence of the flavonoids delivery rate on their initial concentration in the working composition was established. The effect of dimethyl sulfoxide and the base-forming substances in the working composition on the delivery of phytocomplex flavonoids during phonophoresis was studied. An almost 1.5-2 times increase in the delivery rate of the active substances from working compositions containing dimethyl sulfoxide into the model medium during the first ten minutes of the experiment (approximate duration of the phonophoresis procedure) were recorded. Technological methods for the increase of the efficiency of the phonophoresis method and rational use of the phytocomplex were proposed. The obtained results give grounds for further pharmacological studies of the nature and mechanism of the effect of phytocomplex flavonoids during phonophoresis in the rehabilitation of patients with osteoarthrosis.

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Basic Science

Correlation between Estrogen Levels with Lipid Profile in Menopause Women in West Sumatera

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Keywords: Menopause; Total Cholesterol; LDL; HDL; Triglyceride; Estrogen

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Abstract

BACKGROUND: Menopause is a condition where women had not experienced menstruation for 12 consecutive cycles. At menopause period, there is alteration in women reproductive cycle where estrogen decreased and lipid status altered.

AIM: This study is aimed to understand the association of estrogen level and lipid status in menopause women.

METHODS: This study was an observational study with cross-sectional design underwent in Obstetrics and Gynecology Polyclinic Dr M. Djamil Hospital, Padang to examine estrogen level and lipid level in 107 menopause women aged between 40-60 years old. The study was conducted for 6 months. The relationship between estrogen level and lipid profile was done using correlation test and p-value < 0.05 is significant.

RESULTS: There was no significant correlation between estrogen and total cholesterol (p = 0.146), LDL (p = 0.496), HDL (p = 0.172) and triglyceride (p = 0.296) in menopause women. There was negative correlation between estrogen with total cholesterol (r = -0.141; p = 0.146), HDL (r = -0.133; p = 0.172) and triglyceride (r = -0.1; p = 0.296) and a little positive correlation with LDL (r = 0.06; p = 0.496). Estrogen levels are not related to total Cholesterol, LDL, HDL, and triglyceride in menopause women

CONCLUSION: Estrogen levels did not correlate with lipid status in menopause women in West Sumatera.

Introduction

Human survival rate recently has been increased, both in a developed country and developing country. In the year 2000, there was 35 million (about 12% of the total population) above 65 years old in America and the year 2030, it was estimated that elderly population in America would reach 70 million [1]. In Indonesia, it was estimated that in the year 2020, there would be 27.08 million population 60 years older [2]. There were decreased biological process in the increased elderly population, wherein women; this would significantly affect the reproductive system among other systems. One of them is menopause with all of its subsequent effect [1].

Menopause is a condition where women had not experienced menstruation for 12 consecutive cycles. Menopause onset is varied in each people, ranging between 45 to 55 years. Menopause could occur because at least one process in menstruation mechanism is impaired [1].

At menopause, there is alteration in women reproductive system. Ovary function decreased due to ageing. This is characterised by depleted total follicle, sclerosing blood vessel and decreased sex steroid synthesis (estrogen). Decreased ovary function would cause impaired ovary ability to answer gonadotropin stimulation, which in turn disrupting hypothalamus-hypophysis-ovary interaction. This situation would increase the Follicle Stimulating Hormone (FSH) dan Lituenizing Hormone (LH) levels. Besides, endometrium would be atrophied due to low estrogen

level [3]. This change caused symptoms in menopause women such as vasomotor problem, somatic complaints such as dyspareunia, hair loss, dry vagina, arthralgia, palpitation, and psychological such as sleep disturbance and insomnia, mood alteration, and cognitive problem [4], [5], [6], [7].

Decreased estrogen also could affect lipid profile in menopause women. Endogenous estrogen has a suppressive effect on lipase activity in the liver; low estrogen level before and after menopause is correlated with high lipase activity in the liver [8], [9]. Subsequently, high lipase activity would cause a decrease in HDL2 cholesterol level and a small increase of solid LDL particle, correlated to the increase of CVD risk [10]. Moreover, estrogen also contributed in the regulation of lipoprotein lipase, and lipoprotein lipase is responsible for hydrolysing TG to chylomicrons and VLDL; Therefore, estrogen decrease during menopause could cause dysregulation of lipoprotein lipase [11].

Several studies showed that lipid levels and body weight was correlated with age and total cholesterol level and low-density lipoprotein cholesterol (LDL-C), particularly in menopause status [12]. Stevenson John C and colleagues found that postmenopause women significantly had higher concentration of total cholesterol ($p < 0.00l$), triglyceride ($p < 0.005$), LDL cholesterol ($p < 0.001$) and HDL 3 cholesterol subfraction ($p < 0.00l$), while HDL cholesterol and HDL2 was significantly lower ($p < 0.001$) [13]. Studies about estrogen level and lipid status in menopause women are scarce, and this study was aimed to analyse the correlation of estrogen level with lipid profile of menopause women in West Sumatera.

Methods

This was an observational study with a cross-sectional study design. The study was conducted in Obstetrics and Gynecology Outpatient Clinic Dr M. Djamil Hospital, Padang to collect samples and Biomedicine laboratory to examine estrogen level and lipid profile. The study was conducted for 6 months. The study population were all menopause women aged between40-60 years old who presenting herself to Obstetrics and Gynecology Outpatient Clinic Dr M. Djamil Hospital, Padang. The study sample was part of population satisfying inclusion and exclusion criteria. Inclusion criteria were patients willing to study sample by writing informed consent by the patient and had no hormone replacement therapy. Exclusion criteria were menopause due to ovary extraction, had severe infectious disease, alcoholic, smoker, and labour/ athlete. The minimum sample was set at 107 women. This study used consecutive sampling technique. The patient had their history taken and explained about the examination, which is the clinical examination of obstetrics and gynaecology. Then blood sample was collected in the morning to examine for study variables, i.e. estrogen and lipid status. Blood collected from a cubital vein and preserved in the laboratory until the total sample had been fulfilled, then be examined collectively. Total cholesterol, triglyceride, HDL and LDL were determined using the ELISA method and estrogen using Elisa Kit (Biovision catalogue #K3829-100). Data were calculated using the statistic method, i.e. mean, standard deviation, normality test using Kolmogorov Smirnoff Test and Linearity Test, and correlation test using Pearson's test and Spearman's test correlation. Univariate analysis was done to assess the mean value of study characteristics. Bivariate analysis was aimed to assess the correlation of estrogen level with lipid level. Data analysis was done using computer statistic program.

Results

Mean menopausal age was shown in Table 1 which was 48.89 ± 3.05 years and mean lipid profile consisted of total cholesterol, LDL, triglyceride and HDL were 247.98 ± 46.30 mg/dl, 146.64 ± 37.68 mg/dl, 128.25 ± 106.63 mg/dl and 62.32 ± 19.98 mg/dl, respectively. Whereas mean estrogen level in menopause women was 24.08 ± 16.71 pg/ml.

Table 1: Mean of Menopausal Age, Total Cholesterol, Triglyceride, LDL, HDL, and Estrogen in Menopause Women

Variable	Variable	Mean ± SD
Menopausal age	years old	49.89 ± 3.05
Total cholesterol	mg/dl	247.98 ± 46.30
LDL	mg/dl	146.64 ± 37.68
HDL	mg/dl	62.32 ± 19.98
Triglyceride	mg/dl	128.25 ±106.63
Estrogen	pg/ml	24.08 ± 16.71

Correlation of menopausal age with lipid plasma in menopause women was shown in Table 2. There was no significant relation between menopausal age with total cholesterol ($p = 0.893$), LDL ($p = 0.801$), HDL ($p = 0.414$) and triglyceride ($p = 0.740$) and there was negative correlation between menopausal age and total cholesterol levels ($r = -0.013$), LDL ($r = -0.025$) and triglyceride ($r = -0.032$) and little positive correlation with HDL level ($r = 0.08$).

Table 2: Relationship of Menopausal Age and Lipid Plasma Level

Variable	Correlation Coefficient	p value
Menopausal age vs Total Cholesterol	-0.013	0.893
Menopausal age vs LDL	-0.025	0.801
Menopausal age vs HDL	0.08	0.414
Menopausal age vs Triglyceride	-0.032	0.740

Correlation between estrogen level and lipid plasma level was shown in Table 3.

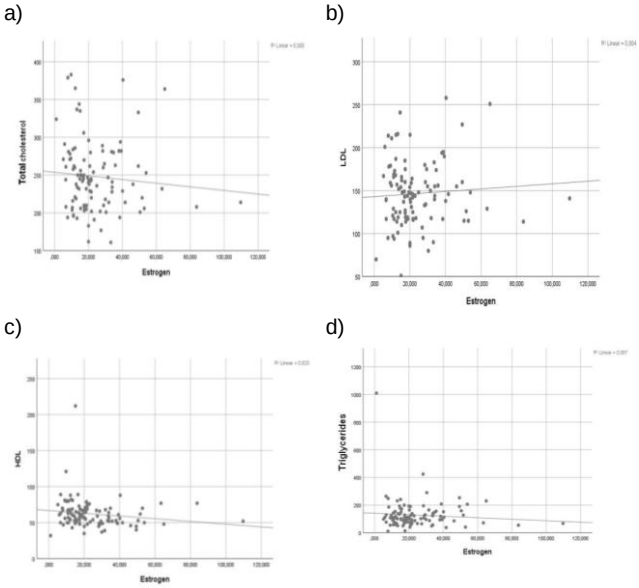

Figure 1: Correlation between estrogen and total cholesterol (a), LDL (b), HDL (c), and Triglycerides (d) concentration in menopause women

There was no significant correlation between estrogen and total cholesterol (p = 0.146), LDL (p = 0.496), HDL (p = 0.172) and triglyceride (p = 0.296) in menopause women (p > 0.05) and there was negative correlation between estrogen and total cholesterol level (r = -0.141), HDL (r = -0.133) and triglyceride (r = -0.1) and small positive correlation with LDL level (r = 0.06).

Table 3: Correlation of Estrogen and Plasma Lipid Level of Menopause Women

Variable	Correlation Coefficient	p value
Estrogen vs Total Cholesterol	-0.141	0.146
Estrogen vs LDL	0.06	0.496
Estrogen vs HDL	-0.133	0.172
Estrogen vs Triglyceride	-0.1	0.296

Discussion

Lipid profile alteration was correlated with cardiovascular risk in menopause women. Increased cholesterol, triglyceride, LDL, apolipoprotein B levels and decreased HDL dan apolipoprotein A levels are the characteristics of menopause women [14]. This study found that there was an increased mean total cholesterol level despite no mean increase of LDL and triglyceride levels and there was no decrease in mean HDL level in menopause women. Prisilia and colleague in 2016 studying 30 menopause women in Manado found that mean total cholesterol, LDL cholesterol was above reference range while mean triglyceride and HDL was according to a reference range.

In this study, there was no correlation between menopausal age with total cholesterol, LDL, HDL and triglyceride (p > 0.05). There were various results for lipid profile study in menopause women. Study conducted by Domenico de Aloysio showed that there was significant increase in total cholesterol (6.9%, P < 0.001), LDL (7.5%, P < 001), and triglyceride (9%, P < 0.002) in post-menopause women compared with premenopause women [15]. Several studies showed that lipid level and weight was correlated with age while total cholesterol level and low-density lipoprotein cholesterol (LDL-C) was associated with menopause status [12]. Women in the transition period of menopause and post-menopause had LDL level (p < 0.002), total cholesterol level (p < 0.0001) and triglyceride (p < 0.004) higher than premenopausal women [9]. While HDL level had no significant difference between premenopausal women, a transitional period of menopause and post-menopause. In this study there was negative correlation between menopause age with total cholesterol level (r = -0.013), LDL (r = -0.025) and triglyceride (r = -0.032) and little positive correlation with HDL level (r = 0.08) [9]. This means that the older onset of menopause the smaller lipid level in the body between women who had early onset of menopause.

This study had no comparison like other studies, i.e. between premenopausal women, transitional period, and post-menopause. LDL and triglyceride levels, which were not increased and HDL level, which was not decreased, could be caused by estrogen levels in menopause women, which was not decreased.

Decreased estrogen level at menopause had affecting lipid profile in menopause women. Endogenous estrogen had a suppressive effect in lipase activity in the liver; low estrogen level before and after menopause was correlated with high lipase activity in the liver [8], [16]. Subsequently, high lipase activity in the liver caused a decrease in HDL2 cholesterol level and a small increase in solid LDL particle, which was correlated with increased CVD risk [8]. Moreover, estrogen also contributed to regulation of lipoprotein lipase, and lipoprotein lipase is responsible for hydrolysing TG to chylomicrons and VLDL [11]; Therefore, estrogen decrease in menopause period could cause dysregulation of lipoprotein lipase. Mean estrogen level in menopause women in this study was 24.08 ± 16.71 pg/ml. This was similar to the study by Ycui, where mean estrogen level was 20.84 ± 29.23 pg/ml [17]. In this study, there was no significant correlation between estrogen and total cholesterol, LDL, HDL and triglyceride (p > 0.05) in menopause women. There was negative correlation between estrogen and total cholesterol level (r = -0.141), HDL (r = -0.133) and triglyceride (r = -0.1) and little positive correlation with LDL level (r = 0.06) (Figure 1).

Swarnalatha PK showed that there was a significant correlation between estrogen level and decreased HDL cholesterol (p < 0.01) in post-menopause women compared with premenopausal

women ($p < 0.01$) [18]. Meslaic L found that estrogen level in menopause women had positive significant correlation with HDL ($p < 0.05$) and negative significant correlation with triglyceride ($p < 0.05$) [14]. G Berg also found negative significant correlation between estrogen and total cholesterol ($r = -0.332$ $p = 0.015$), LDL ($r = 0.301$, $P = 0.023$) [16]. Since this study had a different result with several studies, therefore the future study was needed about lipid profile in premenopausal women, in a transitional period and post menopause and their correlation with estrogen level and another associated hormone such as follicle-stimulating hormone (FSH) and luteinizing hormone (LH).

In conclusion, estrogen levels did not correlate with lipid status in menopause women in West Sumatera.

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Basic Science

Epidermal Growth Factor and Adenosine Triphosphate Induce Natrium Iodide Symporter Expression in Breast Cancer Cell Lines

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Abstract

AIM: This study aims to investigate the effect of ATP, EGF and combination of those two to the Natrium Iodide Symporter (NIS) expression in MCF7, SKBR3 and HaCaT cell lines.

METHODS: MCF7, SKBR3 and HaCaT cell lines were treated with ATP, EGF and combination of those two for 6, 12 and 24 hours. The expression of NIS mRNA was measured through quantitative-reverse transcription-polymerase chain reaction (qRT-PCR). The NIS protein expression was confirmed by immunocytofluorescence.

RESULTS: NIS mRNA was expressed in SKBR3 and HaCaT cell lines but not in MCF7. The levels of NIS mRNA expression, after treatment by epidermal growth factor (EGF), adenosine Tri-Phosphate (ATP) or the combination of both for 6 and 12 hours were not significantly different from those of untreated cells. However, the treatment by a combination of ATP and EGF for 24 hours increases the level of NIS mRNA expression by 1.6 fold higher than that of the untreated cells (1.6241 ± 0.3, $p < 0.05$) and protein NIS expression increase significantly by the treatment than untreated cells ($P < 0.05$).

CONCLUSION: The level of NIS expression varies among the different subtypes of breast cancer cell lines. MCF7 cell line is representing the luminal A subtype of breast cancer does not express NIS. Only SKBR3 cell line express NIS and this subtype might be suitable to receive radioiodine therapy as those cells expressing NIS. A combination treatment of EGF and ATP increases the expression of NIS mRNA and protein at the membrane in SKBR3 cells.

Introduction

Natrium Iodide Symporter is a plasma membrane glycoprotein. It transports two ions of sodium (Na^+) and one of iodide (I^-) and maintained by Na^+/K^+ ATPase [1], [2], [3]. In thyroid cells, NIS plays a pivotal role to accumulate iodine. Additionally, NIS is also expressed in breast cancer tissue and has been considered as a potential target for radioiodine therapy. However, NIS expression in breast cancer tissues had been reported to be varied between studies.

Furthermore, the molecular mechanism of NIS expression in breast cancer remains unclear [4]. Interestingly, it has been reported that NIS is highly expressed in invasive breast cancer tissues. A study by Tazebay *et al.* reported that indeed, there is a correlation between NIS expression and malignant transformation of human breast tissue [5], [6].

Other than the variation of NIS expression level between molecular subtypes of breast cancer, the location of NIS expression is another factor that may influence the uptake of radioiodine by breast cancer cells. Natrium Iodide Symporter supposed to be localised at membrane cell instead of in the cytoplasm to be fully functional as iodine co-transporter [7], [8]. Several agents were used to increasing NIS expression in breast cancer cells, and the effect of those agents had been proven could increase radioiodine uptake and cells susceptibility toward the treatment. A study reported, EGF

increased NIS expression at membrane cell in the T47D cell line [9]. Another study reported that a combination of ATP with all-trans-Retinoid Acid (tRA) and hydrocortisone had increased NIS protein expression and cell membrane targeting in MCF7 cells [10].

In this study, we examined the effect of EGF and ATP for NIS mRNA and protein expressions in SKBR3 and MCF7 breast cancer cell lines and HaCaT a normal cell line. MCF7 and SKBR3 cell lines are representing the luminal A and HER2 type of breast cancer, respectively [11]. HaCaT cell line consists of normal human keratinocyte cells. It is used as a control cell line.

Material and Methods

Cell lines and culture conditions

SKBR3, MCF7 and HaCaT cell lines were used in this study. SKBR3 was obtained from the American Type Culture Collection (ATCC). MCF7 and HaCaT cell lines from faculty of Medicine, Universitas Padjadjaran, Bandung-Indonesia. MCF7 and HaCaT cells were cultured in RPMI 1640 medium (Sigma-Aldrich). SKBR3 was cultured in McCoy's 5A medium (Sigma-Aldrich). Both cell culture mediums were supplemented with 10% fetal bovine serum (Sigma-Aldrich), 1% Penicillin, 1% Streptomycin and 1% amphotericin B. The cells were incubated at 37^{o} C and supplied with 5% of carbon dioxide (CO_2) until 80% confluence. The cells were incubated in serum-free medium overnight and followed by treatment of EGF 50 ng/ml (Abcam #ab9697), ATP 100μM (Sigma-Aldrich # 1388), and a combination of EGF and ATP, for 6, 12 and 24 hours. Each type of cell lines divided into four groups based on the type of treatments, such as ATP, EGF, combination ATP and EGF groups and without any treatment as a control group.

Quantitative real-time reverse transcriptase –PCR (qRT-PCR)

The cells were harvested at the appropriate time points and then followed by centrifuging at 1000 rpm for 4 minutes. The total RNA was isolated by using the RNeasy mini kit (Qiagen #74106) following the manufacturer's instructions. RNA was quantified using Nanodrop 2000, and 5 ng of RNA was reverse transcribed and analysed by one-step real-time quantitative PCR using Rotor-Gene Quantitect probe RT-PCR (Qiagen # 204443) using NIS and beta-actin primers. The mRNA NIS expression fold change of treated cells to those untreated was analysed using a method described by Livak et al., ($2^{-\Delta\Delta C}{}_{T}$) [12]. Three independent qPCR assays were conducted in triplicate.

Immunocytofluoresence

The cells were seeded on coverslips in a well of a 24-wells culture plate and fixated by 4% paraformaldehyde for 15 minutes. This was followed by incubation with protein blocking agent fluorescein-isothiocyanate (FITC) for 15 minutes. The cells were rinsed twice with ice-cold phosphate-buffered saline (PBS) and followed by overnight incubation with 2μg/ml rabbit polyclonal antibody anti-NIS (Abcam, #ab83816) at 4°C. The cells were rinsed three times with PBS and incubated at room temperature with Goat anti-Rabbit IgG secondary polyclonal antibody, with dilution 1:1500 (Abcam, #ab6716) for one hour. The cells were rinsed three times with PBS. The coverslips were placed over slides and mounted with fluoroshield containing 4', 6-diamidino-2-phenylindole (DAPI). The slides were inspected under immunofluorescence microscope (Olympus BX51) with 200 x magnification. Cells that only incubated with secondary antibody were used as a negative control.

Statistical analysis

Collected results are expressed as mean ± SD. Responses of treatments to NIS mRNA expression were calculated in numeric data. They were analysed by ANOVA method to compare means between groups and followed by the Turkey test. $P < 0.05$ was considered statistically significant when compared to controls.

NIS protein expression was analysed using the Her2/neu category. The results were divided based on the level of staining from 0 to 3. Level 0 was no staining, 1 was non-complete staining at the membrane and pale of > 10% tumour cells, 2 was complete staining around the membrane of ≤ 10% tumour cells, and 3 was complete staining at membrane of > 10% tumour cells. Level 0 and 1 were considered as negative, and level 2 to 3 were considered as positive [8], [13]. Mann-Whitney test was used to compare differences between treatment and control groups, $P < 0.05$ was considered statistically significant.

Results

Effect of EGF and ATP treatment on NIS mRNA expression by qRT-PCR

We treat all cell lines with ATP, EGF and the combination of both to induce the level of NIS mRNA expression. The data of NIS mRNA expression was calculated from the cycle threshold (C_T) target toward C_T of reference (beta-actin) and C_T of control genes. The NIS mRNA expression fold change of treated

cells to untreated was analysed.

The expression NIS mRNA is found in SKBR3 and HaCaT cells but not in treated or untreated of MCF7 cells. The levels of NIS mRNA expression in SKBR3 cells after treatment by EGF, ATP or the combination of both for 6 and 12 hours were not significantly different from those of untreated cells. However, the treatment by a combination of ATP and EGF for 24 hours increases the level of NIS mRNA expression by 1.6fold higher than that of the untreated cells (1.6241 ± 0.3, $p < 0.05$) (Figure 1). Furthermore, the treatments in the HaCaT cell line do not change the expression of NIS mRNA in treated and untreated cells.

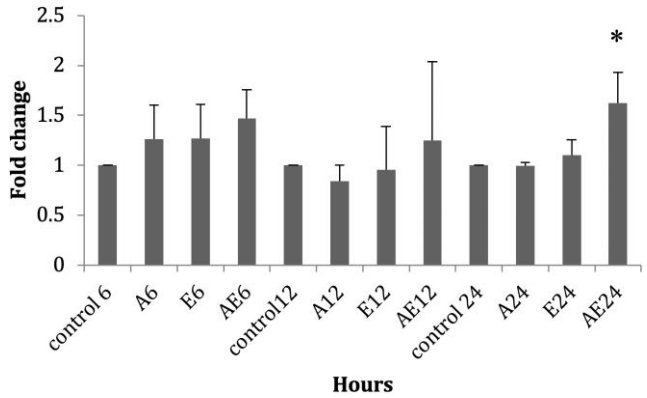

Figure 1: (A) represents the effect of ATP (A); EGF (E) and a combination of ATP and EGF (AE) for 6, 12, 24 hours treatment of SKBR3 cell; The combination of ATP and EGF (AE) for 24 hours treatment increases NIS mRNA expression of SKBR3 cell significantly when compared to untreated (control) cells, P < 0.05

Effect of EGF and ATP treatment on NIS protein expression by immunocytofluorescence

Results of immunocytofluorescence showed that NIS protein express in SKBR3 (Figure 2).

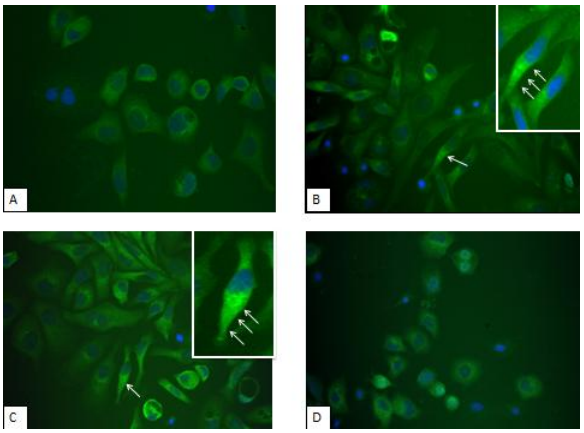

Figure 2: Immunocytofluorescence staining using primary antibody against NIS in the SKBR3 cell line (green) and merging with DAPI staining (blue) at 200 x magnification. The NIS protein expression after 24 hours of treatments with ATP (A), EGF (B) and the combination of both ATP + EGF (C) and untreated (D). The expression of NIS protein increase both in cytoplasm and membrane after 24 hours of treatment by EGF and combination ATP + EGF (arrows)

The NIS expression in those cells is mostly found in the cytoplasm. The treatments of EGF and the combination of both for 12 and 24 hours increase NIS protein expression at membrane cell twofold and threefold respectively, $p < 0.05$ (figure 2B-C). The cells that receive ATP only did not show increasing of NIS protein expression.

Our study shows, NIS protein expression in HaCaT cell line only detects in the cytoplasm. Treatment of ATP, EGF and the combination of both do not induce the NIS protein expression at membrane cell as well as do not increase staining level in the cytoplasm (Figure 3). Furthermore, we cannot detect NIS protein expression in MCF7 cell line in treated and untreated cells.

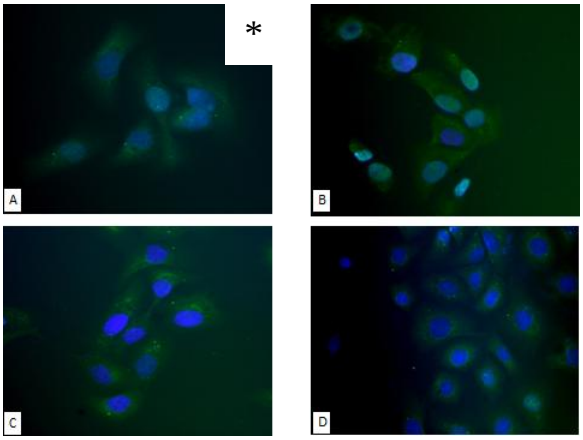

Figure 3: Immunocytofluorescence staining using primary antibody against NIS in HaCaT cell line (green) and merging with DAPI staining (blue) at 200 x magnification. NIS protein expression after 24 hours of treatment by ATP (A), EGF (B) and the combination of both ATP and EGF (C), untreated (D). NIS protein expressions show only in the cytoplasm. NIS protein expressions in a treat and untreated cells do not have a significant different p > 0.05

Discussion

The expression of NIS in extra-thyroid tissues has been reported in some previous studies with varying intensity. These findings have been considered as a potential for radioiodine therapy in the extra-thyroid tumour, including breast cancer [4], [14], [15]. A study by Tazebay et al. reported that NIS was expressed in more than 80% of invasive breast cancer tissues. Another study by Wapnir et al. reported that the expression was found in 76% of invasive breast cancer tissues. Studies by other groups reported that 34% out of 44 breast cancer tissues express NIS. Furthermore, they reported that 65.5% out of 23 triple-negative breast cancer tissues expressed NIS [16], [17], [18], [19].

In this study, we find that NIS mRNA and protein are expressed only in SKBR3 cells, and the expression is not detected in MCF7 cells. Previously published studies on MCF7 cells reported that adding

retinoid acid (RA) is mandatory for NIS expression [10], [19], [20]. Thus, it can safely be assumed that NIS expression in MCF7 is RA-pathway dependent. Till date, the molecular mechanisms of NIS expression in breast cancer are not clearly defined. Meanwhile, it is important to determine the molecular breast cancer subtypes which express NIS as a potential candidate to receive radioiodine.

MCF7 cell line has been established as a breast cancer model for luminal A subtype. It has estrogen and progesterone receptors positive (ER^+/PR^+), HER2 receptor negative (HER2-). Furthermore, the SKBR3 cell line as the HER2 subtype model with estrogen and progesterone receptors negative (ER^-/PR^-) and HER2 receptor-positive ($HER2^+$) [11], [21]. A study by Oh et al. reported that NIS expression is not correlated with the level of hormonal receptors, such as ER/PR. Hormones that regulate NIS expression in lactating breast stage may not play a role in breast cancer [22].

Moreover, a study by Dohan et al. reported that prolactin, oxytocin and estrogen did not induce NIS expression [10]. Instead, another study reported that NIS expresses significantly higher in ER-negative tumour compared to normal breast tissue [15]. Our finding also shows NIS expression is higher in SKBR3 cell compare to MCF7 cell. In this condition, the SKBR3 cell will be appropriate to receive radioiodine therapy. Further studies are needed to examine the effectiveness of radioiodine to breast cancer based on subtypes.

Besides the expression of NIS itself, membrane localisation of the NIS expression also influences radioiodine uptake by the breast cancer cell. The cell will be likely dependent on the level of functional NIS expression at the membrane cell [23]. In vitro study by Elliyanti et al., reported, SKBR3 cell showed higher uptake of radioiodine compare to MCF7 cell [24]. It seems, stimulating of the NIS expression at membrane cell is needed to increase radioiodine uptake. Several agents were used to increasing NIS expressions, such as retinoic acid, hormones and proliferative agents such as EGF and ATP [3], [9], [19], [25]. Binding of EGF with EGF receptor will stimulate the tyrosine kinase activity and will induce cell proliferation [26]. There is a correlation between NIS expression with cell proliferation and malignant transformation in human breast tissue [5], [6]. This leads us to hypothesise that inducing cell proliferation by EGF-EGFR pathway will increase NIS expression at the membrane. In this study, we observed that NIS mRNA expression and protein localisation at membrane cell increase by the combination of ATP and EGF treatment in SKBR3 cells (Figure 2). Treatment of ATP and EGF alone do not increase the level of NIS mRNA; but they increase NIS protein expression at membrane after 12, 24 hours of treatments. Another study reported closed to ours that EGF treatment augmented NIS mRNA and protein expression in T47D cell line, which transfected with NIS [9].

We are unable to detect NIS expression in treated and untreated MCF7 cell. This cell is negative or expresses a low level of EGF receptor [27]. This condition may explain the absence of NIS expression under EGF induction in MCF7 cells. However, EGF can augment NIS expression in MCF7 cells, which are transfected NIS, even though the cells do not have EGF receptors. Further studies are still required to be performed to elucidate the mechanism of NIS expression between breast cancer subtypes.

Additionally, NIS expression was detected in HaCaT cell, even though the expression is within the cytoplasm. Neither ATP nor EGF treatments increase NIS expression in HaCaT cell. Interestingly, radioiodine was taken up by HaCaT cell, but it showed very minimal toxic effect compare to SKBR3 and MCF7 cells [24].

In conclusion, the level of NIS expression varies among the different subtypes of breast cancer cell lines. MCF7 cell line is representing the luminal A subtype of breast cancer do not express NIS as well as SKBR3 cell line. Further studies are needed to cover the differences of NIS expression among breast cancer subtypes. It seems SKBR3 cell which represents the HER2 subtype of breast cancer might be the most suitable to receive radioiodine therapy. In vivo investigations are needed to prove it. Furthermore, a combination treatment of EGF and ATP in SKBR3 cell increases the expression of NIS mRNA and protein at membrane cells.

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Cranioplasty: A New Perspective

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Abstract

AIM: This work aims to present the different indication, benefits, possible complications and methods used for fixation of methyl methacrylate in cranioplasty. Also, 50 cases will be presented demonstrating the different aetiologies of the defects, and the different techniques used for fixation of methyl methacrylate in cranioplasty.

METHODS: This investigation included a prospective study to be carried out on 50 patients with cranial defects of different aetiologies, sites and sizes to be operated upon in Cairo University Hospitals starting from August 2016 to April 2017.

RESULTS: The principal aims of cranioplasty in this study are to restore aesthetic contour and to provide cerebral protection. However, it has been noted that a great improvement occurs in cerebral blood flow and cerebral perfusion after cranioplasty.

CONCLUSION: Ball and socket technique appear to be a simple, safe economic and efficient method for fixation of cranioplasty flap. The high incidence of development of postoperative seroma suggests the necessity of-of a subgaleal drain placement for 48 hours.

Introduction

The repair of skull bone defects is known as cranioplasty. Historically, such procedures date as early as BC 3000, where the Incas used gold to cover cranial defects resulting from trauma. There are several techniques for the repair of the cranial vault defects that can be broadly divided according to the graft used into autologous bone cranioplasty and allograft cranioplasty.

Material and Methods

This was a prospective study carried out on 50 patients with cranial defects of different etiologies, sites and sizes to be operated upon in Cairo University Hospitals starting from August 2016 to April 2017.

Methods

Clinical history was taken. The aetiology of the skull defect was determined from the history given by the patient. General and neurological examination was done to all patients. The general examination includes an examination of the defect to determine the site and size of the defect and to detect any signs of inflammation in the overlying skin. Complete neurological assessment of the patients was done with particular emphasis on motor power.

A preoperative CT scan with a bone window was done for all patients to demonstrate the defect and complementary MRI brain to detect any underlying brain pathology. A preoperative and postoperative CT with 3D reconstruction was done when available for patients with sphenoid wing and orbital lesions.

Operative details including surgical technique, operative time and blood loss.

Inclusion Criteria: - Patients with symptomatic supratentorial skull defects more than 1 cm causing cosmetic problems or large defects causing liability to cerebral injury or syndrome of the trephined; - Cases

of different etiologies of vault defects as neoplastic, traumatic, and inflammatory; - Patients older than 3 years.

Exclusion Criteria: - Patients with skull defects less than 1cm in diameter; - Patients with overlying skin defects that couldn't be approximated with primary sutures; - Patients who are not fit for surgery; - Presence of any sign of infection; - Patients younger than 3 years.

Preparation of the patients

The scalp was scrubbed with disinfectant soap. The entire head was shaved immediately before surgery. Prophylactic 3rd generation cephalosporin was given to all patients, active against Staphylococcus species because the mesh and acrylic are foreign materials.

Surgical Procedures

After induction of general anaesthesia, the head was positioned according to the site of the defect, with the plane of the defect parallel to the ground. Scalp incision was designed to be outside the defect, behind the hairline, never parallel to previous incisions or scars to avoid ischemic necrosis, and with a broad flap base to accommodate the vascular supply to the flap. Proper sterilisation of the skin using povidone iodine was done. Injection of adrenaline 1:200000 (5 mcg: ml) mixed with 10 ml of 0.5% xylocaine to minimise bleeding from the skin was administered. After injection, the wound was re-sterilised. In case of frontal defects (anterior to the hairline), a bicoronal scalp incision was done.

The methods of repair of the defects were methylmethacrylate alone (ball and socket technique). Methylmethacrylate was reinforced with titanium mesh in some of our patients, or fixed with mini plates, and method of repair was randomly selected.

Ball and socket technique

After obvious bone edges were obtained and watertight closure of any dural openings or tears, several notches were buried in the edge of the surrounding cranium, preserving the inner table (Figure 1).

The notches are designed in a way to be slightly wider at the level of the diploe by manual rotation of the burr used in an angulated clockwise manner when the level of the diploe is reached. This notch shape is necessary for fixation of the cranioplasty flap and to prevent flap extrusion being supported by the part of the outer table left above the drilled diploe.

PMMA flap is designed and applied to the cranial defect preserving the normal cranial contour. Overflow of PMMA into the notches ensures solid fixation with the surrounding cranium (Figure 2). No mesh, mini plates, wires or sutures are required.

Figure 1: Notches buried in the margins of the surrounding cranium

Twenty-two cases had a posttraumatic defect in the form of compound depressed fracture 12 cases, intracranial bullet following defect 5 cases and orbital fracture 5 cases. Twenty-one cases had tumour invading bone, one case post infectious with osteomyelitis of the bone flap that is removed and repair was done after 6 months, 3 cases had bony lesion in the form of one case with interosseous haemangioma and 2 cases of aneurysmal bone cyst, 2 cases following acute subdural haematoma treated with decompressive craniectomy with bone flap removal and repair was done after 6 month, and 1 case with fits due to extra-axial lesion with bone hyperostosis treated by excision with skull defect after surgery.

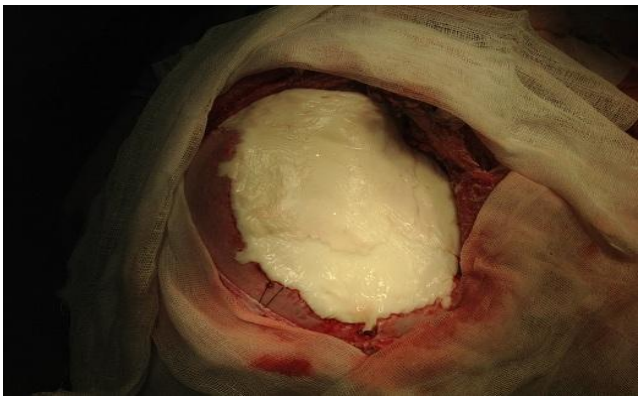

Figure 2: Overflow of PMMA into the notches

Methylmethacrylate was used alone in the repair of the skull defects in 28 of the patients. In these patients, a piece of gel foam and sterile gauze were placed over the dura before placing the methylmethacrylate and moulding it insitu as it was continuously irrigated with saline to protect the brain from the heat produced. The gauze was then removed before fixing the graft in place using ball and socket technique.

In 18 cases, the skull defects were repaired

using methylmethacrylate enforced with titanium mesh, and 4 cases with methylmethacrylate fixed with mini plates (Figure 3).

Figure 3: Intraoperative picture of methyl methacrylate graft enforced with a titanium mesh

Four cases are presenting with a growing skull fracture. Intraoperative, the dural tear was found to be larger than the bone defect, so a craniotomy including the defect was done. Neuroplasty was done using a temporalis fascia graft then repair of the defect was done using methylmethacrylate fixed by mini plates.

Postoperative care

Surgical wound care was done using povidone iodine daily for 10 days. All patients received postoperative antibiotics in the form of third generation cephalosporin (ceftriaxone). Stitches were removed for all patients within two weeks from surgery (stitches were done using prolene sutures and in mattress manner). The drain was left for 48 hours following surgery.

Follow-up

Follow-up CT scan was done two days following surgery. Clinical follow up was done for all patients up to 6 months.

Results

This study included 50 patients with skull defects of different aetiologies and sites.

Age and duration of symptoms

Regarding the age distribution of our cases, it ranged between (3 and 60 years), the largest proportion of cases encountered during 3 rd. A decade of life, twenty cases is constituting 40%. Regarding the duration of symptoms, it ranged from just one month to a maximum of 10 years.

Table 1: Age and duration of symptoms

	mean	Standard deviation	median	minimum	maximum
Age (years)	27.67	18.65	30	3	60
Duration (month)	12.24	16.96	8.5	1	120

Gender distribution

We found that 30 cases were males (60%), and 20 cases were females (40%).

Table 2: Sex of the patients

Sex	Number	Per cent %
Male	30	60%
Female	20	40%

The aetiologies of the skull defects

As shown in Table 3, the aetiology of the skull defect was:

Table 3: Different Etiologies of Cranial Defects

Aetiology	Number of patients	% per cent
Tumour	21	42 %
Infection	1	2 %
Trauma	22	44 %
Fits for 10 years	1	2 %
Bony lesion	3	6. %
Decompressive craniectomy	2	4 %
Total	50	100 %

Twenty-two cases had a posttraumatic defect in the form of compound depressed fracture twelve cases, intracranial bullet following defect five cases and orbital fracture five cases. Twenty-one cases had tumour invading bone (meningioma en plaque), one case post infectious with osteomyelitis of the bone flap that is removed and repair was done after 6 months, three cases had bony lesion one case was interosseous haemangioma, and two cases were aneurysmal bone cyst, two cases following acute subdural haematoma treated with decompressive craniectomy with bone flap removal and repair was done after 6 month, and one case presented with fits due to extra-axial lesion with bone hyperostosis treated by lesion and bone removal.

The sites of the skull defects

As shown in Table 4, 17 cases were with frontal defect due to compound depressed fractures ten cases were on the right side and seven cases were in the left side of which 5 cases with orbital fracture, 21 cases with frontotemporal defect due to meningioma en plaque of which thirteen cases were in the right side and eight cases were in the left side, 10 cases with parietal defect, 7 were in the rt side and 3

were left, and 2 cases with front temporal parietal due to decompressive craniectomy, one in the right side and the other in the left side.

Table 4: Sites of skull effects

Site	Number	Per cent %
Frontal	17	34 %
Parietal	10	20 %
Fronto-Temporal	21	42 %
Fronto tempro parietal	2	4 %

Methods of repair

Twenty-eight cases were done using PMMA by ball and socket technique done, while eighteen patients titanium mesh was used to enforce PMMA and only four cases we used mini plates to fix PMMA cranioplasty flap (Table 5).

Table 5: Methods of repair

Method of repair	Number of patients
PMMA + Ball and socket technique	28
Methylmethacrylate + titanium mesh	18
Methylmethacrylate + mini plates	4
Total	50 patients

Investigations

As regarding investigations 19 cases were diagnosed with MRI pre CT brain pre and post with 3D reconstruction, 20 cases with CT brain pre and post with 3D reconstruction, 9 cases with CT brain pre and post without 3D reconstruction, and 2 cases with MRI pre CT brain pre and post without 3D reconstruction, MRI was used in tumor cases to diagnose soft tissue lesions, and CT 3D was helpful to delineate outline of the flap and to assess in alignment and contour of flap (Table 6).

Table 6. Table of investigations

Investigation	Number	% percent
MRI pre, CT brain pre and post with 3D reconstruction	19	38 %
MRI pre, CT brain pre and post without 3D reconstruction	2	4 %
Ct brain pre and post with3D reconstruction	20	40 %
Ct brain pre and post without 3D reconstruction	9	18 %

Clinical picture

As regarding clinical picture 17 cases present with skull defect, 10 cases presented with proptosis, One case with visual affection due to meningioma en plaque, 10 cases with just headache, 9 cases with swelling either due to growing skull fracture or due to hyperostosis of bone secondary to intracranial lesion, one case with fits due to presence of an extraxial lesion and bone hyperostosis treated by excision of the lesion and bone, one case with disturbed conscious level due to acute subdural haematoma that was treated by decompressive craniectomy and then cranioplasty to repair defect, and one case with post infectious osteomyelitis following previous brain tumor surgery that was treated by debridement and removal of bone and then repair of the defect after six month (Table 7).

Table 7: Table of the clinical picture

Clinical picture	No.	Per cent
Wound infection and cerebritis	1	2 %
Swelling and visual affection	1	2 %
Swelling	9	18 %
Skull defect	17	34 %
Proptosis	10	20 %
Headache	10	20 %
Fits for 10 years	1	2 %
DCL	1	2 %

Complications

As regarding to the complication three cases with infection, one case with swelling and fever patient treated conservatively by broad spectrum antbiotics (intravenous cefoperazone + sulbactame and amikacin for ten days and oral antibiotics in the form of amoxicillin + clavulanate, ofloxacin for two weeks) and passed without need for intervention, two cases were complicated by wound infection and bone cement exposure following meningioma en plaque surgery and needed removal of PMMA and repeated dressing, antibiotics and then treated by PMMA again after 6 months one patient with collection as reaction to bone cement treated consevatively and the collection is absorbed within two weeks, one patient complicated with skin necrosis due to edge of titanium mesh and patient treated by repeated dressing for about two weeks, four cases complaint of skin irritation (unpleasent sensation) due to the edge of mesh and just reassurance, two cases complicated by mesh broken that need replacement by another one, one case complicated by CSF leak that was treated by lumbar drain and medical treatment (cidamex and Lasix), and one case complicated with fracture of bone cement that was fixed with miniplates as he was a child liable for trauma, and this explains why titanium mesh is better for children and was replaced with titanium mesh (Table 8).

Table 8: Table of complications

Complications	Methods used	Number of patients	% per cent
Swelling (subgaleal collection)	Ball and socket technique	1	2 %
Skin necrosis	Titanium mesh	1	2%
Skin irritation	Titanium mesh	4	8%
Mesh broken	Titanium mesh	2	4%
CSF Leak	Ball and socket technique	1	2%
Infection	Ball and socket technique	3	6%
Fracture of MMA	Mini plates	1	2%

The outcome of the patients

About 45 cases (90%) have good outcome cosmetically and functionally, two cases (4%) needed removal of bone cement, they were meningioma en plaque treated with excision of the tumour, orbit decompression and cranioplasty. Later on, they came back within one month with wound infection and one of them with bone cement exposure. Both were treated by debridement, antibiotics, removal of bone cement and cranioplasty again after six months. Three cases (6%) needed replacement by another

prosthesis. Two cases of titanium mesh were replaced by another because they were broken and one case of a child that was PMMA fixed with mini plates which were broken; hence, it was replaced with titanium mesh (Table 9).

Table 9: Table of outcome

Outcome	Number	Per cent
Replaced by another	3	6 %
Removal of bone	2	4 %
Good	45	90 %

Review of Selected Cases

Case 1: A 43 years old female patient with a history of 11-month protrusion of Lt eyeball, was operated by excision of soft tissue and decompression of the orbit (roof, lat wall and floor of the orbit). And the bone defect was replaced by PMMA (ball and socket technique). There were no neurological deficits present, and pathology revealed meningioma plaque. The drain was removed after two days in the third postoperative day, and there was no collection, the patient received intravenous antibiotic (cefoperazone) for 5 days and discharged on an oral antibiotic (amoxicillin, clavulanate) for ten days, follow up CT with 3D done in the third day post-operative (Figure 4 and 5).

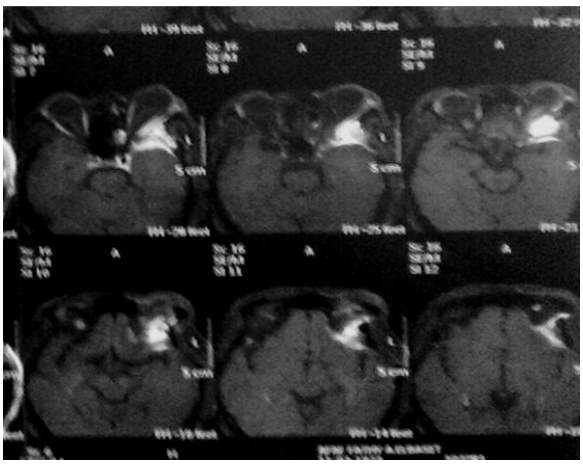

Figure 4: Preoperative MRI of the patient showing lt sided (meningioma plaque)

There was no evidence of infection, and the patient was discharged on the 5th postoperative day.

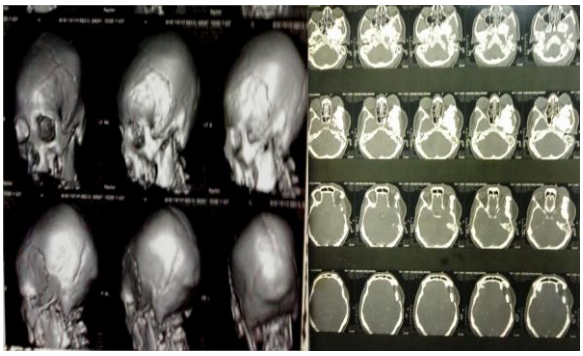

Figure 5: Postoperative CT brain with 3D reconstruction

Case 2: A 40 years old female patient that was complaining of headache for 9 months CT brain and MRI was done showing parasagittal and suprasellar meningiomas, the parasagittal was operated first, the invaded bone was also removed, and the bone defect was repaired using bone cement that was fixated by ball and socket technique. There was no neurological deficit pre and post-operative. The drain was removed in the third post-operative day.

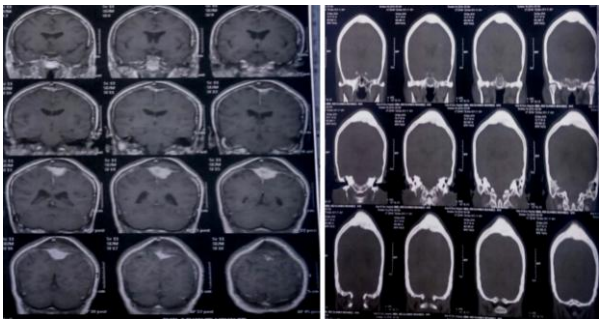

Figure 6: Preoperative MRI and CT bone window of the brain showing parasagittal meningioma

There was no collection and, the patient received an intravenous antibiotic for five days (cefoperazone) and discharged on oral antibiotics (amoxicillin + clavulanate) for one week. CT post was done after removal of the drain (Figure 6 and 7).

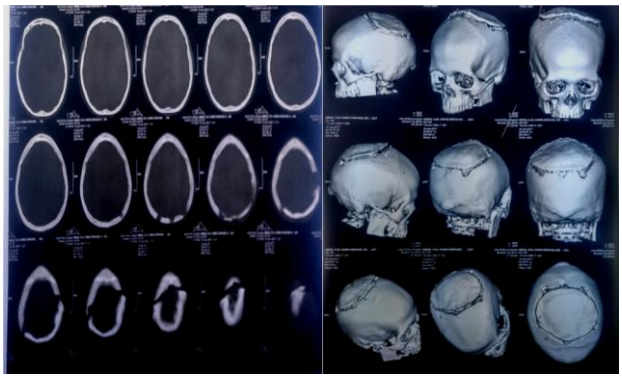

Figure 7: Postoperative CT brain with 3D reconstruction

Case 3: A 10 years old male patient presented in the outpatient clinic with disfigurement and defect in the front orbital region due to the history of trauma 2 years ago when the patient came with a compound depressed fracture.

Figure 8: Preoperative CT of the brain showing Orbital defect

Debridement and removal of bone fragments were done, and closure of skin for later cranioplasty CT bone window and 3D reconstruction was done to outline the defect and show the orbit he was operated through biclonal skin incision and reconstruction was done using PMMA that was fixed by ball and socket technique. There was no neurological deficit pre and post-operative. The drain was removed in the third day, and postoperative CT with 3D reconstruction was done. The patient received intravenous antibiotics (cefoperazone) for 5 days then discharged on oral antibiotics (amoxicillin + clavulanate for ten days. The post-operative recovery was smooth and the patient was discharged in the 5th post-operative day (Figure 8 and 9).

Figure 9: Postoperative CT brain showing repair of fronto orbital defect with PMMA

Case 4: A 43 years old female patient that was complaining of lt eye protrusion for 3 months before presentation CT brain and MRI was done showing meningioma en plaque with soft tissue intradural.

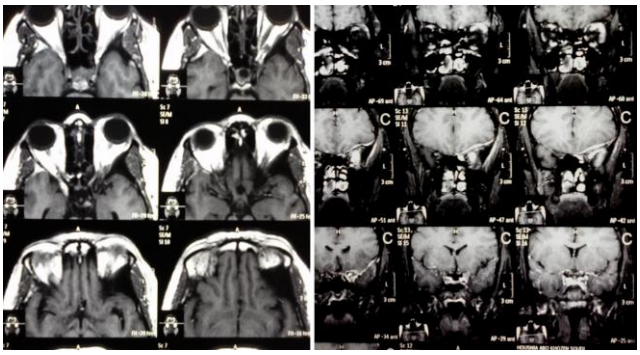

Figure 10: Preoperative MRI showing lt sided meningioma en-plaque

The patient was ttt by excision of soft tissue and decompression of the orbit (lat wall, floor and medial wall) then reconstruction of bone defect was done using bone cement that was fixed using ball and socket technique there was no neurological deficit pre and post-operative (Figure 10 and 11).

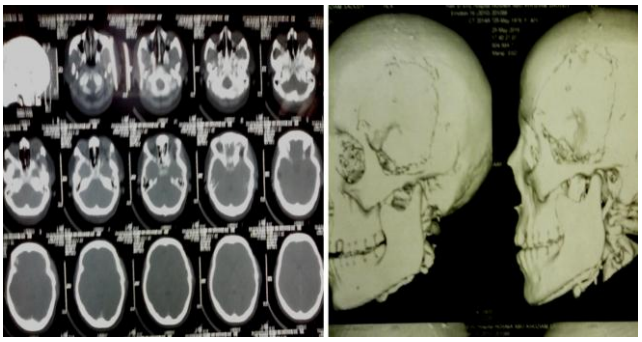

Figure 11: Postoperative CT brain showing excision of tumour and repair of the defect using methyl methacrylate

Case 5: A3-year-old child patient that was presenting with swelling for 6 months CT and MRI brain was done showing growing skull fracture treated by repair of the dural defect and repair of the bony defect by bone cement that was fixed by mini plates a.

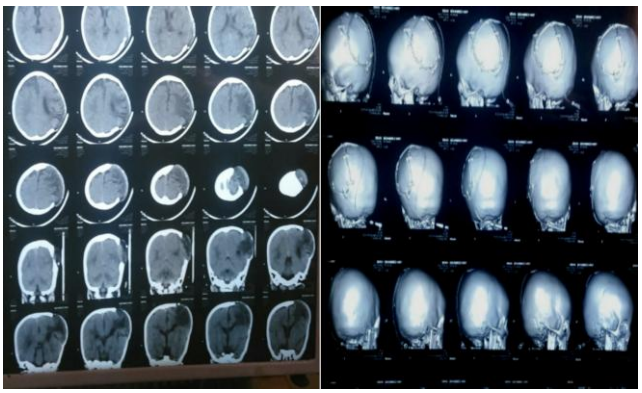

Figure 12: Preoperative CT brain showing growing skull fracture and post-operative ct brain showing repair of the defect by bone cement and mini plates

After 6 months follow up CT brain was done showing a fracture of bone cement that was replaced by titanium mesh (Figure 12 and 13).

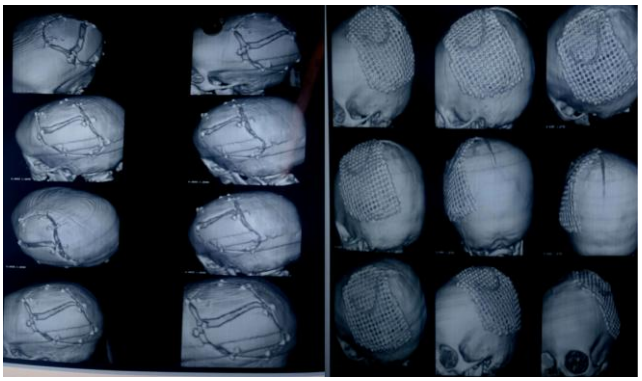

Figure 13: Follow up CT showing fracture of CT brain showing titanium mesh replacing Bone cement

Case 6: A 40-year-old male patient that was complaining of swelling and visual affection in the form of visual acuity 6/36.

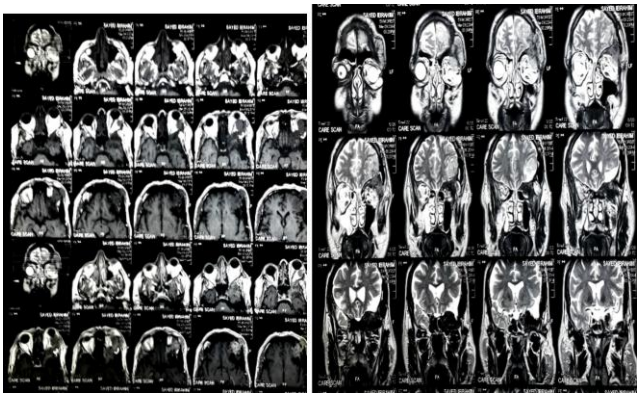

Figure 14: Preoperative MRI showing lt sphenoid wing meningioma

CT brain and MRI was done showing sphenoid wing meningioma that was ttt by surgical excision with removal of invaded dura and its repair by pericranium and removal of invaded bone and its replacement by bone cement fixed by ball and socket technique.

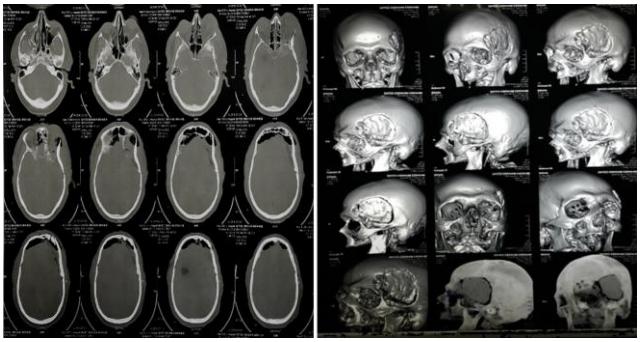

Figure 15: Postoperative CT brain showing tumour excision and replacement of bone by methyl methacrylate using ball and socket technique

The patient did not have any neurological deficits except visual affection (Figure 14, 15, and 16).

Figure 16: An intraoperative picture showing the details of the ball and socket technique before and after insertion of bone cement

Discussion

The principal values of cranioplasty are to restore aesthetic contour and to provide cerebral protection. Thirty of our patients (60%) were males, and the remaining twenty patients (40%) were females. The male prevalence had also been noted in one study by Andrea Mareira et al., [1] carried out over 312 patients where 54.5% of the patients were males while the remaining 45.5% were females. However, this is purely random as the study was directed towards a surgical procedure used for repair of skull defects caused by a wide variety of pathologies and therefore, we cannot use it to make statistical findings regarding age and gender distribution.

In the study mentioned above by Andrea Mareira et al., [1] listed post-tumour resection to be the most common cause of the defect, it was in 32.4% of the cases. Another study carried out by Alexander VanGool et al., on 45 patients [2] listed trauma as the leading aetiology as was found in 46.7% of their cases.

The most common aetiology of the defect in this study was post-traumatic (44% of the patients) whereas post-tumour resection was the second aetiology of the skull defect in (42%) of our patients.

In the two previously mentioned studies the commonest site was frontal (53.2% of the cases in the study by Andrea Mareira et al., [1] and 46.7% of the cases in the study by Alexander VanGool et al., [2].

The most common site of the skull defect noted in our study was found to be frontotemporal (42%), as related to meningioma en plaque, frontal (34%) related to compound depressed fractures and orbital fractures, parietal (20%) and front tempo parietal (4%) due to acute subdural haematoma treated by decompressive craniectomy.

In our study, the highest number was frontotemporal defect related to meningioma en plaque cases followed by frontal defect related to trauma cases

In the study mentioned above by Alexander VanGool et al., [2], the authors mentioned that 33 of their 45 patients (73.3%) mentioned cerebral protection as their main indication for surgery while only 7 patients (15.6%) mentioned cosmetic appearance as their main concern. The remaining 5 patients (11.1%) presented by both cosmetic problem and cerebral protection for which they sought surgery. In our study, the main indication of surgery was cerebral protection alone in 20 of our 50 patients (40%) and a cosmetic problem in 30 patients (60%).

In our study the main indication for cranioplasty was cosmetic (60%) as most of our patients were either tumour cases or post-traumatic with small disfiguring defects, while in the Alexandre

VanGool et al., the main issue was cerebral protection as most cases were traumatic with large defects.

As in all studies carried out on cranioplasty, the main preoperative imaging done for all patients was a CT-scan with a bone window to show the defect. But in our study, 3-D reconstruction was done for 39 patients (78%) because it is better showing the outline of defects [3].

In the study by Alexander VanGool et al., [2] that included 45 cases, 2 cases were reported to be infected and required flap removal. All cases were operated upon using polymethylmethacrylate for the repair. In another study by Victor Chang et al., [4] involving 212 cases over 13 years, the infection was reported in 15 cases.

Of the 15 cases, 8 (4.6%) out of a total of 175 were operated upon by repair using autologous bone, while 7 cases (18.9%) out of a total of 37 patients, had repaired by other materials as methylmethacrylate or titanium mesh.

We believe that our overall infection rate (6%) is not high compared to most studies. One case with swelling and fever patient treated conservatively by broad-spectrum antibiotics(intravenous cefoperazone and amikacin for ten days and oral antibiotics in the form of amoxicillin + clavulanate, ofloxacin for two weeks) and passed without need for intervention, two cases were complicated by wound infection and bone cement exposure following meningioma en plaque surgery and need removal of PMMA and repeated dressing, antibiotics and then treated by PMMA again after 6 month.

One case was complicated by CSF leak that was treated conservatively by lumbar drain left for three days, and another one complicated by collection treated conservatively.

The three cases that complicated by broken bone cement and titanium mesh required replacement by titanium mesh (one case of bone cement and two cases of titanium mesh), this complication is only reported in our study, and they did not mention it in their study.

A case of cranial defect due to infection reason must not be tackled operatively before complete resolution of the infection. Repair must not be done before at least 6 months. Also, it is important to ensure the application of all aseptic precautions. The use of antibiotic-impregnated methylmethacrylate has proved to be more superior than using one not impregnated with antibiotics that were used in the second case complicated by infection in our study; this issue is not discussed in the above two studies.

Even following perfect hemostasis, a subgaleal drain must be placed and left for 48 hours due to the possibility of developing the subgaleal collection. This observation was also noted in several studies made. In the study by Victor Chang et al., [4] covering a total of 212 patients, a drain was left in 84 patients and not in the remaining 128. In the 84 patients with a drain, a subgaleal collection was noted in 2 patients only (2.4%), while 11 of the 128 patients (8.6%) without drains developed the subgaleal collection. In the study by Andrea Mareira et al., [1], a seroma was noted in 9 out of 312 cases (7.9%). However, the mentioned study did not correlate the results to the use of a subgaleal drain.

In our study, the subgaleal drain was left for two days routinely, and this was reflected in the number of cases that develop collection (only one case).

The use of titanium mesh has the advantage of being more resistant to fracture especially for large skull defects, and less likely to develop infection when compared to methylmethacrylate and stainless, thus titanium alloy surfaces are thought to minimize bacterial adhesion and therapy lower the rate of infection, recently immediate titanium mesh cranioplasty has been proposed for the treatment of post-craniotomy infection and is widely used in Japan. However, the use of antibiotic-impregnated methylmethacrylate markedly reduces the risk of infection.

Our surgical technique (ball and socket technique) was a meticulous technique regarding hemostasis and watertight dural closure with subgaleal drains in all patients to avoid the possible compromisation of the structural integrity of theMMA when exposed to CSF and blood. Methylmethacrylate also has the advantages of being cheaper and more readily available for cases where cranioplasty was not planned (e.g., in intraoperative surprise at finding the bone flap infiltrated with a tumour or in the unfortunate event of the bone flap falling on the floor) [5].

Also, our technique does not need to fixate PMMA with stitches or manipulates, and it is suitable for difficult places as orbital defects that need special contouring and configuration, where titanium mesh is difficult to attain this contour.

In conclusion, the principal aims of cranioplasty in this study are to restore aesthetic contour and to provide cerebral protection. However, it has been noted that a great improvement occurs in cerebral blood flow and cerebral perfusion after cranioplasty, most probably due to the protection from the atmospheric pressure [6].

Thorough history taking and clinical evaluation to determine the cause of the defect when considering cranioplasty. A case of cranial defect due to an inflammatory reason must not be tackled operatively before complete resolution of the infection. Also, in cases where the defect was following a decompressive craniotomy, care must be taken not to perform the cranioplasty before complete normalisation of the intracranial pressure.

Ball and socket technique appears to be a

simple, safe economic and efficient method for fixation of cranioplasty flap. Methylmethacrylate has several advantages when compared to titanium mesh. It's more malleable and therefore easier to place at areas of skull convexities and orbital walls, more easily available in the operating theatre and doesn't have to be prepared in advance. The high incidence of development of postoperative seroma suggests the necessity of a subgaleal drain placement for 48 hours.

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Clinical Science

Carotid Artery Disease and Lower Extremities Artery Disease in Patients with Chronic Obstructive Pulmonary Disease

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Abstract

AIM: To assess the frequency of carotid artery disease (CAD) and lower extremities artery disease (LEAD) in patients with chronic obstructive pulmonary disease (COPD) and their relation to the severity of airflow limitation and the level of C-reactive protein (CRP).

METHODS: We performed a cross-sectional study including 60 patients with COPD (52 male, 8 female), aged 40 to 80 years, initially diagnosed according to the actual criteria. Also, 30 subjects in whom COPD was excluded, matched to COPD patients by sex, age, body mass index and smoking status, served as controls. All study subjects completed questionnaire and underwent pulmonary evaluation (dyspnea severity assessment, baseline and post-bronchodilator spirometry, gas analyses, and chest X-ray), angiological evaluation by Doppler ultrasonography and measurement of serum CRP level.

RESULTS: We found a statistically significant difference between the frequency of carotid plaques in COPD patients as compared to their frequency in controls (65% vs 30%; P = 0.002). The mean value of intima-media thickness (IMT) in COPD patients with CAD was significantly higher than its mean value in controls (0.8 ± 0.2 vs. 0.7 ± 0.2; P = 0.049). IMT value in COPD patients with CAD was significantly related to the degree of airflow limitation, i.e. to the degree of FEV1 decline (P = 0.000), as well as to the serum CRP level (P = 0.001). We found a statistically significant difference between the frequency of COPD patients with LEAD as compared to the frequency of LEAD in controls (78.3% vs 43.3%; P = 0.001). According to the Fontaine classification, COPD patients with LEAD were categorized in the stages I, IIA and IIB (53.3%, 30% and 16.7%, respectively), whereas all controls with LEAD were categorized in the Fontaine stage I. Among COPD patients with LEAD there was significant association between disease severity and clinical manifestations due to the vascular changes (P = 0.001) and serum CRP level (P = 0.001).

CONCLUSION: Our findings suggest higher prevalence and higher severity of vascular changes in COPD patients as compared to their prevalence and severity in non-COPD subjects. Prevalence and severity of vascular changes in COPD patients were significantly related to the severity of airflow limitation and serum CRP levels.

Introduction

COPD is a systemic disease with many comorbidities like: cardiovascular disease (CVD), anemia, polycythemia, malnutrition, muscle disorder, osteoporosis, metabolic syndrome, diabetes, gastroesophageal reflux, anxiety, depression, hormonal imbalance, infections, lung cancer, thrombosis [1], [2], [3], [4], [5]. The major cause for hospitalisation and mortality in COPD patients are CVD: heart failure, ischemic heart disease, arrhythmias, peripheral artery disease (PAD) and hypertension [1], [3], [4], [6], [7], [8], [9].

Cigarette smoking is a common risk factor for both diseases, but there are other predictors such as inflammation, oxidative stress, hypoxia, endothelial dysfunction, prosthesis/antiprotease imbalance etc. [10], [11]. COPD is characterised by chronic, low-grade, systemic inflammation that leads to atherosclerosis [12]. In a review of 14 relevant studies, Gan et al. demonstrated that levels of systemic inflammatory markers are increased in patients with COPD compared to smokers without COPD [10], [13]. Elevated C-reactive protein (CRP) as a marker of systemic inflammation is present in stable

COPD, as well as in COPD exacerbations [6]. The prevalence of PAD in COPD patients is wide-ranging. Lyn et al. reported the prevalence of asymptomatic PAD in Taiwan of 8.4%, Pecci in a Hispanic study of 36.8%, Pizzaro in a German study of 80% and Castagna in a French study of 81%. The common conclusion in all these studies is that patients with COPD and PAD, have worse pulmonary function [14], [15], [16], [17], [18], [19].

We aimed to assess the frequency of carotid artery disease (CAD) and lower extremities artery disease (LEAD) in patients with chronic obstructive pulmonary disease (COPD) and their relation to the severity of airflow limitation and the level of C-reactive protein (CRP).

Material and Methods

Study design and setting

A cross-sectional study aimed at comparison of frequency and severity of carotid and lower limb arteries changes in initially diagnosed COPD patients and non-COPD controls was performed at the General Hospital "8-th September", Skopje, Macedonia in the period January – May 2018. The study was approved by the Ethics Committee of the Medical Faculty at University Ss. "Cyril and Methodius" of Skopje, Skopje, Republic of Macedonia (03-2237/5/21.05.2018).

Study subjects

The study population included 60 patients with COPD initially diagnosed according to the actual Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria and 30 subjects without COPD matched to the COPD group by sex, age, body mass index (BMI), and smoking status. All enrolled subjects gave their written informed consent before entering the study.

Inclusion criteria for COPD group were: both genders, age 40-80 years, newly diagnosed COPD according to the actual GOLD (Global Initiative for Chronic Obstructive Lung Disease) criteria, and history of current or former smoking (equal or more than 10 pack-years).

Exclusion criteria for COPD group were: age less than 40 years and more than 80 years, BMI higher than 35, other chronic respiratory diseases (asthma, bronchiectasis, active tuberculosis, sarcoidosis, lung carcinoma, pulmonary fibrosis, sleep apnea syndrome), other chronic diseases (valvular and congenital heart disease, left ventricular hypertrophy, diabetes mellitus, hepatic and renal failure, anaemia, electrolyte imbalance, and immunosuppressive therapy), contraindication for spirometry, and patients who did not agree to participate in the study.

Inclusion criteria for the non-COPD group were: Age 40-80 years, current or former smoking (equal or more than 10 pack-years), BMI higher than 35, no pulmonary abnormalities, normal spirometric finding, clinically stable condition, and signed consent for participation in the study.

Study protocol

All study subjects completed questionnaire, as well as underwent a pulmonary and angiological evaluation and measurements of serum CRP.

Questionnaire

The questionnaire included questions on demographics (sex, age, weight and height, working history, socio-economic status), smoking history, respiratory and other symptoms in the last 12 months, as well as medical history and medication use.

The BMI as a measure of body fat based on height and weight that applies to adult population was determined in all study subjects by computed calculation using BMI calculator [20].

Classification of smoking status was done by the World Health Organization (WHO) recommendations [21].

Respiratory symptoms in the last 12 months (cough, phlegm, dyspnea, wheezing, and chest tightness) were documented using the European Community for Coal and Steel questionnaire (ECCS-87), and the European Community Respiratory Health Survey (ECRHS) questionnaire [22], [23].

Pulmonary evaluation

The pulmonary evaluation included: dyspnea severity assessment, baseline and post-bronchodilator spirometry, arterial gas analysis, and chest X-ray.

Dyspnea severity was assessed by the British Medical Council Dyspnea Scale [24].

The baseline spirometry, including measures of forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), FEV_1/FVC, and maximal expiratory flow at 75%, 50%, 25%, and 25-75% of FVC (MEF_{75}, MEF_{50}, MEF_{25}, and MEF_{25-75}, respectively), was performed in all subjects using electronic spirometer Spirobank G USB Spirometer (Medical International Research, Roma, Italy) with recording the best result from three measurements the values of FEV_1 of which were within 5% of each other. The results of spirometry were expressed as percentages of the predicted values according to the

actual recommendations of the European Respiratory Society (ERS) and ATS [25].

Bronchodilator test was performed by spirometric measurements before and 20 minutes after administration of 400 μg salbutamol by metered dose inhaler through the spacer. Post-bronchodilator value of the FEV_1/FVC ratio less than 0.70 indicated persistent airflow limitation [25].

According to the actual GOLD recommendations, COPD was considered by finding of a post-bronchodilator FEV_1/FVC ratio less than 0.70 in symptomatic subjects (dyspnea, chronic cough and/or sputum production) with a history of exposure to risk factors for the diseases (noxious particles and gases). In addition, according to the FEV_1 value, airflow limitation in the subjects with COPD, i.e. severity of the disease, was classified as mild (FEV_1 value higher than 80% of the predicted value), moderate (FEV_1 value higher than 50% but lower than 80% of the predicted value), severe (FEV_1 value higher than 30% but lower than 50% of the predicted value), and very severe (FEV_1 value lower than 30% of the predicted value) [1].

The gas analysis was performed with SIEMENS RAPIDPOINT 405 System (Siemens Healthineers, Australia).

Angiological evaluation

Doppler ultrasonography of carotid arteries was performed with high-resolution General Electric Vivid 7 (GE Healthcare, Milwaukee, USA), B mode ultrasonography, 5-10MHz multifrequency linear probe. Carotid intima-media thickness (IMT), as an indicator of subclinical atherosclerosis, was measured in three points: at the site of the largest thickening, at the proximal and distal point. The mean value in these three points was calculated for each carotid artery, and the highest value was taken for IMT. Lesions with IMT greater than 1.2 mm were defined as atheromatous plaques [26].

Doppler ultrasonography of lower limb arteries was performed with the same General Electric Vivid 7 ultrasound system receiving information about the localisation, extensiveness and severity of vascular lesions [27]. LEAD may be asymptomatic or symptomatic. Fontaine classification is a classification based on clinical symptoms of the disease, which includes five stages: I, IIa, IIb, III, IV. Stage I refers to asymptomatic or subtle symptoms such as paraesthesia, stage IIa to intermittent claudication after walking more than 200 m, stage IIb to claudication at walking less than 200 m, stage III to resting pain, especially during the night, and stage IV to ischemic ulceration or gangrene [28].

Serum CRP measurements

All study participants underwent blood sampling and measuring of CRP in serum by latex-enhanced immunonephelometric assay (ABX Pentra CRP CP, HORIBA GROUP, Montpellier, France), reference value 0-10mg/L.

Statistical analysis

Statistical analysis was done using the SPSS Statistics 17 software package (SPSS. Inc., Chicago, IL, USA). The results of the tests were usually expressed with numerical values, so the comparison between them was performed using a correlation with the Pearson Correlation test. To test hypotheses involving multiple samples, a standard Student *t*-test for two or more samples was used. The Mann-Whitney *U*-test was used to test two independent samples. In the case of more than two samples, a Kruskal-Wallis H test of *K*-independent samples was used, which is a one-way analysis of the variants of independent samples (one-way ANOVA on ranks). The level of statistical significance was set at *P* value less than 0.05.

Results

Demographic and other characteristics of the study subjects are given in Table 1. The two groups were similar regarding the sex and age distribution of the included subjects, as well as regarding their smoking status and mean BMI. The mean values of spirometric parameters (FVC, FEV_1 and FEV_1/FVC ratio) were significantly lower in COPD patients than in non-COPD controls. Also, the mean value of serum CRP was significantly higher in COPD patients than in non-COPD (10.2 *vs* 5.9; *P* = 0.04), suggesting a low-grade systemic inflammation in these patients.

Table 1: Demographics and other characteristics of the study subjects

Characteristic	COPD patients (n = 60)	Non-COPD subjects (n = 30)
Sex		
Males	52 (86.7%)	23 (76.7%)
Females	8 (13.3%)	7 (23.3%)
Mean age (years)		
Males	65.9 ± 7.5	64.8 ± 8.6
Females	67.9 ± 6.1	66.7 ± 7.8
Smoking status		
Active smokers	35 (58.3%)	18 (60%)
Former smokers	25 (41.7%)	12 (40%)
Pack-year smoked	66.1 ± 25.8	67.4 ± 25.5
Mean BMI value	25.8 ± 4.9	24.9 ± 2.1
Mean baseline values of spirometric parameters		
FVC (% pred)	78.8 ± 12.3	115.2 ± 16.8
FEV_1 (% pred)	47.5 ± 17.9	92.3 ± 14.7
FEV_1/FVC ratio	0.6 ± 0.07	0.8 ± 0.05
Level of serum CRP		
< 3,14 mg/L	10 (16.7%)	15 (50%)
3,14 - 10mg/L	27 (45%)	10 (33.3%)
> 10mg/L	23 (38.3%)	5 (16.7%)

COPD: chronic obstructive pulmonary disease; BMI: body mass index; FVC: forced vital capacity; FEV_1: forced expiratory volume in one second; %pred: percentage of the predicted value; CRP: C-reactive protein; mg: milligram; L: litre.

According to the severity of airflow limitation, i.e. to the post-bronchodilator value of FEV_1, COPD patients were categorised in four stages: mild, moderate, severe and very severe COPD.

Table 2: Distribution of the COPD patients by degree of airflow limitation

COPD severity	COPD patients (n = 60)
GOLD 1 - mild (FEV_1 ≥ 80% pred)	4 (6.7%)
GOLD 2 – moderate (FEV_1 = 50% – 79% pred)	21 (35%)
GOLD 3 – severe (FEV_1 = 30% – 49% pred)	22 (36.7%)
GOLD 4 – very severe (FEV_1 < 30% pred)	13 (21.7%)

COPD: chronic obstructive pulmonary disease; GOLD: Global Initiative for Chronic Obstructive Lung Disease; FEV_1: forced expiratory volume in one second; %pred: percentage of the predicted value.

Doppler-ultrasonography of carotid arteries detected a statistically significant difference between the frequency of carotid plaques in COPD patients as compared to their frequency in controls (65% *vs* 30%; $P = 0.002$). Distribution of COPD patients with carotid plaques by the degree of airflow limitation is presented in Table 3.

Table 3: Distribution of COPD patients with carotid plaques by the degree of airflow limitation

COPD patients (n = 60)	Plaques without stenosis	Plaques with stenosis up to 40%	Plaques with stenosis 40-60%
GOLD 1 (n = 4)	0 (0%)	0 (0%)	0 (0%)
GOLD 2 (n = 21)	7 (33.3%)	2 (9.5%)	1 (4.7%)
GOLD 3 (n = 22)	11 (50%)	2 (9%)	5 (22.7%)
GOLD 4 (n = 13)	3 (23%)	5 (38.4%)	3 (23%)

COPD: chronic obstructive pulmonary disease; GOLD: Global Initiative for Chronic Obstructive Lung Disease.

The mean value of IMT in COPD patients with detected carotid plaques was 0.8 ± 0.2, whereas its mean value in controls was 0.7 ± 0.2 ($P = 0.049$). IMT value in COPD patients with CAD was significantly related to the degree of airflow limitation, i.e. to the degree of FEV_1 decline ($P = 0.000$), as well as to the serum CRP level ($P = 0.001$).

We found a statistically significant difference between the frequency of LEAD in COPD patients as compared to their frequency in controls (78.3% *vs* 43.3%; $P = 0.001$). The distribution of COPD patients with LEAD by the degree of airflow limitation is presented in Table 4.

Table 4: Distribution of COPD patients with LEAD by the degree of airflow limitation

COPD patients (n = 60)	Initial atherosclerotic plaques without stenosis	Diffuse atherosclerotic plaques without stenosis	Plaques with stenosis up to 40%	Plaques with stenosis 40-60%
GOLD 1 (n = 4)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
GOLD 2 (n = 21)	5 (23.8%)	3 (14.3%)	3 (14.3%)	2 (9,5%)
GOLD 3 (n = 22)	4 (18.2%)	7 (31.8%)	6 (27.3%)	4 (18.2%)
GOLD 4 (n = 13)	0 (0%)	3 (23%)	5 (38.4%)	5 (38.4%)

COPD: chronic obstructive pulmonary disease; GOLD: Global Initiative for Chronic Obstructive Lung Disease.

According to the Fontaine classification, COPD patients with LEAD were categorised in the stages I, IIA and IIB (53.3%, 30% and 16.7%, respectively), whereas all controls with LEAD were categorised in the Fontaine stage I.

Among COPD patients with LEAD, there was a significant association between disease severity, i.e. FEV_1 value, and clinical manifestations due to the vascular changes ($P = 0.001$). The relation between clinical manifestations of LEAD and serum CRP level was also statistically significant ($P = 0.001$).

Discussion

Vascular changes are one of the cardiovascular complications of COPD, probably due to the chronic, low-grade, systemic inflammation that leads to atherosclerosis in carotid and other arteries [29]. The inflammatory cascade that initially arises from exposure to noxious substances, mainly tobacco smoke, accelerates atherogenesis at all stages by formation, destabilisation and rupture of plaque as well as by platelet activation and clotting that lead to atheroma formation and atherothrombosis [18]. Nevertheless, the association between COPD and vascular comorbidities, i.e. CAD and LEAD, is still poorly understood [30].

Our study aimed to assess the frequency of CAD and LEAD in patients with initially diagnosed COPD and their relation to the severity of airflow limitation and the serum level of CRP. We performed a cross-sectional study, including 60 patients with COPD (52 male, 8 female), aged 40 to 80 years, diagnosed according to the actual criteria. Also, 30 subjects in whom COPD was excluded, matched to COPD patients by sex, age, BMI, and smoking status, served as controls. Airflow limitation in over half of the COPD patients were classified as severe and very severe, suggesting the delayed diagnosis, as well as the late onset of appropriate treatment of the disease.

Doppler-ultrasonography of carotid arteries detected a statistically significant difference between the frequency of carotid plaques in COPD patients as compared to their frequency in controls. Also, the mean value of IMT in COPD patients with detected carotid plaques was significantly higher as compared to its mean value in controls with detected carotid plaques. IMT value in COPD patients with CAD was significantly related to the COPD severity, i.e. to the post-bronchodilator value of FEV_1, as well as to the serum CRP level.

In the study on subclinical cardiovascular changes in COPD patients, Sadeka et al. found that patients with COPD had **a** higher frequency of carotid plaques and the higher mean value of IMT compared to non-COPD controls, but they did not find a

significant association between these findings and the severity of COPD [31]. On the other side, the MESA Lung Study on the link between subclinical atherosclerosis and emphysema confirmed the presence of higher mean IMT value in smokers compared to non-smokers [32]. Furthermore, unlike the results of the Sadeka's study, Kim et al., reported results similar to our findings, i.e. significantly higher frequency of CAD in COPD patients than in non-COPD controls and its significant relation to COPD severity and serum level of CRP as a marker of systemic inflammation [31], [33].

We found a statistically significant difference between the frequency of LEAD in COPD patients as compared to their frequency in controls. According to the Fontaine classification, COPD patients with LEAD were categorised in the stages I, IIA and IIB, whereas all controls with LEAD were categorised in the Fontaine stage I suggesting more expressed clinical manifestations of LEAD in COPD patients as compared to their manifestations in controls. Among COPD patients with LEAD, there was a significant association between disease severity, i.e. the post-bronchodilator FEV_1 value, and clinical manifestations due to the vascular changes. The relation between clinical manifestations of LEAD and serum CRP level was also statistically significant.

Similarly to our findings, in a Spanish cross-sectional study, Pecci et al., found that LEAD is present with a high prevalence in patients with COPD [34]. The same, i.e. a high frequency of LEAD in COPD patients (80.4%), was also demonstrated by Pizzaro et al., [18]. On the other side, Watz et al. demonstrated a lower frequency of LEAD in COPD patients (25.3%) [35]. These differences are mainly due to the different methodology used for detection of LEAD, i.e. its diagnosis in the study performed by Watz et al., was based on ankle-brachial measurements, whereas in the studies performed by Pecci et al., and Pizzaro et al., the diagnosis was based on the colour duplex sonography of lower extremity arteries [18], [34], [35].

The present study must be interpreted within the context of its limitations. First, a relatively small number of the study subjects could have certain implications on data obtained and its interpretation. Also, the unequal distribution of COPD patients by degree of the disease severity could have certain implications on data obtained and its interpretation. On the other hand, detection of the vascular changes in newly diagnosed COPD patients is the strength of the study.

In conclusion, our findings suggest higher frequency and higher severity of vascular lesions in newly diagnosed COPD patients as compared to their prevalence and severity in non-COPD subjects. Frequency and severity of vascular changes in COPD patients were significantly related to the severity of airflow limitation and serum CRP levels. Our findings also suggest a need for early screening for vascular comorbidities in COPD patients to detect them and to obtain an integrated-care approach in the management of these patients.

Authors Participation

DBI participated in the study design, writing the protocol, data collection, managing the analyses of the study, and writing all versions of the manuscript. JM and NKK participated in the study design, writing the protocol, managing the analyses of the study, as well as writing all versions of the manuscript. IG, AD and MB participated in the data collection and managing the analyses of the study.

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Clinical Science

Investigating the Manifestation of Coronary Artery Disease and Determining the Role of Effective Factors in the Need for Pacemaker Insertion in These Patients

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Abstract

BACKGROUND: Many patients who are candidates for a pacemaker are also at the same time risk factors for coronary artery disease such as high blood pressure, hypertension, diabetes, and hyperlipidemia, and therefore the probability of having coronary artery disease is significant. Effective diagnostic measures can be taken to prove the factors affecting the incidence of CAD in patients undergoing pacemakers at high-risk, including angiography. Therefore, it can prevent complications during and after pacemaker implantation, which leads to an increase in the quality of treatment in patients requiring pacemaker implantation.

AIM: Therefore, the purpose of this study was to determine the predictive factors of significant coronary artery disease in patients with pacemaker implantation to identify patients in need of coronary angiography at the time of pacemaker implantation.

METHODS: This retrospective study was carried out to examine the patients' files that were placed at the heart of Imam Reza Hospital during the period between March 2017 and September 2017. Demographic data, risk factors, echocardiography findings, and angiography, were collected and then recorded using a checklist. Statistical analysis was performed using SPSS software version 22 and Chi-square, and Mann-Whitney tests were used for determining significates variables.

RESULTS: A group of 102 patients who had undergone a permanent cardiac pacemaker insertion due to an atrioventricular (AV) Block were included in the study, and also coronary anatomy was determined coronary angiography. Based on the results, 13.7% of patients with cardiac pacemaker had obstructive coronary artery disease (stenosis > 70%). Factors affecting coronary artery stenosis on angiography include gender, chest pain, history of myocardial infarction, angioplasty, diabetes, smoking, history of aspirin intake, calcium blocker and Plavix, high hematocrit, ST elevation and ST depression in the ECG, and severe mitral regurgitation.

CONCLUSION: It seems that in most patients requiring permanent pacemaker insertion because of the atrioventricular (AV) Block, angiography does not change the patient's fate, and so can be ignored. However, in patients who have several risk factors from the listed above, coronary angiography is recommended during admission.

Introduction

Coronary Artery Disease (CAD) is the leading cause of death in industrialised countries [1]. In addition to its impact on mortality rates, CAD also causes disability and reduced productivity [2]. Over the past few decades, with the advancement of CAD diagnosis, prevention and treatment, and the mortality rate of this disease has decreased. However, in the United States alone, about 1.2 million cases of myocardial infarction or fatal heart attacks occur annually [3]. Nearly half of all deaths in industrialised countries and 25% of deaths occur in developing countries due to coronary artery disease [4]. By 2020, the number of deaths expected to exceed the number of deaths from infectious diseases [5].

Many patients who are candidates for pacemakers have a simultaneous risk factor for coronary artery disease, such as high age, hypertension, diabetes, hyperlipidemia; so, the probability of having coronary artery disease is significant, with a 30% risk [6], [7].

Also, many patients with bradyarrhythmias, especially the atrial fibrillation block, have symptoms such as chest pain and shortness of breath that overlaps with the symptoms of coronary artery disease, and at the same time, patients may suffer from acute coronary syndrome and bradycardia of the heart [8], [9]. Finally, the pacemaker insertion can be implemented in the cardiac catheterisation room, where there is the possibility of performing coronary angiography simultaneously. However, coronary angiography is not routinely found to indicate in patients with Bradyarrhythmia.

However, if the prediction of patients with significant coronary artery disease is performed, coronary angiography can also be done concurrently with pacemaker insertion and identified the presence and severity of coronary artery disease. Additionally, coronary angiography can be avoided in unnecessary cases, which is an invasive procedure with inherent complications, especially in older patients [12].

Effective diagnostic measures including angiography can be performed by proving the factors influencing the incidence of CAD in high-risk patients who undergo pacemaker implantation to prevent complications during and after placement of the pacemaker and to increase the quality of therapy in patients requiring pacemakers. Several studies have investigated the location of coronary artery involvement in patients with heart block, or have examined the effect of revascularization on the removal of the heart block removal and the implantation of a permanent pacemaker [6], [7], [8], [13], [14], [15].

Therefore, the current study was aimed to evaluate factors associated with coronary artery disease in patients with pacemakers, which can help choose patients who simultaneously require coronary angiography.

Material and Methods

This retrospective study was carried out using the medical record of all patients who had permanent pacemaker implantation from the beginning of March 2017 to September 2017 at the cardiology department, Imam Reza Hospital of Mashhad University of Medical Sciences. The coronary anatomy of patients was determined by angiography during admission or at least one month before or after the pacemaker insertion, and then subjects were included in the study. The location and extent of involvement for all coronary arteries and their branches were determined by the cardiologist via examining the angiographic film, or according to available and reliable written reports.

Based on the results of coronary artery angiography, patients were divided into two groups. The first group was patients with significant coronary artery disease. (There is at least one vessel involved with stenosis of up to 70% in a major vessel or in the main branch of a vessel that needs revascularisation). Group 2: Patients with no significant coronary artery involvement.

The variables studied included: Age, sex, BMI, diabetes, hypertension, dyslipidemia, smoking, serum troponin levels, CK and CK-MB, symptoms in referral time (syncope, chest pain, dyspnea), serum creatinine, left ventricular ejection fraction (LVEF), Mitral valve failure, pulmonary artery pressure, coronary calcification in fluoroscopy during pacemaker implantation, mitral annular calcification in fluoroscopy or echocardiography, cause of pacemaker implantation.

Statistical analysis

Data from patients' demographic and clinical observations were analysed using SPSS software version 22. To describe the data, descriptive statistical methods, including central indicators, distribution, and frequency, were employed. T-test or its nonparametric equivalents were used to compare quantitative variables in two independent groups. Furthermore, the Chi-square test was applied to compare qualitative variables in two groups. In all tests, $p < 0.05$ was considered as a significant level.

Results

This study was performed on 102 patients under coronary angiography and pacemaker insertion. Among all enrolled subjects, 53 (52%) men and 49 (48%) women were present. In this study, individuals with permanent pacemaker implantation were searched form the medical records, and people who had coronary angiography were also examined. Also, subjects who included both of the measures mentioned above were enrolled in the study. The mean age of the patients was determined as 71.25 ± 11.32 year, and the patients were in the range of 44 to 93 years old. Among all 102 enrolled patients, 88 (86.3%) were not found to suffer from coronary artery disease, and 14 (13.7%) had coronary artery involvement.

Table 1: Average and frequency of demographic and clinical variables in patients with pacemaker implantation based on coronary artery involvement

Coronary artery disease / Disorder		No	Yes	Total	P-value
Age(year)		71.1±11.87	72.21±7.14	71.25±11.3	0.733
Sex	41 (%77.4)	12 (%22.6)	53 (%100.0)	0.008	0.008
	47 (%95.9)	2 (%4.1)	49 (%100.0)		
Smoking		16 (%72.7)	6 (%27.3)	22 (%100.0)	0.037
Type of referral symptoms	50 (%89.3)	6 (%10.7)	56 (%100.0)	0.787	0.787
	35 (%87.5)	5 (%12.5)	40 (%100.0)		
Type of referral symptoms	53 (%89.8)	6 (%10.2)	59 (%100.0)	0.047	0.047
	21 (%95.5)	1 (%4.5)	22 (%100.0)		
Underlying disease	10 (%66.7)	5 (%33.3)	15 (%100.0)		
	2 (%100.0)	0	2 (%100.0)		
	4 (%50.0)	4 (%50.0)	8 (%100.0)	0.012	0.012
	1 (%50.0)	1 (%50.0)	2 (%100.0)	0.257	0.257
	1 (%20.0)	4 (%80.0)	5 (%100.0)	0.001	0.001
	2 (%100.0)	0	2 (%100.0)	1	1
	1 (%100.0)	0	1 (%100.0)	1	1
	17 (%68.0)	8 (%32.0)	25 (%100.0)	0.005	0.005
	52 (%83.9)	10 (%16.1)	62 (%100.0)	0.38	0.38
	15 (%75.0)	5 (%25.0)	20 (%100.0)	0.102	0.102
	22 (%81.5)	5 (%18.5)	27 (%100.0)	0.399	0.399

As shown in Table 1, no significant difference was found in the mean and standard deviation of age in patients with pacemaker implantation based on coronary artery involvement (P > 0.05). However, the frequency of coronary artery disease in men was significantly higher than that of women (22.6% vs 4.1%) (P = 0.008). The frequency of coronary artery disease in patients with cigarette smoking was significantly higher as compared to non-smokers (27.3% vs 10%), (P < 0.05). Also, the frequency of referral type in patients with pacemaker implantation, based on coronary artery disease, was not significantly different (P > 0.05). Moreover, the incidence of coronary artery disease in patients with chest pain was significantly higher when compared with other patients (33.3%) (P = 0.47).

Frequency of coronary artery disease in patients with myocardial infarction (50% versus 10.6%), angioplasty (80% vs 10.3%) and diabetes (32% versus 7.8%) was significantly higher than other patients without aspects (P < 0.05).

Table 2: Frequency of medication use in patients with pacemaker implantation based on coronary artery involvement

Coronary artery disease / Drug			No	Yes	Total	P-value
Beta blocker	No	Number	44	4	48	
		Percent	%91.7	%8.3	%100.0	0.136
	Yes	Number	44	10	54	
		Percent	%81.5	%18.5	%100.0	
Digoxin	No	Number	86	14	100	
		Percent	%86.0	%14.0	%100.0	0.569
	Yes	Number	2	0	2	
		Percent	%100.0	%0.0	%100.0	
Aspirin	No	Number	63	3	66	
		Percent	%95.5	%4.5	%100.0	0.001
	Yes	Number	25	11	36	
		Percent	%69.4	%30.6	%100.0	
Calcium blocker	No	Number	56	5	61	
		Percent	%91.8	%8.2	%100.0	0.048
	Yes	Number	32	9	41	
		Percent	%78.0	%22.0	%.100	
Plavix	No	Number	87	8	95	
		Percent	%91.6	%8.4	%100.0	<0.001
	Yes	Number	1	6	7	
		Percent	%14.3	%85.7	100.0	

The frequency of coronary artery disease in patients with history of aspirin use (30.6% vs. 4.5%), calcium blocker (22% vs. 8.2%) and Plavix (85.7% vs. 8.4%) %) was significantly higher as compared to other patients without the history of using the drugs (Table 2).

Table 3: Mean and standard deviation of laboratory variables of patients with pacemakers based on coronary artery involvement

Variable	No	Yes	P-value
FBS	105.06±41.52	103.5±36.22	0.834
Cr	1.04±0.17	1.11±0.14	0.103
Na	139.98±9.97	138.64±2.02	0.633
K	4.77±0.44	4.87±0.49	0.351
Ca	8.85±0.32	9±0.51	0.203
Uric Acid	32.9±8.15	32.07±8.94	0.701
CTn.1	0.004±0.018	0.033±0.05	0.142
CTn.2	0.0007±0.004	0	0.825
Chol	146.21±31.64	153.23±33.88	0.364
TG	118.55±29.78	114.69±57.27	0.327
LDL	103.4±30.6	102.15±18.13	0.812
HDL	42±8.7	39±8.27	0.215
WBC	7.45±1.61	7.22±1.29	0.605
RBC	4.2±1.09	4.35±0.63	0.282
Hgb	13.47±3.22	13.97±1.12	0.569
Hct	40.15±7.23	41.72±2.8	0.042
MCV	89.71±21.86	88.21±2.08	0.949
Plt	200.81±51.72	203.31±69.74	0.456

LDL (Low-density lipoprotein), HDL (High –density lipoprotein ;RBC (Red blood cell), WBC (White blood cell); MCV (mean cell volume).

As indicated in Table 3, the only hematocrit in patients with coronary artery disease was significantly higher compared with patients without coronary artery disease (41.72 vs 40.15) (P = 0.42).

Table 4: Frequency of ECG abnormalities in patients with pacemaker implantation based on coronary artery involvement

Coronary artery disease / Disorder			No	Yes	Total	P-value
AF	No	Number	85	13	98	
		Percent	%86.7	%13.3	%100.0	**0.451**
	Yes	Number	3	1	4	
		Percent	%75.0	%25.0	%100.0	
AT	No	Number	88	13	101	
		Percent	%87.1	%12.9	%100.0	**0.137**
	Yes	Number	0	1	1	
		Percent	%0.0	%100.0	%100.0	
AVB 2	No	Number	87	14	101	
		Percent	%86.1	%13.9	%100.0	**1**
	Yes	Number	1	0	1	
		Percent	%100.0	%0.0	%100.0	
AVB 2.1	No	Number	67	12	79	
		Percent	%84.8	%15.2	%100.0	**0.731**
	Yes	Number	21	2	23	
		Percent	%91.3	%8.7	%100.0	
CAVB	No	Number	25	2	27	
		Percent	%92.6	%7.4	%100.0	**0.266**
	Yes	Number	63	12	75	
		Percent	%84.0	%16.0	%100.0	
RBBB	No	Number	71	13	84	
		Percent	%84.5	%15.5	%100.0	**0.454**
	Yes	Number	17	1	18	
		Percent	%94.4	%5.6	%100.0	
LBBB	No	Number	68	11	79	
		Percent	%86.1	%13.9	%100.0	**0.914**
	Yes	Number	20	3	23	
		Percent	%87.0	%13.0	%100.0	
Narrow QRS	No	Number	48	10	58	
		Percent	%82.8	%17.2	%100.0	**0.264**
	Yes	Number	40	4	44	
		Percent	%90.9	%9.1	%100.0	
No Intrinsic QRS	No	Number	87	14	101	
		Percent	%86.1	%13.9	%100.0	**1**
	Yes	Number	1	0	1	
		Percent	%100.0	%0.0	%100.0	
ST Elevation	No	Number	81	6	87	
		Percent	%93.1	%6.9	%100.0	
	Ant	Number	1	3	4	
		Percent	%25.0	%75.0	%100.0	**<0.001**
	Inf	Number	0	4	4	
		Percent	%0.0	%100.0	%100.0	
	Lat	Number	1	0	1	
		Percent	%100.0	%0.0	%100.0	
ST Depression	No	Number	82	9	91	
		Percent	%90.1	%9.9	%100.0	**<0.001**
	Ant	Number	0	4	4	
		Percent	%0.0	%100.0	%100.0	
T wave Inversion	No	Number	79	13	92	
		Percent	%85.9	%14.1	%100.0	
	Inf	Number	2	0	2	
		Percent	%100.0	%0.0	%100.0	**0.782**
	Lat	Number	1	0	1	
		Percent	%100.0	%00.0	%100.0	

AF (Atrial fibrillation); AVB (Atrioventricular block); CAVB (Complete atrioventricular block); RBBB (Right bundle branch block); LBBB (Left bundle branch block).

According to Table 4, the incidence of coronary artery disease in patients with ST-segment elevation was significantly lower in the lower wall (100%) and the front wall (75%) than in other patients (P < 0.001). Furthermore, the frequency of coronary artery disease in patients with ST-segment depression in the front part (100%) was significantly higher as compared to patients without ST-segment depression (p < 0.001).

Table 5: Frequency of angiographic impairment in patients with pacemaker implantation based on coronary artery involvement

Coronary artery disease / Angiography			No	Yes	Total	P-value
Vessels involved in angiography	No	Number	85	0	85	<0.001
		Percent	%97.7	%0.0	%84.2	
	LM	Number	0	4	4	
		Percent	%0.0	%28.6	%4.0	
	LAD	Number	1	4	5	
		Percent	%1.1	%28.6	%5.0	
	LCX	Number	0	1	1	
		Percent	%0.0	%7.1	%1.0	
	OM	Number	1	0	1	
		Percent	%1.1	%0.0	%1.0	
	RCA	Number	0	4	4	
		Percent	%0.0	%28.6	%4.0	
	PLV	Number	0	1	1	
		Percent	%0.0	%7.1	%1.0	
Angioplasty after angiography	No	Number	86	6	92	<0.001
		Percent	%97.7	%42.9	%90.2	
	Yes	Number	2	8	10	
		Percent	%2.3	%57.1	%9.8	
CABG after angiography	No	Number	88	12	100	0.018
		Percent	%100.0	%85.7	%98.0	
	Yes	Number	0	2	2	
		Percent	%0.0	%14.3	%20.0	

LM (Left main artery); LAD (Left anterior descending artery); LCX (Left circumflex artery) OM(Obtuse marginal); RCA (Right coronary artery) PLV (Posterior left ventricular).

As summarised in Table 5, the most involved vessels in the patients simultaneously included LM, LAD, and RCA (each of 28.6%). Also, patients experienced angioplasty after angiography (57.1%), and CABG after angiography (14.3%). Regarding the results presented in Table 6, patients with severe mitral regurgitation (100%) had coronary artery disease after implantation of the pacemaker (P < 0.05).

Table 6: Frequency of echocardiography in patients with pacemaker implantation based on coronary artery involvement

Coronary artery disease Echocardiography			No	Yes	Total	P-value
Mitral insufficiency	No	Number	61	6	67	**0.003**
		Percent	%91.0	%9.0	%100.0	
	Mild	Number	16	1	17	
		Percent	%94.1	%5.9	%100.0	
	Moderate	Number	5	3	8	
		Percent	%62.5	%37.5	%100.0	
	Severity	Number	0	1	1	
		Percent	%0.0	%100.0	%100.0	
Mitral annulus calcification	No	Number	82	11	93	**0.128**
		Percent	%88.2	%11.8	%100.0	
	Yes	Number	0	1	1	
		Percent	%0.0	%100.0	%100.0	
Tricuspid insufficiency	No	Number	52	4	56	**0.123**
		Percent	%92.9	%7.1	%100.0	
	Mild	Number	21	4	25	
		Percent	%84.0	%16.0	%100.0	
	Moderate	Number	6	3	9	
		Percent	%66.7	%33.3	%100.0	
	Severity	Number	3	1	4	
		Percent	%75.0	%25.0	%100.0	
Aortic valve position	Normal	Number	73	10	83	**0.721**
		Percent	%88.0	%12.0	%100.0	
	Calcification	Number	1	0	1	
		Percent	%100.0	%0.0	%100.0	
	Failure	Number	8	2	10	
		Percent	%80.0	%20.0	%100.0	

Discussion

Many patients who are candidates for pacemaker implantation, due to the atrioventricular (AV) Block, have high age and at the same time are at risk for coronary artery disease. Coronary artery blockage can be an etiological factor in the impairment of the cardiac conduction system and require the use of a pacemaker. On the other hand, in patients requiring pacemakers, non-invasive tests, such as exercise tests, are not possible for diagnosis of coronary artery disease, or their diagnostic value is lower than other patients [18], [16]. Therefore, the study of factors associated with coronary artery disease in these patients can help choose patients who simultaneously need coronary angiography.

This study was performed on 102 patients with pacemaker implantation. Among all included patients, 53 (52%) were men, and 49 (48%) were women. The mean age of the patients was calculated to be 71.11 ± 25.32 years old, and the patients were in the range of 44 to 93 years old. Of the 102 patients who were admitted, 88 (86.3%) were without coronary artery disease, and 14 (13.7%) had coronary artery disease. Based on the result presented herein, the mean and standard deviation of age, frequency of f referral type in patients with pacemaker implantation based on coronary artery involvement was not found to be significantly different (P > 0.05). However, it was found that the frequency of coronary artery disease in men was significantly higher as compared to women (22.6% vs 4.1%). The findings revealed that the frequency of coronary artery disease in patients with chest pain was significantly higher while comparing with other patients (33.3%). Frequency of coronary artery disease in patients with myocardial infarction (50% versus 10.6%), angioplasty (80% vs 10.3%) and diabetes (32% versus 7.8%) was significantly higher than other patients without aspects (P < 0.05). However, other diseases did not have a significant relationship with the incidence of coronary artery disease. The frequency of coronary artery disease in patients with cigarette smoking was remarkably higher than non-smokers (27.3% vs 10%). The frequency of coronary artery disease in patients with a history of aspirin use (30.6% vs 4.5%), calcium blocker (22% vs 8.2%) and Plavix (85.7% vs 8.4%) %) was markedly higher as compared to other patients without the history of using the drugs. Regarding laboratory variables, it was found that only hematocrit in patients with coronary artery disease was significantly higher in comparison with patients without coronary artery disease.

Our findings demonstrated that the incidence of coronary artery disease in patients with ST-segment elevation was significantly lower in the lower part (100%) and the front part (75%) as compared to in other patients. The frequency of coronary artery disease in patients with ST-segment depression in the front part (100%) was found to be remarkably

increased as compared to patients without ST-segment depression.

The findings indicated that the most involved vessels in the patients simultaneously included LM, LAD, and RCA (each of 28.6%). Furthermore, patients experienced angioplasty after angiography (57.1%), and CABG after angiography (14.3%).

Regarding the results presented in Table 6, patients with severe mitral regurgitation (100%) had coronary artery disease after implantation of the pacemaker. In a study conducted by Brueck et al., in 2008, investigated the incidence of coronary artery disease in pacemaker insertion in 507 patients, where 212 (42%) with permanent pacemakers underwent coronary angiography with two months before or after the pacemaker insertion. They indicated that severe conduction disturbances or sinus node dysfunction could probably be associated with CAD, where myocardial revascularisation is needed. In this study, diabetes and hypercholesterolemia were predictors of coronary artery disease. The data show that patients with severe conduction disturbances or sinus node dysfunction have coronary artery disease, with at least one risk factor for atherosclerosis [5].

Based on the data presented herein, out of 102 cases, 88 cases (86.3%) were without the involvement, and 14 (13.7%) had coronary artery disease, which is much lower than study above. The reason for this difference may be due to the sample size and sampling methods, patient demographics, inclusion, and exclusion criteria. The study mentioned above-demonstrated diabetes as a cause of coronary artery disease, which was in concurrence with our observations.

Another study by Alai et al. was conducted to examine the incidence of coronary artery disease in patients requiring pacemaker implantation. They indicated that 45% of patients showed CAD, of which 29% revealed obstructive CAD, reaming 16% exhibited non-obstructive CAD. Furthermore, 53.3% of patients revealed SVD, followed by DVD (15.6%) and TVD (31.1%).

Type I (6.9%), II (34.5%), III (10.3%), and IV (48.3%) coronary anatomies were found in enrolled subjects. The presence of CAD is linked to the history of smoking, dyslipidemia, and family history of CAD. CAD angiography has been found in most subjects with symptomatic bradyarrhythmias and risk factors for CAD, as reported by Alai et al.

It can be said that such patients should undergo coronary monitoring before undergoing pacemaker surgery. Simultaneous CAD treatment is likely to improve the long-term prognosis of these patients [19]. The findings of the mentioned study on coronary artery disease are more than our study. Regarding effective risk factors, only smoking had a significant correlation with coronary artery disease. The reason for this difference may be due to the difference in the sample size, the difference in patient demographics, the difference in the sampling method and inclusion and exclusion criteria, as well as leaving the study. However, similar to our study, it was found that diabetes is a factor in the incidence of coronary artery disease.

Hsueh et al. investigated the incidence of coronary artery disease and its risk factors in patients requiring pacemakers. They found the following results: Regarding coronary angiography, the incidence rate of CAD has been determined as 20%.

The node-related artery was rarely seen among CAD patients with symptomatic bradyarrhythmia (9%), and the majority of subjects showed stenosis for LAD (74%). The baseline features and symptoms presented in patients with or without CAD were not statistically different.

Hypercholesterolemia and DM were found to be two independent predictors of CAD. Finally, it was concluded that hypercholesterolemia and DM were one of the most important predictors of CAD in these patients.

The node-related artery is rarely involved in patients with CAD and symptomatic bradyarrhythmia [14]. Meanwhile, in our study, it was found that 88 patients (86.3%) had no coronary artery disease, and 14 (13.7%) had coronary artery disease that was less than the study above. By our findings, it became clear that diabetes is a factor in the incidence of coronary artery disease. However, in contrast to the study, hypercholesterolemia in our study did not correlate with CAD incidence [14]. Ciaroni et al., investigate the incidence of coronary artery disease and its risk factors in patients requiring permanent transvenous pacemaker using dobutamine stress echocardiography, thallium-201 myocardial CT, and coronary arteriography during 8 days. None of these patients experienced myocardial infarction. Sixteen (55%) of patients was diagnosed as CAD in angiography. Diagnostic sensitivity for CAD was determined to be 94% for tomography and 88% for echocardiography, but this difference in CAD diagnosis was not found to be significant.

Among 13 enrolled subjects without CAD, 9 patients exhibited positive findings using tomography, where the specificity of 31% was achieved, on the other hand, one patient exhibited positive result based on the echocardiography, e.g., 92%. Therefore, dobutamine stress echocardiography was capable of increasing the rate of false-positive results in these patients and also maintains diagnostic sensitivity for CAD, consistent with myocardial tomography. In the study above, it was also found that male gender, diabetes, and positive family history were among the factors affecting the incidence of CAD in patients under pacemaker implantation (20). Meanwhile, our findings revealed that, among 102 included patients, 88 (86.3%) patients didn't show coronary artery disease, and 102 cases (13.7%) had coronary artery

disease, that was less than mentioned study The reason for this difference may be due to the sample size, demographic characteristics of the patients, the difference in the sampling method and inclusion and exclusion criteria and leaving the study. In line with our study, it appeared that diabetes and male gender were involved in the incidence of coronary artery disease.

Regarding the fact that some factors are positive in predicting the incidence of coronary artery disease in patients with pacemaker therapy, it is possible to reduce complications and reduce hospitalisation time by performing coronary angiography in essential cases without implementation in unnecessary cases, thus leading an acceptable impact on cost and patient satisfaction.

In conclusion, by proving the factors influencing the incidence of coronary artery disease in patients with pacemaker insertion can perform effective diagnostic procedures for high-risk patients, including angiography to prevent complications during and after pacemaker implantation and increase the quality of treatment in patients requiring a pacemaker.

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Clinical Science

Role of Matrix Metalloproteinase-9 in Neonatal Hypoxic-Ischemic Encephalopathy

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Abstract

BACKGROUND: Neonatal encephalopathy is a heterogeneous syndrome characterised by signs of central nervous system dysfunction in the newborn. Matrix metalloproteinase-9(MMP-9) increases the blood-brain barrier permeability, and their inhibitors can reduce its damage. MMP-9 has been implicated specifically in cerebral ischemia.

AIM: To measure serum MMP-9 in neonatal hypoxic-ischemic encephalopathy and evaluate its correlation to the severity of early prediction and treatment.

METHODS: its case-control study. The serum concentration of MMP-9 was determined by ELISA in 100 hypoxic neonates and 50 healthy neonates of matched age and sex who served as controls.

RESULTS: In our present study the serum MMP-9 level was significantly higher at p = 0.0001 in hypoxic-ischemic full-term newborns (176.7 ± 68.7 ng/ml)as compared to control newborn (69.4 ± 34.85 ng/ml)and it was significantly higher at p = 0.0075 in hypoxic-ischemic preterm newborn (171.2 ± 132.9 ng/ml) when compared to control newborn (72.54 ± 36.74 ng/ml),also MMP-9 was significantly higher at Sarnat stage III at p = 0.0001.

CONCLUSION: Serum MMP-9 level was significantly higher in hypoxic-ischemic newborns, and significantly increased with severity, so we suggest that serum MMP-9 level is important for predicting neurological sequel and severity in neonatal encephalopathy.

Introduction

Neonatal encephalopathy is a syndrome that has signs of central nervous system dysfunction in newborns and infants. Clinical suspicion of neonatal encephalopathy should be considered in any infant showing convulsions, an abnormal level of consciousness, feeding problems, apnea, aspiration, tone and reflex abnormalities, and hearing abnormalities [1], [2].

Perinatal asphyxia happens in 2 to 10 per 1000 newborn that are born at term, and neurological dysfunctions are present in 25% to 28% of the affected infants. Asphyxia is inversely related to gestational age and birth weight. It occurs in 9% of infants below thirty-six weeks of gestation and 0.5% of infants more than 36 weeks of gestation [3].

The incidence of hypoxic-ischemic encephalopathy in developed countries is 1.5 per 1000 live births, while it varies between 2.3-26.5 per 1000 live births in developing countries [4]. However, in most developing countries, including Egypt, the incidence and outcomes of hypoxic-ischemic encephalopathy are not well documented. One of the studies estimated the rate of neonatal mortality in Egypt as 25 per 1000 live births, almost all of the mortality occurring in the first week of life. Prematurity and its complications were responsible for the largest percentages of deaths (39%), followed by birth asphyxia and birth trauma as major causes [5].

Neonatal encephalopathy was once automatically referred to hypoxia-ischemia, but now it is well known that hypoxia-ischemia is one of many other possible mechanisms of neonatal encephalopathy. Whether a particular newborn's encephalopathy can be attributed to hypoxic-ischemic

brain injury is often not clear [6].

Stringent criteria were needed by some investigator for using the term neonatal encephalopathy, as ≥ 2 symptoms of encephalopathy lasing over twenty-four hours [7]. However, others need only a low 5-minute Apgar score [8]. Using of Apgar scores alone is problematic, as it may be low due to analgesia used by the mothers or premature labour or falsely normal in some cases of acute hypoxia-ischemic insult, or can be inflated in actual clinical practice [9].

Neonatal encephalopathy is mostly because of dysfunction of the central nervous system in full and preterm infants when neonatal encephalopathy is due to hypoxic-ischemic brain injury (anoxic brain insult) it is preferred to use the term hypoxic-ischemic encephalopathy (HIE) [10].

Early Estimation of the severity degree of HIE is important for tailoring the medical intervention to decide whether or not to perform potentially neuroprotective therapies [11].

Matrix metalloproteinases are a group of proteases that physiologically adjust the extracellular matrix and basement membrane reconstruction [12].

Matrix metalloproteinase-9 increases the permeability of the blood-brain barrier. Matrix Metalloproteinases inhibitors can reduce blood-brain barrier damage [13].

Matrix metalloproteinase-9 has been included specifically in cerebral ischemia [14].

Neuroprotection following of MMP-9 inhibitors activation has been demonstrated previously in the brain of an adult after cerebral ischemia by using MMP-9 inhibitors [15]. To our knowledge, there are no studies evaluating the level or role of MMP-9 in neonatal hypoxic-ischemic encephalopathy.

Our study aimed to measure the serum level of Matrix metalloproteinases-9 in neonatal hypoxic-ischemic encephalopathy and evaluate its correlation to the severity for early prediction and treatment, which in turn will reduce the financial burden of the country.

Subjects and Methods

Subjects

The present study was conducted 150 newborns admitted to neonatal intensive care units in Nasser general hospital.

They were divided into 2 groups

Group I:

Included 100 hypoxic neonates 70 of them are full-term, and 30 are preterm with a mean gestational age of ± 38 wks for full-term and ± 34 wks for preterm, 54 are males, and 46 are females, regarding the mode of delivery 37 were delivered with caesarean section and 63 by spontaneous vaginal delivery. They were diagnosed as having HIE as evidenced by the presence of at least 2 of the following criteria:

- Evidence of fetal distress (abnormal fetal heart patterns and meconium-stained amniotic fluid).

- Apgar score < 3 at one minute or < 6 at 5 minutes.

- Evidence of neonatal respiratory distress.

- Umbilical cord arterial pH < 7.2 with base deficit > 10 mmol/l.

- Abnormal neurological signs on examination are denoting HIE according to Sarnat staging [16].

Exclusion criteria

- Neonatal sepsis.

- Blood group incompatibility.

- Babies born to preeclamptic mother.

- Infant of diabetic mothers (IDM).

Group II

Included 50 healthy neonates 35 of them were full-term and 15 were preterm, 20 of them were males, and 30 were females with a mean age of ± 39 wks for full-term and 35 wks for preterm. Regarding mode of delivery, 28 were delivered with cesarean section and 22 by normal vaginal delivery, without signs of perinatal asphyxia.

Methods

All newborns included were subjected to the following:

- *Full maternal history taking*

- *Detailed antenatal and perinatal history.*

- *Clinical examination including* 1. Gestational age assessment: modified Ballard scoring system [17]; 2. Apgar scores estimation at 1 & 5 minutes to assess the presence of perinatal asphyxia [18]; 3. Vital signs*:* Blood pressure; 4. Head circumference; 5. Anthropometric measures; 6. Full cardiac, chest, abdominal and neurological examination and 7. Detailed neurological examination including A) Level of consciousness; B) Activity; C) Neuromuscular examination: Tone, power, position and stretch reflex; D) Primitive reflexes (suckling, Moro, grasp, rooting, glabellar and neck rigidity reflexes); E) Irritability; F) Seizures (type, responses to anticonvulsant drugs) and G) Sarnat and Sarnat staging according to [16].

Laboratory investigations

Venous or capillary blood samples were withdrawn on heparinised tubes for blood gas assessment. Moreover, peripheral blood samples were collected three ml venous blood sample was collected from each patient into a plain tube. After clotting for 30 minutes, serum was separated by centrifugation for 15 minutes at approximately 1000 x g, and samples were stored at -20°C till the assay.

Methods of assay

Blood gases were analysed by the GEMpremier3000 system analyser.

Determination of serum MMP-9

The quantitative determination of serum MMP-9 was done using the commercially available ELISA kit supplied by R&D Systems.

Statistical analysis

Standard computer program SPSS for Windows, release 23 (SPSS Inc, USA) was used for data entry and analysis. All numeric variables were expressed as mean ± standard deviation (SD). Comparison of different variables in various groups was made using student t-test followed by Duncan's multiple range tests with $P < 0.05$ selected as the level of the statistical significance. Comparisons of multiple subgroups were made, and Data are presented as M ± SEM and analysed by one-way ANOVA followed by Tuckey Kramer post-test using Graph Pad Prism software. For all tests, a probability (p) less than 0.05 was considered significant.

Results

As mentioned before this study included 100 neonates with HIE and 50 healthy neonates of matched age and sex who served as controls.

Table 1: Descriptive Data of group I (HIE) (N = 100)

	Full Term N = 70		Preterm N = 30		P Value
Mean age (weeks)	38.8	± 1.436	33.9	±0.852	0.078
Mean weight (gram)	3220	± 142	2205	±279.3	0.254
Mean apgar 1 min	2.9	± 0.9679	2.8	±0.8333	0.0987
Mean apgar 5 min	5.8	± 0.894	5.65	±0.8751	0.254
Mean PH	7.173	± 0.0233	7	±0.067	0.667
Mean MMP-9 (ng/ml)	176.7	± 168.7	171.2	±132.9	0.98

P < 0.05 was significant. Data are presented as mean ± SD; MMP-9: Matrix metalloproteinases-9; HIE: hypoxic-ischemic encephalopathy.

Descriptive, demographic and laboratory data of HIE patients and controls were shown in (Table 1, 2 and 3).

Table 2: Deceptive data of group II (control) (N = 50)

	Full Term N = 35	Preterm N = 15	Pvalue
Mean age (weeks)	39.93±11.335	35.25 ± 9.541	0.45
Mean weight (gram)	3981 ± 311	2002 ± 258	0.142
Mean apgar 1 min	7.133 ± 0.3519	6.65 ± 0.299	0.871
Mean apgar 5 min	9.267 ± 0.4577	8.787 ± 0.398	0.5412
Mean PH	7.373 ± 0.0045	7.11 ± 0.0023	0.0854
Mean MMP- 9 (ng/ml)	69.41 ± 34.85	72.54 ± 36.74	0.145

P < 0.05 was significant. -Data are presented as mean ±SD ;MMP-9: Matrix metalloproteinases-9.

Table 4 showed that serum MMP-9 was significantly higher in full-term cases than in controls.

Table 3: Descriptive data of both group regarding gender and mode of delivery

	HIE N (%) = 100			Control N (%) = 50		
	Full Term N (%) = 70	Preterm N (%) = 30	P Value	Full Term N (%) = 35	Preterm N (%) = 15	P Value
Gender (Male/Female	38(54.2)/32(45.7)	16(53.3)/14(46.6)	0.95	11(31.4)/24(68.5)	9(60)/6(40)	0.214
Mode of delivery (Normal/C-section)	56(80)/14(20)	7(23.3)/23(76.6)	0.458	14(40)/21(60)	8(53.3)/7(46.6)	0.121

P < 0.05 was significant. -Data are presented frequency (percentage); HIE: hypoxic-ischemic encephalopathy.

Also, there was a significant difference in mean PH and Apgar scores at 1 and 5 minutes between full-term cases and controls as PH was significantly lower in cases than controls, while there was an increase in Apgar score at 1 and 5 minutes in controls.

Table 4: Comparison between Full Term cases and control

	Cases (Full Term) N = 70	Control (Full Term) N = 35	P value
Mean age (weeks)	38.8 ± 18.436	39.93 ± 19.335	0.087
Mean weight (gram)	3220 ± 142	3981 ± 311	0.74
Mean apgar 1 min	2.9 ± 0.9679	7.133 ± 0.3519	0.0001
Mean apgar 5 min	5.8 ± 0.894	9.267 ± 0.4577	0.0001
Mean PH	7.173 ± 0.0233	7.373 ± 0.0045	0.0001
Mean MMP-9 (ng/ml)	176.7 ± 68.7	69.41 ± 34.85	0.0001

P < 0.05 was significant; Data are presented as mean ±SD; MMP-9: Matrix metalloproteineases-9.

Table 5 showed that serum MMP-9 was significantly higher in preterm cases than in controls. Also, there was a significant difference regarding mean PH and Apgar scores as PH was significantly lower in cases than controls while there was an increase in Apgar score at 1 and 5 minutes in controls.

Table 5: Comparison between Preterm cases and control

	Cases Preterm N = 30	Control Preterm N = 15	P value
Mean age (weeks)	33.9 ± 0.852	35.25 ± 9.541	0.98
Mean weight (gram)	2205 ± 279.3	2002 ± 258	0.254
Mean apgar 1 min	2.8 ± 0.8333	6.65 ± 0.299	0.0001
Mean apgar 5 min	5.65 ± 0.8751	8.787 ± 0.398	0.0001
Mean PH	7 ± 0.067	7.11 ± 0.0023	0.0001
Mean MMP-9 (ng/ml)	171.2 ± 132.9	72.54 ± 36.74	0.0075

P < 0.05 was significant; Data are presented as mean ± SD.; MMP-9: Matrix metalloproteinases-9.

According to Sarnat stages of HIE, There was a significant difference in mean MMP-9 in different stages as it was significantly higher in stage 3 when compared with stage 2 and 1 (Table 6).

Table 6: Comparison between MMP-9 levels in HIE group regarding Sarnat stages

	Stage 1	Stage 2	Stage 3	P-value
Mean MMP-9 (ng/ml)	123.5 ± 74.54	134.2 ± 89.39	189.2 ± 139.8	0.0001

P < 0.05 was significant; Data are presented as mean ± SD; MMP-9: Matrix metalloproteinases-9; HIE: hypoxic-ischemic encephalopathy.

Discussion

Neonatal encephalopathy is a syndrome that has signs of central nervous system dysfunction in newborns and infants. Clinical suspicion of neonatal encephalopathy should be considered in any infant showing convulsions, an abnormal level of consciousness, feeding problems, apnea, aspiration, tone and reflex abnormalities, and hearing abnormalities [1], [2].

Prematurity and its complications were responsible for the largest percentages of deaths (39%), followed by birth asphyxia and birth trauma as major causes [5].

Early Estimation of the severity degree of HIE is important for tailoring the medical intervention to decide whether or not to perform potentially neuroprotective therapies [11].

Matrix metalloproteinases are a group of proteases that physiologically adjust the extracellular matrix and basement membrane reconstruction [12].

Matrix metalloproteinase-9 increases the permeability of the blood-brain barrier. Inhibitors of matrix Metalloproteinases can reduce the damage to the blood-brain barrier [13].

Matrix metalloproteinase-9 has been implicated specifically in cerebral ischemia [14].

Neuroprotection following inhibition of MMP-9 activation has previously been demonstrated in the adult brain after cerebral ischemia by using MMP-9 inhibitors [15].

The present study was conducted 150 newborns admitted to neonatal intensive care units in Nasser general hospital.

Included 100 hypoxic neonates 70 of them are full-term, and 30 are preterm with a mean gestational age of ± 38 wks for full-term and ± 34 wks for preterm, 54 are males, and 46 are females, regarding the mode of delivery 37 were delivered with cesarean section and 63 by spontaneous vaginal delivery.

Fifty healthy neonates 35 of them were full-term, and 15 were preterm, 20 of them were males, and 30 were females with a mean age of ± 39 wks for full-term and 35 wks for preterm. Regarding mode of delivery, 28 were delivered with cesarean section and 22 by normal vaginal delivery, without signs of perinatal asphyxia.

Apgar score is one of the essential criteria for the diagnosis of perinatal asphyxia. Moreover, Apgar scores at 1 and 5 minutes were not significantly different between the cases and control in group l and group 2 newborns (Table 1 and 2). Table 4 & 5 showed that Apgar score at 1 minute (2.9 ± 0.9679) in full-term HIE newborn which was significantly lower at p = 0.0001 compared to control newborn (7.1 ± 0.351) ,and (2.8 ± 0.83) in preterm HIE newborn which was significantly lower compared to control newborn (6.6 ± 0.29), also at 5 minutes, it was (5.8 ± 0.89) in full-term HIE newborn which was significantly lower at p =0.0001 compared to control full-term newborn (9.26 ± 0.45) and it was (5.65 ± 0.87) in preterm HIE newborn who was significantly lower compared to control preterm newborn (8.78 ± 0.39). These results are in agreement with Mostert al., [19] who reported that Apgar score could differentiate between the control groups and hypoxic groups.

In our study pH was significantly different in preterm HIE newborns (7.0 ± 0.06) compared to control newborns (7.1 ± 0.0023) at p = 0.0001, also there was significant difference in HIE full-term newborns PH (7.1 ± 0.02) compared to control newborns (7.3 ± 0.0045) at p = 0.0001.These results are in a disagreement with khan et al., [20] who reported the insignificant difference in pH level in hypoxic and control groups. On the other hand this agrees with *Johnston et al.,* [21]*,Ferriero,* [22] *and Fahey and King,* [23] as they reported that low arterial umbilical cord pH had a strong, consistent, and temporal association with neonatal mortality and morbidity composite of hypoxic-ischaemic encephalopathy, seizures, and intraventricular haemorrhage or periventricular leucomalacia and long term outcome in the form of cerebral palsy.

In our present study the mean serum MMP-9 level was significantly higher at p = 0.0001 in HIE full-term newborns (176.7 ± 68.7 ng/ml)as compared to control newborn (69.4 ± 34.85 ng/ml)and level of serum MMP-9 was significantly higher at p = 0.0075 in HIE preterm newborn (171.2 ± 132.9 ng/ml) when compared to control newborn (72.54 ± 36.74 ng/ml). These results are in agreement with *Ashai et al.,* [15] who reported that on studying animal models of cerebral ischemia MMP-9 is regulated up early in injured tissue suggesting its involvement in neuronal death and brain damage. Moreover, MMP-9 inhibition through knockout models or drug treatments reduces infarction volume. On the other hand, this disagrees with *Sunagawa et al.,* [24] who studied serum levels of MMP-9/TIMP on the day of birth in asphyxiated newborns and they demonstrated no statistically significant difference in MMP-9 levels between asphyxiated newborns and controls.

According to Sarnat staging of HIE serum level of MMP-9 was 123.5 ± 74.54 ng/ml, 134.2 ± 89.39 ng/ml and 189.2 ± 139.8 ng/ml in stages I, II and III respectively, which was significantly higher at p = 0.0001 with worsening of symptoms and signs in hypoxic newborns. These results are in agreement with *Sunagawa et al*., [24] who reported that MMP-9 levels in asphyxiated newborns with neurological insult on birthday were significantly higher than those in asphyxiated newborn without sequelae also he suggested that high concentrations of MMP-9 on day of birth could injure the blood-brain barrier and causes

irreversible brain damage. Also, *Tsuji et al.,* [25] who tested MMP-9 upregulation after focal cerebral ischemia, found that patients with high plasma levels of MMP-9 experience more brain injury with poor outcomes after cerebral ischemia.

In conclusion, serum MMP-9 level is significantly higher in hypoxic-ischemic newborns, and significantly increased with severity, so we suggest that serum MMP-9 level is important for predicting neurological sequel and severity in neonatal encephalopathy.

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Clinical Science

The Difference of Angiopoietin-2 Levels between Dengue Hemorrhagic Fever Patients with Shock and without Shock

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Keywords: Angiopoietin-2; Dengue Hemorrhagic Fever (DHF); Shock

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Abstract

BACKGROUND: Dengue infection is one of the problems in the national health sector in Indonesia because the incidence and mortality in Indonesia due to dengue infection is still very high. In 2005 Indonesia became the highest contributor to dengue infection in Southeast Asia (53%) with 95.270 people and 1.298 deaths.

AIM: This study aims to analyse differences in angiopoietin-2 levels in dengue hemorrhagic fever (DHF) with and without shock.

METHODS: This study was a consecutive sampling design with the research subject was obtained based on the order of admission to the hospital — the serum Angiopoietin-2 levels using the ELISA method. The statistical test used is the independent t-test. The value of $p < 0.05$ was said to be statistically significant.

RESULT: The result showed that the mean of Angiopoietin-2 levels in DHF patients with shock was higher than in DHF ($p < 0.05$).

CONCLUSION: This study concluded that there was a difference in the average level of Angiopoietin-2 among DHF patients with shock compared to without shock.

Introduction

Dengue infection is one of the problems in the national health sector in Indonesia because the incidence and mortality in Indonesia due to dengue infection is still very high. In 2005 Indonesia became the highest contributor to dengue infection in Southeast Asia (53%) with a total of 95.270 people and 1.298 deaths [1]. Manifestations of bleeding, enlargement of the liver and increase in SGOT and SGPT were significantly more common in dengue shock syndrome (DSS) than in the DHF group [2], [3].

In dengue infection, the liver is also involved. Liver cell damage, in addition to the inflammatory process and the formation of antigen-antibody complexes, also because of the process of dengue infection. Infected hepatocytes increase the activity of SGOT and SGPT, release VEGF and reduce the synthesis of clotting factors [4], [5], [6]. Hepatomegaly in dengue infection results from the direct influence of the dengue virus on the liver. Data from previous studies also show that hepatomegaly plays an important role as a risk factor for severe dengue virus infection [7]. Angiopoietin-1 and Angiopoietin-2 have functions in maintaining vascular function and integrity. Angiopoietin-1 is reported to not only play a role in blood vessel stabilisation during angiogenesis but also plays a role in inhibiting vascular permeability and has anti-inflammatory effects [8]. m-RNA molecules Ang-1 expressed in per endothelial cells

play a role in maintaining blood vessel stability, inhibiting plasma leakage, suppresses the expression of inflammatory genes and prevents recruitment and migration of leukocytes. Unlike the case of Ang-1, Ang-2 which is selectively expressed in endothelial cells when binding to the Tie 2 receptor will result in signalling disorders of Ang-1 / Tie 2 resulting in impaired vascular permeability [9], [10].

This study aims to analyse differences in angiopoietin-2 levels in dengue hemorrhagic fever with and without shock.

Materials and Methods

This study was an observational study with a comparative cross-sectional design. The angiopoietin-2 examination was carried out in the Biomedical Laboratory, Faculty of Medicine, Andalas University, Padang.

Study Population

The study population was patients with dengue virus infection (DHF and DSS) who were hospitalised at Dr M. Djamil Central General Hospital, according to WHO 2011 criteria [11]. Subjects were part of the population that met the inclusion and exclusion criteria. The inclusion criteria were patients with dengue hemorrhagic fever who had received informed consent from parents to participate in the study with the age of 1-15 years. Exclusion criteria were patients suffering from other viral or bacterial infections based on clinical and laboratory examinations, receiving corticosteroid therapy, malnutrition and obesity.

Examination of Angiopoietin-2 Levels

Blood samples ± 2-3 cc (which is checked in the critical phase) that were inserted into the serum tube were sent to the Biomedical Laboratory, Faculty of Medicine, Andalas University using media transport at 4°C. Samples must be stored at freezing temperature if not checked directly after taking. Provide 8 strips for inspection. Dissolve serum and plasma with the Standard Diluent Buffer ratio 1:10. Then, add 25 ul Incubation Buffer to each sample. After that, add 100 ul Standard Diluent Buffer to the standard and add 100 ul standard, controls, or sample to the appropriate microtiter tube. Pipette a solution of 50 ul biotinylated Hu Ang-2 Biotin Conjugate into each reagent plate. Mix for 30 seconds. Cover the plate with a cover plate and incubate for 2 hours at the temperature. Pull or pour the solution from the plate and separate the solution, and then wash 4 times (according to washing instructions). After that, add 100 ul HRP Streptavidin solutions for each sample except empty chromagen (according to instructions for reagent preparation and storage). Cover the plate with a cover plate and incubate for 30 minutes at room temperature. Pull or pour the solution from the plate and separate the solution and wash 4 times (according to washing instructions). Then, add 100% stabilised chromogen in each case. The solution will turn blue. Incubate for 30 minutes at ambient room temperature and add 100 ul stop solution into each sample. Mix slowly. The solution will change colour to yellow. Then, read the absorbance at 450 nm on the readromagen device. The plot of results obtained into the standard.

Statistical analysis

Bivariate analysis was performed to see the difference in mean Angiopoietin-2 in DHF patients with shock and without shock. First, the data are analyzed using normality test to determine the normality of the data using the Shapiro Wilk test ($n < 50$), then followed by bivariate analysis, if the data is normally distributed then the analysis is done using the dependent test t-test, but if it is known to be not normally distributed then Mann-Whitney test was done with confident interval (CI) 95% and $\alpha = 0.05$. The conclusion of the test results if the value of $p \leq 0.05$ then H_0 is rejected, meaning that there is a difference in the mean between the independent variables and the dependent variable.

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.

Results

The difference in the results of Ang-2 examination between dengue sufferers with shock compared to those without shock can be seen as follows.

Table 1: Differences in the results of Ang-2 examination between dengue sufferers with shock compared to without shock

	DHF		
Variable	DSS (n = 62) mean ± SD	DHF (n = 48) mean ± SD	p-value
Ang-2 (pg/ml)	739.66 ± 55.21	497.90 ± 220.68	0.036*

Table 1 showed that the average level of Ang-2 in DHF patients with shock 739.66 ± 55.21 pg/ml while DHF 49790 ± 220.68 pg/ml. The results of statistical tests showed that there were differences in

the mean levels of Ang-2 among DHF patients with shock compared to those without shock ($p < 0.05$).

The cut-off point for Ang-2 levels as a predictor of dengue sufferers with shock

The cut-off point of Ang-2 levels as a predictor of dengue patients with shock is shown in Figure 1.

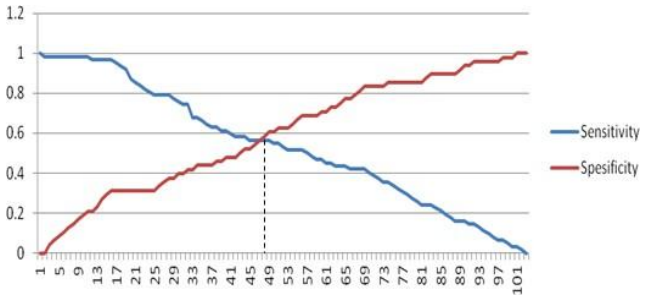

Figure 1: Point cut-off of Ang-2 levels as a predictor of DHF patients with shock

Figure 1 showed that the optimal cut-off points on the intersection of sensitivity and specificity lines to determine the cut-off point of Ang-2 levels as a predictor of DHF patients with shock is between point 48. The cut-off point of Ang-2 levels as a predictor of DHF patients with shock can be explained as follows subjects experienced DSS if the level of Ang-2 ≥ 427.56 pg/ml and subjects experience DHF if the level of Ang-2 < 427.56 pg/ml.

The cut-off point of the sensitivity of Ang-2 levels was 56.4%, and specificity was 58.3%. Accuracy of cut-off point Ang-2 levels as predictors of dengue patients with shock is shown in Figure 2 below.

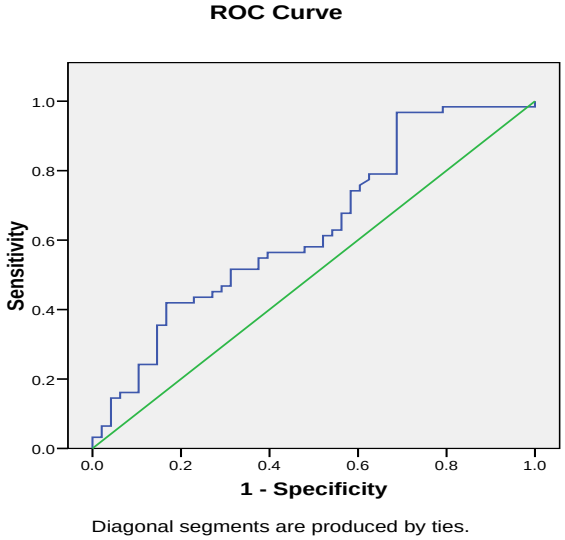

Figure 2: Accuracy of Cut-off point Ang-2 levels as predictors of DHF patients with shock

Based on the receiver operating curve analysis (ROC) it is known that it has an area under the curve (AUC) of 63.4%, which means that the cut-off points of Ang-2 which is ≥ 427.56 pg/ml have good accuracy in predicting DSS events.

Discussion

The difference in the results of Ang-2 examination between dengue sufferers with shock compared to without shock

Haematological parameters are considered as potential predictors, one of which is Angiopoietin. This study obtained Ang-2 levels of DHF patients with bigger shock than without shock ($p < 0.05$). This indicates that the higher the level of Ang-2, the more severe the disease due to dengue virus infection. The main complications of dengue infection mainly affect the vascular system and include plasma leakage, which will cause hypovolemic shock and potentially continue to become DSS. Angiopoietin can be considered a potential predictor for DSS in patients suffering from dengue infection. This is by the research conducted by Rampengan *et al*., (2015) who found that an increase in Ang-2 levels could trigger blood vessel instability, causing an increase in vascular permeability and plasma leakage. In this study, we found an increase in Ang-2 levels in DSS patients compared to DHF patients and dengue fever [12].

The mechanism for increasing Ang-2 levels and plasma leakage is a complex cascade. Binding of Ang-1 with the Tie-2 receptor via the phosphatidylinositol-3-kinase (PI3K) Akt pathway will cause blood vessel stability by recruiting pericytes into new blood vessels and maintaining intercellular contact. Ang-1 also has anti-inflammatory properties by regulating surface molecular adhesion. The binding of Ang-2 to the Tie-2 receptor can inhibit Ang-1 signalling to Tie-2 and increase blood vessel instability [13]. Mitchell *et al.* (2012) also found that DSS and DHF patients also had higher serum Ang-2 levels at baseline. Treatment compared to home care and compared to the control group. Release of Ang-2 from WPB is caused by activation of endothelial cells by proinflammatory cytokines, direct interaction of the virus with endothelial cells, the release of mast cell products and procoagulant factors such as thrombin [14].

Literature that has written normal levels of Ang-2 in children as long as the knowledge of researchers has not yet existed. The study by Rampengan *et al.,* (2015) obtained the highest Ang-2 level on DSS compared to DHF and dengue fever with a result of 2,486.21 pg/dl in dengue fever, 3,194.95 pg/dl in DHF and 4,005.32 pg/dl on DSS. In the research conducted by researchers concluded that if the level of Ang-2 ≥ 427.56 pg/ml, then the subject will experience DSS (12).

Increased levels of Ang-2 are also associated with death in sepsis. In a study by David *et al.* (2012), it was found that the concentration of Ang-2 has been reported as a predictor of mortality in critically ill patients. Ang-2 cut-off value of 2.86 ng/mL predicts the development of severe sepsis with a sensitivity of 75% and specificity of 56%, the cut-off value of Ang-2 3.56 ng/mL predicts the development of septic shock with a sensitivity of 73% and specificity 68%, and the Ang-2 cut-off value of 5.1 ng/mL predicted mortality with 80% sensitivity and 76% specificity. This research is important because it can estimate the level of Ang-2 can be used as a biomarker in clinical practice so that it can be used in clinical decision-making regarding prognosis [15]. The cut-off point illustrates the accuracy in predicting DSS events. The cut-off point value is taken from the optimal intersection point of the sensitivity and specificity line intersections used in predicting the occurrence of shock in DHF patients.

This study concluded that there was a difference in the average level of Angiopoietin-2 among DHF patients with shock compared to without shock.

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Clinical Science

The Levels of Vitamin D, Metalloproteinase-9 and Tissue Inhibitor Metalloproteinase-1 in COPD Patients, Healthy Smokers and Non-Smokers of Indonesian Citizens

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Keywords: Vitamin D; MMP9; TIMP1; COPD

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Abstract

BACKGROUND: Exposure to cigarette smoke may stimulate the inflammatory response and activate polymorphonuclear leukocytes, thus resulting in secretion of cellular proteases. Vitamin D has the potential to modulate the inflammatory response to harmful particles in patients with Chronic Obstructive Pulmonary Disease (COPD).

AIM: This study aimed to determine the levels of vitamin D, MMP-9, and TIMP-1 in COPD subjects, healthy smokers and nonsmokers of Indonesian citizens

METHODS: Seventy-eight male subjects took part in this study. They comprised three groups, i.e. COPD (n = 26), healthy smokers (n = 25) and healthy non-smokers (n = 27). Serum 25(OHD) levels, MMP-9, and TIMP-1 concentrations measured by electrochemiluminescence binding assay (ECLIA) and enzyme-linked immunosorbent assay (ELISA).

RESULTS: The levels of vitamin D in COPD (21.96 ± 6.62ng/mL) and healthy smokers (27.87 ± 7.08 ng/mL) were significantly ($p < 0.001$) lower compared to that in healthy non-smokers (31.71 ± 9.24 ng/mL). On contrary, the levels of MMP-9 in COPD (11.98 ± 41.54 ng/mL) was significantly ($p = 0.003$) higher compared to that in healthy smokers (2.23 ± 3.39 ng/mL) and healthy non-smokers (0.89 ± 1.12 ng/mL). Whereas the levels of TIMP-1 in healthy smokers (24.64 ± 57.77 ng/mL) was significantly ($p < 0.001$) lower compared to that in COPD (58.40 ± 77.53 ng/mL) and healthy non-smokers (46,54 ± 71,48 ng/mL).

CONCLUSION: The present study showed the lowest level of vitamin D, the highest level of MMP-9 and TIMP-1 in the COPD subjects.

Introduction

Vitamin D has reported playing a role in various body functions outside of the musculoskeletal system [1], [2] including pulmonary maturation [3]. While vitamin D deficiency can increase the risk of many diseases such as the musculoskeletal, cardiovascular and respiratory systems [3]. The Southeast Asia Nutrition Survey (SEANUTS) for children conducted during the 2010 / 2011 period in Indonesia, Malaysia, Thailand, and Vietnam [4], found that vitamin D levels did not differ between countries except Indonesia, which has a much lower level. In all countries except Vietnam, girls have vitamin D levels lower than boys after correcting age and area of residence. Urban children have lower levels of vitamin D, except in Indonesia [4]. Similarly, for adult women in Indonesia, the level of vitamin D for the rural group (20.24 ± 4.43 ng/mL) was greater than the urban group (14.9 ± 3.64 ng/mL), [5]. Various reports mentioned that patients with chronic obstructive pulmonary disease (COPD) have a low level of vitamin D [6], [7], [8], [9], [10] including the last reported by British researchers [11]. How do vitamin D levels in people with COPD in Indonesia?

Vitamin D has the potential to modulate the inflammatory response to harmful particles in patients with COPD [6]. It relates vitamin D deficiency to disease severity based on the assessment of lung function FEV1 [7], [9], [12]. In the study of Lange et

al., 2012, pulmonary function FEV1 decreased twice in smokers with vitamin D deficiency compared to smokers without vitamin D deficiency. Chronic obstructive pulmonary disease (COPD) is more common in smokers and former smokers than in non-smokers [13].

Cigarette smoking is a major risk factor for COPD. Exposure to cigarette smoke may stimulate the inflammatory response and activate polymorphonuclear leukocytes, thus resulting in secretion of cellular proteases [14]. Neutrophils produce MMP-8 and MMP-9 enzymes, while macrophages produce MMP-9 as the main proteolytic enzyme that can degrade extracellular matrix and elastin fibres [15], [16]. Many MMPs activated by smoking and oxidative stress [13]. In basal conditions, polymorphonuclear leukocytes of COPD patients released significantly more MMP-9 compared with polymorphonuclear leukocytes of healthy controls ($P = 0.016$) [14].

However, MMP-9 activity inhibited by tissue metalloproteinase (TIMP) inhibitors under normal circumstances, especially by TIMP-1, which shows it can bind to the active form and precursor form MMP-9. In smokers of COPD patients, the possibility of TIMP production does not inhibit the action of MMP-9 that occurs emphysema [15]. Therefore, COPD characterised by an imbalance between MMP-9 and TIMP-1, which may play an important role in the pathogenesis of tissue remodelling and airway obstruction [13].

MMP-9 exploded in COPD patients, and healthy smokers compared to healthy non-smokers. While TIMP-1 increases more in healthy non-smokers than COPD patients and healthy smokers [13]. The previous report showed that MMP-9 and TIMP-1 significantly increased in the serum of patients with COPD [17]. The results of the meta-analysis show that high MMP-9 and TIMP-1 protein levels can correlate with the pathogenesis of COPD, and both proteins can be important biological markers for the initial diagnosis of COPD [17].

Vitamin D can inhibit MMP-9 production, and thus, a deficiency of vitamin D can cause an increase in lung parenchymal degradation by MMP-9 [18].

Therefore, it is necessary to research how vitamin D, MMP-9, and TIMP-1 levels in COPD, healthy smokers and nonsmokers of Indonesian citizens.

Material and Methods

We conducted this case-control study after being approved by the Ethics Committee of the University of North Sumatra, and after study, participants gave written approval.

Seventy-eight male subjects of an Indonesian citizen, aged 40-65 years, took part in this study. They comprised three groups, namely: 1, COPD group, 26 subjects were stable COPD outpatients [using (GOLD 2013) criteria], based on chest X-rays and spirometry results of at least 200 cigarettes had been smoked throughout their lives; 2, healthy smokers, 25 healthy subjects who had no abnormalities in the lungs, were known by spirometry examination, and 3, healthy non-smokers, 27 subjects were healthy people who did not have abnormalities in the lungs, known with spirometry examination, and not smoking;

We exclude subjects if they have other lung diseases, hypothyroidism, or if they have a history of taking drugs that can affect calcium and vitamin D metabolism.

We performed spirometry examination using the Minispir New spirometer (MIR-Medical International Research, Italy) MIR Spirobank II spirometer. Serum vitamin D was assessed by quantitative determination of 25-hydroxyvitamin D (25-OHD) by the electrochemiluminescence method using Elecsys Cobas® total vitamin D reagent according to the manufacturer's instructions. Serum 25-OHD concentrations of less than 20 ng/ml are considered as deficiencies, insufficiency 20-29 ng/ml and 30 ng/ml and more sufficiency [6].

MMP-9 and TIMP-1 levels were examined by human Qayee-Bio® MMP-9 ELISA (enzyme-related immunosorbent test).

Statistical analysis

We express all data as mean ± standard deviation. We analysed comparisons between groups with Kruskal Wallis (one-way); $p < 0.05$ was considered significant.

Results

We can see the normality of data for each variable level of vitamin D, in Table 1. Based on the Shapiro-Wilk test for normality test, we found that only vitamin D levels in the COPD group, they normally distributed only vitamin D levels in the COPD group.

Table 1 above also shows a significant difference ($p < 0.05$) for the mean level of vitamin D, MMP-9, and TIMP-1 among subjects with COPD, healthy people who smoke and healthy people who are not smokers.

Healthy smokers followed low levels of vitamin D in COPD subjects and the highest in healthy non-smokers (Table 1). We showed the opposite in

MMP-9 levels, where the highest MMP-9 levels in COPD subjects followed by healthy smokers and the lowest in healthy non-smokers (Table 1) whereas a group of healthy non-smokers followed the highest TIMP-1 level in the COPD group and the lowest in healthy smokers (Table 1).

Table 1: Normality of vitamin D, MMP-9 and TIMP-1 levels in COPD subjects, healthy smokers and healthy non-smokers

	COPD (n = 26)	Healthy Smoker (n = 25)	Healthy Non-Smoker n = 27)	p-value
Vitamin D 25(OH)D (ng/mL)				
Mean	21.96	27.87	31.71	< 0.001
Median	21.35	27.44	30.21	
SD	6.62	7.08	9.24	
Minimum	3.38	17.19	19.97	
Maximum	32.72	53.80	60.60	
95% CI	19.28 – 24.63	24.95-30.79	28.06-35.37	
p (Shapiro-Wilk)	0.35	0.001	0.02	
MMP-9(ng/mL)				
Mean	11.98	2.23	0.89	0.003
Median	1.74	0.99	0.50	
SD	41.54	3.94	1.12	
Minimum	0.25	0.21	0.08	
Maximum	214	19.7	4.5	
95% CI	-4.80-28.76	0.61-3.86	0.44-1.33	
p (Shapiro-Wilk)	< 0.001	< 0.001	< 0.001	
TIMP-1(ng/mL)				
Mean	58.40	24.64	46.54	< 0.001
Median	17.43	5.66	12.6	
SD	77.53	57.77	71.48	
Minimum	10.66	1.66	0.28	
Maximum	297.2	290	327	
95% CI	27.09-89.72	0.80-48.49	18.27-74.82	
p (Shapiro Wilk)	< 0.001	< 0.001	< 0.001	

Discussion

Although previous studies have suggested that vitamin D levels in Indonesian society are insufficient in children and adult women, in the group of healthy non-smoker Indonesian men, the level of vitamin D is sufficient. Sari et al., (2017) found that the level of vitamin D in adult Indonesian women from rural area (20.24 ± 4.43 ng/mL) is higher than those from urban area (14.9 ± 3.64 ng/mL), it turns out this does not apply to however all urban male subjects took part in the present study, ie: healthy nonsmokers (31.71 ± 9.24 ng/mL) and COPD patients (21.96 ± 6.62 ng/mL) are not deficient. The difference in vitamin D levels in adult Indonesians is like that reported in Indonesian children [4].

The low level of vitamin D in the COPD group aged between 40-65 years in this study is under other previous research reports, apart from the influence of age, as stated by Heidari et al. (2015). Many studies suggest that vitamin D deficiency is common in all COPD patients [6], [7], [8], [9], [10] including the last report from British researchers [11]. Also, there is a dose-response relationship between vitamin D levels and pulmonary function FEV1 [6], [9].

People with COPD have a high risk of vitamin D deficiency for low intake of vitamin D from food, decreased synthesis of vitamin D along with aging of the skin, lack of outside activity and lack of sun exposure, increased glucocorticoid catabolism, disorders activation because of renal dysfunction, and decreased muscle mass [8] while Indonesian researchers found that predictive factors independently related to the risk of low levels of vitamin D were sun exposure, work, and intake of vitamin D [5].

Matrix metalloproteinases (MMPs) are a large group of calcium-dependent zinc-containing endopeptidases it mainly concerns which with the remodelling of tissue along with degradation of the extracellular matrix. In the clinical trials sectors, we examine various MMPs along with to import the properties of being a high biomarker in various disorders such as COPD [16].

MMP-9 is the main elastolytic enzyme produced by stromal cells such as alveolar and neutrophil macrophages, which play a major role in lung diseases such as COPD. Increased serum MMP-9 concentrations in COPD subjects illustrate increased proteolytic activity associated with disease severity [19]. In the present study, they find the highest MMP-9 levels in the COPD subjects (11.98 ± 41.54 ng/ml) and the lowest in the healthy subjects who did not smoke (0.89 ± 1.12 ng/ml). The findings in the present study are like those reported by Esa et al., (2014). Constantly raised the amount of it may involve MMP-9 in the degradation of extracellular matrix in the lungs as seen in COPD patients [14].

Although the results of our study are in line with those reported by Esa et al., (2014) but the MMP-9 levels in this study were lower than those reported, both in the COPD subjects (11.98 ± 41.54 ng/ml vs 194.4 ± 100.6 ng/ml), healthy smoker (2.23 ± 3.94 ng/ml vs 104.5 ± 42.1 ng/ml) or healthy non-smokers (0.89 ± 1.12 ng/ml vs 34.5 ± 36.1 ng/ml). It is probable that differences in nutritional status, race, genetics, gender, location of the area between the subjects may cause this studied. This finding is in line with the previous report [3], which found that sputum MMP-9 concentrations increased in COPD subjects compared to those who had never smoked but were similar to healthy smokers.

MMP activity is regulated by proteolytic activation of the inactive proenzyme and through inhibition of active enzymes by TIMP. TIMP-1 binds both the active and precursor form of MMP-9 in a ratio of 1: 1 and selectively inhibits enzyme activity [20]. In the study of Esa et al., (2014), it shows that MMP-9 levels may relate to the severity of increased according to COPD severity, while TIMP-1 levels did not change, this may cause MMP-9 / TIMP-1 ratio to be greater than one. Mild COPD has an MMP-9 / TIMP-1 ratio of less than one (Esa et al., 2014). The COPD subjects who took part in our study seemed to classified as mild COPD with a small MMP-9/TIMP-1 ratio. However, the MMP-9 / TIMP-1 ratio value in healthy smoking and non-smoking subjects cannot attribute to the lung function. This study showed that TIMP-1 levels were highest in the COPD subjects

(58.40 ± 77.83 ng/ml) compared with the group of healthy non-smokers (46.54, 71.84 ng/ml) and the lowest in the healthy smoker group (24.64 ± 57.77 ng/ml).

Regarding TIMP-1, we found that the COPD group showed the highest average TIMP-1 compared to the healthy group of non-smokers and the healthy smoker group (table 3). This result is not inherent with the previous report. It shows increased tIMP-1 more in healthy non-smokers (192.7 ± 37.7 ng/ml) than healthy smokers (145.3 ± 35.1 ng/ml) and COPD patients (115 ± 55.5 ng/ml) (Esa et al., 2014). The lowest value of TIMP-1 in the study of Esa et al. (2014) found in COPD subjects may relate to the severity of the disease. While the present study showed the low level of vitamin D in COPD followed by the high level of MMP-9 but it does not reduce the TIMP-1, this might be the severity of COPD subjects took part in the present study is mild.

Vitamin D plays a role in regulating human lung fibroblast functions in wound repair, and tissue remodelling through not only inhibiting IL-1β stimulated MMP-9 production and conversion to its active form but also inhibiting IL-1β inhibition on TIMP-1 production [18]. Vitamin D deficiency can not reduce MMP-9 activity, which results in increased lung parenchymal degradation [18].

In conclusion, the present study showed that the levels of vitamin D were lowest in COPD subjects compared to that in healthy smokers and healthy non-smokers. The levels of MMP-9 and TIMP-1 were highest in COPD subjects of an Indonesian citizen.

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Clinical Science

Echocardiographic Parameters Correlated with Age in Isolated Severe Rheumatic Mitral Stenosis Patients in Indonesia

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Abstract

BACKGROUND: Despite the high prevalence of rheumatic mitral stenosis (MS) in Indonesia, the impact of aging on the anatomical and hemodynamic component of rheumatic MS is not well studied.

AIM: To analyze the association of age with various echocardiographic parameters in patients with isolated severe rheumatic MS in Indonesia.

METHODS: A cross-sectional study was conducted enrolling 263 subjects with isolated severe rheumatic MS who underwent transthoracic echocardiography (TTE) during January 2015 until December 2017 at National Cardiovascular Center of Harapan Kita, Jakarta, Indonesia. Demographic data were collected, and echocardiographic variables were measured based on standard TTE examination using GE Vivid 7 and S6 Doppler Echocardiography System (GE Medical System, Norway).

RESULTS: Of 263 subjects, there are 84 men and 179 women aged 18-80 (mean age 42.9) years old. Most patients had atrial fibrillation (80%), with a higher prevalence of AF in the older group. Age was positively correlated with LA diameter and Wilkin's score ($r = 0.186$, $P = 0.002$; $r = 0.142$, $P = 0.022$; respectively); while mean MVG ($r = -0.304$, $P < 0.001$), TR Vmax ($r = -0.126$, $P = 0.04$), TR maxPG ($r = -0.127$, $P = 0.039$) and TAPSE ($r = -0.125$, $P = 0.044$) were correlated negatively with age. Mean MVG has the strongest correlation with age in our subjects.

CONCLUSION: This is the first study in Indonesia that analyze the association of age and different echocardiographic parameters in isolated severe rheumatic severe MS patients. Age has a significant correlation with mean MVG, LA diameter, Wilkin's score, TR Vmax, TR maxPG, and TAPSE. We assume that the association of age and these parameters were influenced by the normal aging process and progression of chronic MS.

Introduction

Mitral stenosis (MS) is characterized by a decrease in mitral valve (MV) orifice area, causing blood flow obstruction from left atrium to left ventricle. The consequence is stagnation of blood proximal to the MV that results in the elevated pressure of left atrium, pulmonary venous, pulmonary artery, and right heart [1], [2]. Mitral stenosis is most commonly caused by rheumatic heart disease (RHD) with typical rheumatic features such as commissural fusion, leaflet thickening and calcification that primarily affects the leaflet tips, and chordal fusion and shortening [3]. Mitral stenosis is highly prevalent in developing countries because of its association with the prevalence of rheumatic fever, although degenerative MS is now more prevalent in the developed countries [4], [5]. The incidence and prevalence of RHD vary greatly among different age groups and regions of the world. The global prevalence of RHD is around 1 per 1,000 in children aged 5-14 years. There is no current data on the prevalence of rheumatic MS in Indonesia, but based on the study by Carapetis *et al.*, Indonesia was included in the Asia region (East and Southeast Asia, excluding China and Japan) which had a prevalence of 0.8 per 1,000 [4].

The diagnosis of rheumatic MS is made

based on clinical examination and echocardiography findings. However, most symptoms occur at the later stage of the disease, which usually leads to late diagnosis and increased morbidity and mortality. Most RHD cases we face in the clinics in Indonesia are greater than moderate in severity due to low health literacy of the low-income population. Echocardiography has been used widely to confirm the diagnosis, determine the aetiology of MS and its severity, and evaluation of other valve lesions. It can also provide more detailed information which helps to decide for the management, whether it is percutaneous or surgical intervention [6].

Rheumatic MS is more common in female and typically presents in the third or fourth decade of life while degenerative MS in the seventh and eighth decades [7]. Aging itself leads to significant cardiovascular structural changes, which may impact the pathologic process of rheumatic MS. Despite the high prevalence of rheumatic MS in Indonesia, the impact of age and ageing process on the anatomical and hemodynamic component of rheumatic MS is not well studied.

Our study mainly focused on echocardiographic data (anatomical, hemodynamic and heart function) of isolated severe rheumatic MS cases in National Cardiovascular Center Harapan Kita, Jakarta, Indonesia, and the correlation of age with various echocardiographic parameters.

Methods

We conducted a cross-sectional study using data from the echocardiography registry of valvular heart disease at Harapan Kita National Cardiovascular Center, Jakarta, Indonesia. Total of 263 patients with a confirmed diagnosis of isolated severe rheumatic MS that underwent transthoracic echocardiography examination from January 2015 to December 2017 was included in the present study. Diagnosis of rheumatic MS was confirmed based on WHO criteria and 2014 AHA/ACC Valvular Heart Disease Guideline [8], [9]. Severe MS is defined by a mitral valve area ≤ 1.0 cm^2 either by planimetry or PHT method [2]. In this study, we only included severe isolated rheumatic MS patients without significant mitral regurgitation and aortic lesions. Patients with more than mild mitral regurgitation (MR) and / or aortic valve disease and history of previous percutaneous commissurotomy or surgical valve repair or replacement were excluded from this study.

Demographic data recorded was age, sex, body weight and height, body surface area (BSA) and blood pressure. Patients were classified as in sinus rhythm (SR) or atrial fibrillation (AF) based on their baseline electrocardiogram. For display purpose, several data were presented in two age categories, < 50 and ≥ 50 years old. This categorization was to show the value difference between younger and older subjects' group, cutoff age of 50 years old was chosen based on previous studies on aging and cardiovascular structural and functional changes [10].

Transthoracic echocardiography (TTE) examination was carried out in all cases using GE Vivid 7 and S6 Doppler Echocardiography system (GE Medical System, Norway). Echocardiographic examination was performed by experienced sonographers and calculated by two cardiologists. All echocardiographic parameters, including mitral valve area (MVA) by 2D planimetry and pressure half time (PHT) method, mean transmitral valve gradient (mean MVG), left atrial (LA) diameter, LA volume index (LAVI), left ventricular ejection fraction (LVEF), LV end-diastolic diameter (LVEDD), LV end-systolic diameter (LVESD), tricuspid annular plane systolic excursion (TAPSE), tricuspid regurgitation (TR) severity, TR maximal velocity (TR Vmax), tricuspid valve maximum gradient (TR maxP), pulmonary valve acceleration time (PV AccT), right ventricular outflow tract velocity time integral (RVOT VTI), Wilkin's score, presence of spontaneous echo contrast (SEC) and thrombus at LA were assessed based on recommendations from the latest American Society of Echocardiography guidelines [11], [12], [13].

Baseline data, including echocardiographic values, were expressed descriptively, and the correlations between age and all echocardiographic parameters were analysed by Pearson's correlation test. In all statistical analyses, $P < 0.05$ indicated a significant correlation between means.

Results

This study enrolled 263 patients, 84 men and 179 women, with an age range of 18-80 (mean age 42.9) years old. Seventy-five percent of the subjects aged under 50 years old. Most patients had atrial fibrillation (80%), but less than half of them (43%) had SEC at LA, and even only 42 patients (16%) appeared to have thrombus at LA when examined with TTE. Compared to the younger patients, older patients (> 50 years old) had a higher percentage of AF (96.9% *vs* 75.1%). The severity of TR varies with mild TR is the most common (43.3%) followed by moderate and severe TR (29.3% and 20.9%, respectively). All patients had severe MS based on both MVA planimetry and PHT values that ranged between 0.3-1.0 cm^2, with mean MVG as low as 1.7 mmHg and highest of 25 mmHg (mean 12.18 mmHg). Interestingly, 71 patients (27%) had mean MVG lower than 10 mmHg, with a bigger percentage at subjects aged ≥ 50 years old compared to < 50 years old (43.9% vs 21.3%). Baseline characteristics and

echocardiographic values are shown in Table 1.

Table 1: Baseline characteristics and echocardiographic values

Baseline characteristics (Total N = 263)	N (%) or Mean±SD
Age (years), mean ± SD	42.94 ± 10.05
Age (years)	
< 50	197 (75%)
≥ 50	66 (25%)
Gender	
Female	179 (68%)
Male	84 (32%)
Body height (cm)	157.6 ± 7.95
Body weight (kg)	53.2 ± 12.09
Atrial fibrillation	208 (80%)
LASEC	116 (44.1%)
LA Thrombus	42 (16.1%)
TR severity	
Trace	17 (6.5%)
Mild	114 (43.3%)
Moderate	77 (29.3%)
Severe	55 (20.9%)
Echocardiographic parameters	
Mean MVG (mmHg)	12.18 ± 4.08
MVA PHT (cm^2)	0.69 ± 0.18
MVA Planimetry (cm^2)	0.74 ± 0.24
Wilkin's score	7.90 ± 1.47
LAVI (ml/m^2)	126.26 ± 85.28
LA diameter (cm)	5.34 ± 0.89
LV EF (%)	60.02 ± 10.03
LV EDD (cm)	4.28 ± 0.68
LV ESD (cm)	2.93 ± 0.59
TAPSE (cm)	1.7 ± 0.48
TR Vmax (m/s)	3.63 ± 0.77
TR maxPG (mmHg)	55.28 ± 22.69
PV AccT (ms)	88.07 ± 22.15
RVOT VTI (cm)	11.19 ± 3.81

Abbreviations: LASEC, left atrial spontaneous echo contrast; LA, left atrium; TR, tricuspid regurgitation; MVG, transmitral valve gradient; MVA, mitral valve area; PHT, pressure half time; LAVI, left atrial volume index; LV, left ventricular; EF, ejection fraction; TAPSE, tricuspid annular plane systolic excursion; Vmax, maximal velocity; maxPG, maximum pressure gradient; PV AccT, pulmonic valve acceleration time; RVOT VTI, right ventricular outflow tract velocity time integral; EDD, end-diastolic diameter; ESD, end-systolic diameter.

The correlation test showed that age was positively correlated with LA diameter and Wilkin's score (r = 0.186, *P* = 0.002; r = 0.142, *P* = 0.022; respectively), while negatively correlated with mean MVG (r = -0.304, *P* < 0.001), TR Vmax (r = -0.126, *P* = 0.04), TR maxPG (r = -0.127, *P* = 0.039) and TAPSE (r = -0.125, *P* = 0.044) (Table 2).

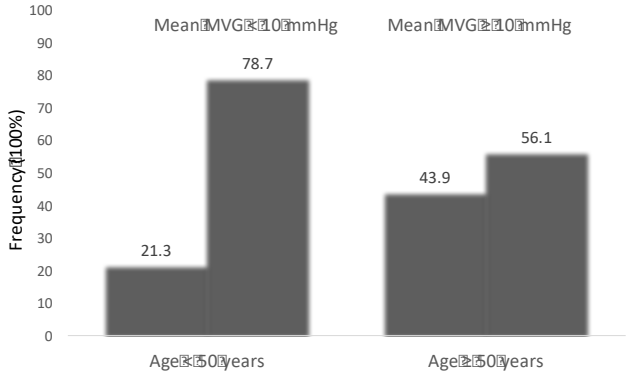

Figure 1: Frequency of subjects with means MVG < 10 mmHg and ≥ 10 mmHg in younger (< 50 years old) and older age (≥ 50 years old) groups

Among these parameters, mean MVG has the strongest correlation with age in our subjects. Majority of the younger subjects (< 50 years old) had higher mean MVG, 155 (78.7%) subjects with mean MVG ≥ 10 mmHg compared to 42 (21.3%) subjects with mean MVG < 10 mmHg. Meanwhile, this proportion shifted among older subjects (≥ 50 years old). The percentage of older patients with lower MVG was higher than the younger subjects, where 29 (43.9%) subjects with mean MVG < 10 mmHg and 37 (56.1%) subjects with mean MVG ≥ 10 mmHg (Figure 1).

Table 2: Correlation of age and various echocardiographic parameters

Parameters	Age (years)	
	r	*P*
Mean MVG (mmHg)	-0.314	<0.001*
MVA PHT (cm^2)	0.093	0.131
MVA Planimetry (cm^2)	0.012	0.853
Wilkin's score	0.142	0.022*
LAVI (ml/m^2)	0.098	0.113
LA diameter (cm)	0.186	0.002*
LV EF (%)	-0.017	0.779
LV EDD (cm)	-0.030	0.623
LV ESD (cm)	-0.008	0.900
TAPSE (cm)	-0.125	0.044*
TR Vmax (m/s)	-0.126	0.041*
TR maxPG (mmHg)	-0.127	0.039*
PV AccT (ms)	-0.046	0.462
RVOT VTI (cm)	0.061	0.333

*Statistically significant.

Discussion

In our study, there were more patients under the age of fifty years, with a percentage of 75% of the total sample. This is in accordance with the results of other studies which showed the highest prevalence of rheumatic MS is in adults aged 20–50 years [4], [14]. Both rheumatic MS and mitral annular calcification have been described in previous studies to be more common in women, two to four times more prevalent in women than men, whereas mitral regurgitation has similar prevalence between men and women [14], [15], [16]. Our study showed a marked female predominance in isolated rheumatic MS with a two-fold higher incidence in women than men. The scientific reasons for this female predominance had not been well explained, but some studies had proposed that it might be associated with social factors such as childbearing, which might increase exposure to group A streptococcus, access to health care, and genetic factors that predispose women to autoimmune diseases [14].

Mitral stenosis is associated with increased LA stiffness, LA remodelling, and abnormal contractility. In the setting of MS, LA enlargement due to pressure overload is usually secondary to increased LA afterload. This causes LA compliance reduction, increased LA and pulmonary pressures and right heart failure [17]. The impact of ageing on LA size had been established at the study by Nikitin *et al.*, LA diameter was increased with age with significantly higher LA diameter in the oldest subjects [18]. This supports our study finding that LA diameter had a significant positive correlation with age in our study, which means that the older the patient, the bigger or more dilated the LA.

Epidemiological studies had shown that generally, the prevalence and incidence of AF increased steeply after 65 years of age [19]. Atrial fibrillation and LA thrombus frequently complicate rheumatic mitral valve disease with 30-40% of patients had AF in long-term follow-up. The occurrence of AF correlates well with LA size; the incidence of AF increases from 3% when LA diameter is < 40mm to 54% if LA diameter is > 40 mm [20]. A study by Kim *et al.,* showed that the annual AF development rate was 3.5% in rheumatic MS patients with sinus rhythm that increased with LA size and MS severity. Meanwhile, MS patients with enlarged LA had an average AF development rate of 6.0% per year [21]. In rheumatic MS, enlarged LA combined with older age leads to very high AF prevalence as shown by our subjects. Eighty-percent of our subjects had AF, which is an even bigger percentage compared to previous studies. Almost all of older patients in our study had AF (96.9%). González-Torrecilla *et al.* found that the prevalence of SEC and thrombus at LA observed by TTE and transesophageal echocardiography (TEE) in MS patients with chronic AF is 52% and 29.5% [22]. In this study, the prevalence of SEC and thrombus is 43% and 16%. This lower prevalence in our study might be due to our method that only included results from TTE since thrombus at LA and LA appendage is easier to observe during TEE study.

Currently, there are no studies on the impact of age on the size of MVA by any measurement methods. MVA is one criterion to determine the anatomical severity of rheumatic MS, and in our study, we only included severe rheumatic MS patients (MVA ≤ 1.0 cm^2). Our study showed no correlation between age and both MVA planimetry and MVA PHT. We assumed that the degree of the valve stenosis is rather fixed throughout life and not being progressive over the years. Interestingly, Wilkin's score, that defines mitral valve score by calculating the individual subcomponent scores, including leaflet thickening and mobility, valve calcification, and subvalvular disease, is significantly correlated with age in our study. Ramakrishna and Kanattu had shown that despite the similarity in MVA between younger and older subjects, total mitral valve score < 8 was more common in the younger group with score > 11 was statistically more common in the older group. Older patients had higher leaflet calcification and subvalvular thickening score (> 2) compared to younger patients [23]. Older patients tend to have higher degenerative changes, although these changes might not have an impact on the degree of the mitral stenosis severity. Thus, older patients might not be suitable for percutaneous commissurotomy due to higher Wilkin's score. For cardiologists, this data will help decide the management of older rheumatic MS patients.

Mean MVG or the transmitral pressure gradient represents hemodynamic severity rather than anatomic severity and more closely associated with the patient's hemodynamic status. Mean MVG had the strongest significant correlation with age in our study (r = -0.314, *P* < 0.001). It is inversely correlated with age. Thus older patients had lower mean MVG. A recent study that supports our finding is by El Sabbagh *et al.,* which showed patients with low gradient severe MS (mean MVG < 10 mmHg) were older when compared to high gradient MS (mean age 65 ± 10 vs 56 ± 13 years old, *P* < 0.001) [24]. Although it had not been studied before, lower mean MVG in older patients might be due to several cardiovascular changes that happen with the normal aging process, including increased LV end-diastolic pressure, decreased LA compliance, higher heart rate and lower cardiac output [10]. Mitral stenosis itself increases LA pressure that leads to a reduction of LA compliance [17]. As chronic rheumatic MS patients get older, these factors will gradually lead to hemodynamic changes that cause lower mean MVG. Other factors that influence mean MVG are MVA, heart rate, cardiac output, LV and LA compliance, and other associated valve lesions. We excluded other significant mitral and aortic valve diseases, and our patients had similar MVA regardless of the age difference. Therefore, the mean MVG was not influenced by these factors in our study.

In patients with severe MS, persistently raised LA pressure results in pulmonary venous hypertension, reflex pulmonary arteriolar constriction, obliterative changes in the pulmonary vascular bed, pulmonary artery hypertension (PAH), RV hypertrophy and dilatation and tricuspid valve dysfunction [25]. Peak TR velocity (TR Vmax) is used to measure the pressure difference between the right atrium (RA) and RV using simplified Bernoulli equation (P = 4 $[TRmax]^2$) [26]. This method correlates well with PASP on right heart catheterisation [27]. Elevated TR Vmax (≥ 2.8 m/s) is highly accurate in correctly identifying pulmonary hypertension [28]. Our findings showed high TR Vmax and TR maxPG with low PV AccT and RVOT VTI, which all support the signs of pulmonary hypertension in rheumatic severe MS patients. Both TR Vmax and TR maxPG had a significant negative correlation with age (r = -0.126, *P* = 0.04; r = -0.127, *P* = 0.039; respectively). Several studies showed that pulmonary hypertension is greater in younger patients. Sinha *et al.* found that mean pulmonary arterial pressure and pulmonary vascular resistance are greater in juvenile MS patients compared to adults [29]. Studies by Tandon *et al.* and Ramakrishna and Kanattu showed that more severe pulmonary vascular changes and significant pulmonary hypertension were more common in younger patients [23], [30]. Lower TR Vmax and TR maxPG in elderly might also caused by greater RA and RV dilation which creates equalisation of RA and RV pressures, leading to underestimation of PA pressure by echocardiography.

Age is also correlated negatively with TAPSE in our study. TAPSE, which represents RV systolic

function, decreases with age [31]. RV hypertrophy and dilation due to chronic MS also leads to reduced RV function [25]. This pathophysiological changes in older rheumatic MS patients explain the mechanism of reduced TAPSE in this study.

In conclusion, this study is the first one that analyzes the correlation of age and different echocardiographic parameters in isolated severe rheumatic MS patients. Mean MVG had the strongest significant correlation with age in our study. Age had a significant positive correlation with LA diameter and Wilkin's score, while mean MVG, TR Vmax, TR maxPG, and TAPSE were found to be negatively correlated with age. The impact of the aging process on cardiovascular changes combined with a progression of chronic MS as the patient gets older leads to the association of those echocardiographic parameters with age. Our study results will help cardiologists, especially in a developing country who deal with more rheumatic MS patients, to understand more on the impact of ageing on the progression of rheumatic MS.

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Clinical Science

The Difference in Maternal Serum Hypoxia-Inducible Factors-1α Levels between Early Onset and Late-Onset Preeclampsia

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Abstract

BACKGROUND: Preeclampsia can be divided into early (EOPE) and late (LOPE) onset preeclampsia. Preeclampsia is related to the failure of placentation. Accumulation of hypoxia-inducible factors (HIF)-1α is commonly an acute and beneficial respond to hypoxia, while chronically elevated is associated with preeclampsia.

AIM: This study aims to evaluate the serum levels of HIF-1α in preeclampsia and normal pregnancy, and to compare the difference between early-onset and late-onset preeclampsia.

METHODS: A cross-sectional comparative study was conducted among a total of 69 pregnant women at ≥ 20 weeks of gestation, were recruited at obstetrics and gynaecology department at Dr M. Djamil Padang Hospital, network hospitals, health centres. They were divided into three groups early-onset preeclampsia, late-onset preeclampsia, and normal pregnancy. Preeclampsia was diagnosed using International Guidelines. Data were analysed by SPSS 24 program; data are presented as median and range or as mean ± standard deviation. One-way ANOVA test was used to determine the relationship between HIF-1α levels with the onset of preeclampsia.

RESULTS: The results showed that the mean maternal serum HIF-1α levels in early-onset preeclampsia (EOPE), late-onset preeclampsia (LOPE), and normal pregnancy were 1366.96 ± 733.40 pg/ml, 916.87 ± 466.06 pg/ml, and 716.77 ± 541.08 pg/ml. Serum HIF-1α levels were higher in early-onset preeclampsia (EOPE), and late-onset preeclampsia (LOPE) compared to normal pregnancy. Among preeclampsia patients, serum HIF-1α was higher in EOPE than LOPE women. Statistical analysis revealed a significant difference in mean maternal serum HIF-1α between early-onset preeclampsia, late-onset preeclampsia, and normal pregnancy ($p < 0.05$).

CONCLUSION: This study concluded that there is a significantly different level of HIF-1α between in early-onset preeclampsia, late-onset preeclampsia and normal pregnancy. Early-onset preeclampsia is the highest levels of serum HIF-1α.

Introduction

Preeclampsia is a multisystem disorder that occurs in about 2-10% of all pregnancies and most often causes maternal and fetal morbidity and mortality [1], [2]. The World Health Organization (WHO) reports 16% of maternal mortality caused by preeclampsia in developing countries [3]. Preeclampsia can be divided into sub-classifications: early-onset preeclampsia (EOPE) and late-onset preeclampsia (LOPE). The risk of maternal and fetal mortality in early-onset preeclampsia (EOPE) is significantly greater than that of late-onset preeclampsia (LOPE), this is due to the large majority of problems with severe placental perfusion and prematurity [4]. Most newborns from EOPE pregnancies experience premature and fetal growth restriction (FGR) [5], [6].

Based on the research which is placenta molecular basically, EOPE has oxygen level more than LOPE. Van der Merwe *et al*., (2010) report that placenta of EOPE is smaller has more infarct, and

unexact maturation, meanwhile placenta of LOPE has enhancement of decidual arteriopathy and placental solution [7].

EOPE occurs in 5-20% of all cases of preeclampsia associated with impaired fetal growth, pathological fetus, impaired uterine blood circulation, small placental size, preterm birth, and neonatal mortality whereas LOPE is around 75-80% of the total cases of preeclampsia associated with maternal mobility (such as metabolic syndrome, impaired glucose tolerance, obesity, dyslipidemia, chronic hypertension), with normal fetal weight and normal placental volume [7]. Although the aetiology of preeclampsia is unclear, abnormalities in the placenta are undoubtedly the pathogenesis of preeclampsia. The presence of abnormal placentations, including incomplete trophoblast spiral arteries invasion, plays an important role as the pathogenesis and pathophysiology of preeclampsia [1], [8].

Preeclampsia is related with failure of placentation, which is begin by the failure of arterial spiralis remodelling to the place of implantation. The pathological change of preeclampsia signed bg anti-angiogenesis, hypoxia, endosteal dysfunction, and interference of immunology's response, which cause disturbance of morphology and histopathology of many organs such as heart, lung, kidney, heart, brain and placenta [9].

Accumulation of hypoxia-inducible factor 1 α (HIF-1α) is commonly an acute and beneficial respond to hypoxia, while chronically elevated HIF-1α is associated with multiple disease conditions, including preeclampsia [10].

The initial phase of placentation happened under the relative hypoxia condition. HIF-1α as the marker of the cellular oxygen deprivation, expressed by its high level in the trophoblast. Persistent hypoxia or the failure to decrease the expression of transforming growth factor β3 (TGF-β3) after the ninth month of the pregnancy cause failure of trophoblast to differentiate from proliferative phenotype become invasive. The expression of HIF-1α is regulated not only by hypoxia but also by inflammation stimulation (for example, thrombin, peptide cytokine, like tumour necrosis factor (TNF), and reactive oxygen species (ROS), especially mediated by nuclear factor κB (NF-κB), as the promotor of HIF-1α contains binding site of NF-κB [9].

The availability of oxygen greatly influences placental function at all stages of pregnancy, and hypoxia-inducible factors (HIF) are the main mediator of placental adaptation [11]. During hypoxia, HIFs are stabilised and act as transcription factors [12]. During low oxygen conditions, HIF-1α is expressed high and helps placental development early in pregnancy. Excessive expression of HIF-1α has been observed in many inflammatory disorders, including cancer and preeclampsia [13].

The role of HIF-1α in the pathogenesis of preeclampsia is evidenced by Cannigia *et al*., (2000) which shows that HIF-1α is expressed more by the placenta with a low oxygen condition at initial gestation, then decreases at week 9 gestational age when oxygen levels begin to increase [14]. Likewise, with the research of Rajakumar *et al*., (2000) which states that HIF-1α plays an important role in the development and function of the placenta [15], [16]. Bobek *et al*. (2015) also researched mice and found that HIF-1α induced by cytokine imbalance, plays a role in the pathogenesis of preeclampsia [17]. Another study by Akhilesh *et al*. (2013) proves that HIF-1α levels in preeclampsia women are higher than controls [18].

Research on the relationship of HIF-1α levels in preeclampsia, especially in EOPE, where there is still not much research done, and the results are still controversial. The goals of this study were to evaluated the serum levels of HIF-1α in preeclampsia and normal pregnancy and to compare the difference between early-onset and late-onset preeclampsia.

Material and Method

Subjects

The study was conducted at Dr M. Djamil Hospital Padang, network hospitals and health centres. The study used a cross-sectional comparative study design by comparing the three study groups, namely early-onset preeclampsia, late-onset preeclampsia, and normal pregnancy to evaluated the serum levels of HIF-1α in preeclampsia and normal pregnancy and to compare the difference between early-onset and late-onset preeclampsia. Preeclampsia is defined as hypertension with minimum criteria for systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg after 20 weeks' gestation, and one or more proteinuria ≥ 300 mg / 24 hours or urine / creatinine protein ratio ≥ 30 mg / dl (1 + urine dipstick), renal insufficiency, hematological abnormalities, liver disorders, cerebral disorders, pulmonary edema. The number of samples was 69 samples that met the inclusion criteria (single pregnancy women, gestational age > 20 weeks, there were no severe medical abnormalities, no chorioamnionitis and no pregnancy with major congenital abnormalities), and exclusion criteria damage to blood samples during the research process and patients drop out during the research process). The study was conducted at Dr M. Djamil Padang Hospital, network hospitals and health centres. In patients who come in the third trimester, into the late-onset sample because sampling is done when the patient first arrives.

Samples

Patients who meet the inclusion and exclusion criteria will be interviewed to obtain characteristic data including name, age, identity number, address, contact number, history of pregnancy, the first day of the last day or ultrasound examination to assess gestational age. The size of the samples is calculated according to the simple formula to estimate the proportion of a population. The sampling formula used in unpaired numerical comparative research [19]. The sampling technique is done by the consecutive method, i.e. the samples are taken sequentially. By the inclusion criteria and exclusion until the required number of samples is fulfilled. After the patient signed a letter of informed consent, venous blood specimens were collected in the median cubital vein by folding the elbow by 10 ml. Next blood is sent to the laboratory for examination HIF-1α levels. Examination of HIF-1α levels using reagents from the Human Hypoxia Inducible Factor-1α ELISA Kit (Catalog No: E-EL-H1277, R & D System, USA). How to measure BMI before pregnancy is by asking the patient the weight and height of the patient before the detection of pregnancy. In this study, patients were not followed up until birth. This baby weight data is obtained from secondary data available.

Examination of HIF-1α Levels (Work protocol based on the Human HIF-1α ELISA Kit)

Prepare all reagents and samples at the examination room temperature. Add 100 ul, blank and sample into each well. Add 100 ul Biotinylated Detection Abworking solution into each well, cover with plate sealer. One-hour incubation at 37°C. Dispose of all fluids in the well. Wash well by adding 350 ul of wash buffer solution. Repeat three items of washing. Add 100 ul Avidin-Horseradish Peroxidase (HRP) Conjugate working solution to all wells. Cover with plate sealer. Incubate for 30 minutes at 37°C. Repeat the washing process as in step 5 five times. Add 90 ul of the Substrate Reagent solution to each well. Cover with a new plate sealer. Incubation for 15 minutes at 37°C. Avoid bright light. Add 50 ul Stop Solution to each well. Read the results of the inspection with the ELISA reader at a wavelength of 450 nm. Optical density (OD) values and concentration values of samples were examined.

Statistical Analysis

Data were analysed by SPSS 24 program; data are presented as median and range or as mean ± standard deviation. One-way ANOVA test was used to evaluate the serum levels of HIF-1α in preeclampsia and normal pregnancy, and to compare the difference between early-onset and late-onset preeclampsia, if a p-value of < 0.05 was obtained, a significant association was found HIF-1α levels with the onset of preeclampsia.

Results

The study was conducted on 69 patients consisting of 23 early-onset preeclampsia, 23 late-onset preeclampsia and 23 normal pregnancies. Sample characteristic of 69 patients, based on age, gestational age, parity, systole and diastole blood pressure, body mass index (BMI), and birth weight was shown in Table 1.

Table 1: Clinical characteristics of the study population

Characteristic	EOPE (n = 23)	LOPE (n = 23)	Normal (n = 23)
Age of pregnant women (year)	32.35 ± 7.33	32.96 ± 6.8	29.30 ± 2.18
Gestational age (week)	29.04 ± 3.37	36.91 ± 2.04	38.22 ± 0.67
Parity (%)			
- Nullipara	34.8	39.1	4.3
- Multipara	65.2	60.9	95.7
Systolic blood pressure (mmHg)	169.35 ± 20.90	160.43 ± 17.18	114.78 ± 5.11
Diastolic blood pressure (mmHg)	103.61 ± 12.32	100 ± 9.53	73.48 ± 4.87
BMI before pregnancy (kg/m^2)	28.52 ± 5.06	28.76 ± 6.04	25.08 ± 1.69
BMI during pregnancy (kg/ m^2)	32.18 ± 4.84	35.10 ± 6.56	31.02 ± 1.96
Birth weight (gr)	1363.48 ± 486.58	2945.00 ± 675.76	2730.43 ± 186.92

Based on the table, it is known that early-onset and late-onset preeclampsia have respondents with almost the same age, namely 32 years, whereas in normal pregnancies the average age is 29 years. In the early onset preeclampsia group, the systolic blood pressure is higher than the late-onset preeclampsia and normal pregnancy (169.35 ± 20.90 mmHg vs 160.43 ± 17.18 mmHg vs 114.78 ± 5.11 mmHg). Likewise, with diastole blood pressure found in the preeclampsia group, early onset is higher than late-onset preeclampsia and normal pregnancy (103.61 ± 12.32 mmHg vs 100 ± 9.53 mmHg vs 73.48 ± 4.87 mmHg). In the late-onset preeclampsia group, the mean IMT before pregnancy was higher than that of early-onset and normal pregnancy (28.76 ± 6.04 kg/m^2 vs 28.52 ± 5.06 kg/m^2 vs 25.08 ± 1.69 kg/m^2). Similar results were found in IMT during pregnancy (35.10 ± 6.56 kg/m^2 vs 32.18 ± 4.84 kg/m^2 vs 31.02 ± 1.96 kg/m^2).

Table 2: HIF-1α levels and the onset of preeclampsia

Variable	Preeclampsia Onset			P-value
	EOPE (n = 23)	LOPE (n = 23)	Normal (n = 23)	
HIF-1α (pg/ml)	1366.96 ± 733.40	916.87 ± 466.06	716.77 ± 541.08	0.003

As described in Table 2, there was the difference in maternal serum HIF-1α between groups early-onset preeclampsia, late-onset preeclampsia, and normal pregnancy, in which the mean level of maternal HIF-1α was 1366.96 ± 733.40 pg/ml, 916.87 ± 466.06 pg/ml and 716.77 ± 541.08 pg/ml. The mean of maternal HIF-1α level was statistically different between early-onset preeclampsia, late-onset preeclampsia and normal pregnancy (p-value < 0.05). There is a significant difference between the levels of HIF-1α and the onset of preeclampsia, as shown in Figure 1.

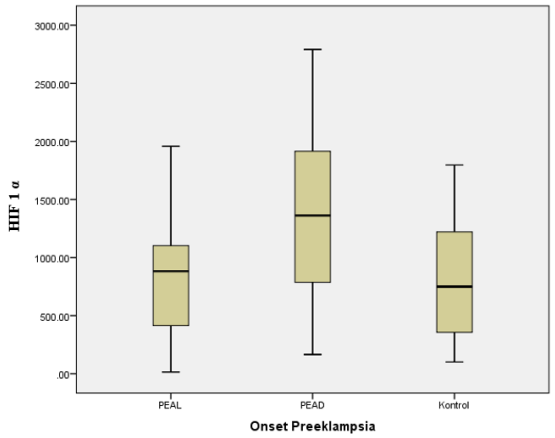

Figure 1: Boxplot Levels of HIF-1α with the Onset of Preeclampsia

From Figure 1, it is known that HIF-1α levels are higher in early-onset preeclampsia than late-onset preeclampsia and normal pregnancy.

Discussion

Although the aetiology of preeclampsia is unclear, abnormalities in the placenta are undoubtedly the pathogenesis of preeclampsia [1], [8]. Accumulation of hypoxia-inducible factor 1 α (HIF-1α is commonly an acute and beneficial respond to hypoxia, while chronically elevated HIF-1α is associated with multiple disease conditions, including preeclampsia [10].

The results of this study showed that the mean HIF-1α levels were significantly higher in both preeclampsia groups (early-onset and late-onset) compared to the normal pregnancy. Statistically, there is a significant difference with a value of p 0.003 ($p < 0.05$) between levels of HIF-1α and the onset of preeclampsia. Whereas research conducted by Rath *et al*., (2014) obtained ROC curve analysis, HIF-1α nucleus expression was significant in the preeclampsia group compared to the control group (P = 0.0001) with 88.9% specificity and 88.9% sensitivity, whereas ELISA obtained HIF-1α serum levels higher (mean = 6,581 pg/ml) in the preeclampsia group than in the control group (mean = 4,947 pg/ml) [13]. Kimura *et al*., (2012) measuring the proportion of positive nuclei of HIF-1α in placental trophoblast cells and found that staining of HIF-1α was significantly higher in both groups of preeclampsia than in the control group. However, the more staining was observed in women with early-onset preeclampsia compared to women with late-onset preeclampsia [20]. HIF-1α is a major transducer of hypoxic signals in several tissues, including the human placenta. HIF-1α is very highly expressed in low oxygen environments in early pregnancy and plays an important role in the development and function of the placenta. HIF-1α also shows overexpression in the placenta of preeclamptic women, together with soluble Fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (SEng) [21]. HIF-1 was transcripted by much dependent oxygen genes that code protein, which correlates to angiogenesis and cell metabolism [13]. HIF-1α plays a role in maintaining homeostatic state, inducing gene transcription such as VEGF and erythropoietin. When the level of oxygen is low, HIF-1α would express much more and helps placenta development on early pregnancies. Chaiworapongsa *et al*., (2014), considerable evidence supports a role for hypoxia in creating an environment that predisposes to implantation disorders, including preeclampsia: expression of HIF-1α and HIF-2α protein is increased in the placentas of women with pre-eclampsia [9]. During low oxygen conditions, HIF-1α is expressed high and helps placental development early in pregnancy. Excessive expression of HIF-1α has been observed in many inflammatory disorders, including cancer and preeclampsia [13]. This theory matches Rajakumar *et al*., (2003) that preeclampsia placenta got more over the expression of HIF-1α and HIF-2α compared to the normal placenta. Even explant villus oxygenation from preeclampsia placenta failed to lower HIF-1α and HIF-2α levels in vitro [15].

In this study it was found that HIF-1α levels were higher in EOPE than others, similar to the theory that the key mediator of this hypoxic condition is HIF-1α. HIF-1α is included in the description by many dependent oxygen genes that encode proteins that are associated with angiogenesis and cell metabolism [13], [22]. Likewise, with research conducted by Roflo *et al*., (2010), it was found that oxygen disorders occur in placenta EOPE, but not in placental LOPE [21]. Hypoxic conditions affect HIF-1α levels. HIF-1α is the main mediator, which is the cellular response to oxygen pressure under pathological and physiological conditions. Other researchers also proved that HIF-1α levels in preeclampsia women were higher than controls [18]. However, it is different from the study by Davutoglu *et al*., (2017) which found no significant difference between HIF-1α levels in preeclampsia and control, and found that HIF-1α levels were greater in LOPE. They suspect that the mechanism of inflammation and independent oxygen is the dominant form of increasing HIF-1α in late-onset preeclampsia [22].

In the condition of early-onset preeclampsia that last a long time, uterine artery blood flow is damaged, and the reduction in placental blood flow and the severity of hypoxic changes and DNA oxidation damage is greater in the placenta, causing fetal growth restriction (FGR), therefore most women preeclampsia with FGR experiencing preeclampsia early-onset and inadequate trophoblast cell invasion and remodelling of incomplete spiral arteries often occur in early-onset preeclampsia women [20].

There are a significant difference level of HIF-1α between in early-onset preeclampsia, late-onset

preeclampsia and normal pregnancy. Early-onset preeclampsia is the highest levels of serum HIF-1α.

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Case Report

A Unique Communicating Arterial Branch between the Celiac Trunk and the Superior Mesenteric Artery: A Case Report

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Keywords: Abdominal Aorta; Celiac Trunk; Superior Mesentery Artery; Anterior Inferior Pancreaticoduodenal Artery

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Abstract

BACKGROUND: Many anatomical variations have been associated with the Celiac Trunk, of which most are classified as being asymptomatic.

CASE PRESENTATION: In this article, we describe yet another anatomical variation involving the Celiac Trunk, Superior Mesenteric artery and the Inferior Pancreaticoduodenal Artery during routine cadaveric dissection. We identified a fourth branch of the Celiac trunk (quadrification) that communicated with the Superior Mesenteric artery at the point of origin of the Inferior Pancreaticoduodenal artery which we concluded to be the Anterior Inferior Pancreaticoduodenal artery.

CONCLUSION: This anastomosis could be essential in the case of occlusion between the Celiac Trunk and the Superior Mesenteric artery.

Introduction

Being well-informed of the common variations that occur in the branches of the abdominal aorta decreases the risk of vascular injuries to them during an invasive surgical procedure or other interventional approaches in the abdomen. Among the several identified branching patterns, the most frequently seen branching variations of the abdominal aorta occur at the Celiac Trunk (CT) and Superior Mesenteric Artery (SMA); they are usually asymptomatic [1], [2]. These variations are generally expressed as branches not arising from their known anatomical location and are later classified as an aberrant or accessory vessel.

The CT and the SMA are unpaired branches of the abdominal aorta which contribute to the abdominal viscera vasculature through a series of anastomoses in the region. The CT, which is the first ventral branch of the abdominal aorta, usually diverge from the level of T12-L1 vertebrae [1], [3] or from the level of T11-T12 vertebrae [4], where it normally gives out three terminal branches also named as Haller's tripod [5]: Common Hepatic Artery, Left Gastric Artery and Splenic Artery. These branches further supply the abdominal viscera derivatives of the foregut. Meanwhile, the SMA, the second ventral branch of the abdominal aorta, emerges from the lower border of the L1 vertebrae to give off branches that supply the abdominal viscera derivatives of the midgut [4].

Common variations of the CT branching pattern have been reported extensively in the

literature [Table 1]. However, another study has shown that the CT and SMA may arise from their normal origins and form a common trunk known as the celiacomesenteric trunk [6].

This case report shows a new branching variation of the CT not yet demonstrated in any literature to the best of our knowledge. It consists of an accessory branch, a communicating artery, arising from the CT and travelling directly into the SMA, in addition to the Haller's tripod. We attempt to explain how this new branching variation may have developmental correlations and impending clinical repercussions.

Table 1: Summary of the Celiac Trunk Branching Patterns Variants reported in the literature

Authors	Branching Pattern Variant	Number of Branches	Year	References
Cicekcibasi AE et al.	Left & Right IPA + LGA + SA + CHA + Left GEA	6	2005	[7]
Nayak SR, et al.	Left IPA + LGA + SA + GDA	4	2008	[7]
Mahajan A, et al.	RPA + LGA + SA + CHA + AHA	5	2009	[7]
Astik RB, Dave UH.	Left SSRA + Left MSRA + LGA + SA + CHA + Right IPA + GDA	7	2011	[7]
Kalthur SG, et al.	LGA + SA + CHA + DPA	4	2011	[7]
D'Souza AS, et al.	LGA + SA + CHA + AHA + APA	5	2012	
Nayak SB, et al.	Left IPA + LGA + SA + CHA	4	2012	[7]
Sathidevi VK, Rahul UR.	LGA + SA + CHA + GDA	4	2013	
Agarwal S, et al.	Left & Right IPA + LGA + SA + CHA + DPA	6	2016	[7]
Prasanna LC, et al.	CHA + GST (CT absent)	2	2016	[8]
Prasanna LC, et al.	CGPT + CHA + SA + LGA (CT absent)	4	2016	[8]
Daescu E et al.	Left IPA + LGA + Right AHA + CHA + SA	5	2017	[9]
Mahajan A, et al.	Left IPA + HGT + SA + CHA	4	2018	[5]
Hemamalini et al.	HMT (CT absent)	1	2018	[10]
Hemamalini et al.	LGA + CHA + SA + MCA	4	2018	[10]
Hemamalini et al.	LGA + CHA + SA + DPA	4	2018	[10]
Hemamalini et al.	LGA + CHA + SA + Left IPA	4	2018	[10]

CT: Celiac Trunk; LGA: Left Gastric Artery; SA: Splenic Artery; CHA: Common Hepatic Artery; IPA: Inferior Phrenic Artery; GEA: Gastroepiploic Artery; GDA: Gastroduodenal Artery; DPA: Dorsal Pancreatic Artery; AHA: Accessory Hepatic Artery; APA: Accessory Pancreatic Artery; SMA: Superior Mesenteric Artery; cA: communicating artery; GST: Gastrosplenic trunk; CGPT: Common Gastrophrenic Trunk; HGT: Hepatogastric Trunk. HMT: Hepatomesenteric Trunk; MCA: Middle Colic Artery; HST: Hepatosplenic Trunk; RPA: Retroportal Artery; SSRA: Superior Suprarenal Artery; MSRA: Middle Suprarenal Artery.

Case Report

A routine cadaveric dissection of the abdomen of a 78-year-old Caucasian male cadaver by medical students at the Anatomy laboratory, University of Medicine and Health Sciences, St. Kitts & Nevis revealed a cadaveric dissection with a CT quadrification anomaly. The dissection revealed the CT branches: Common Hepatic artery, Left Gastric artery and Splenic artery following their normal anatomical courses. Further exploration of the CT vessels revealed an additional branch which continued into the SMA with exploration.

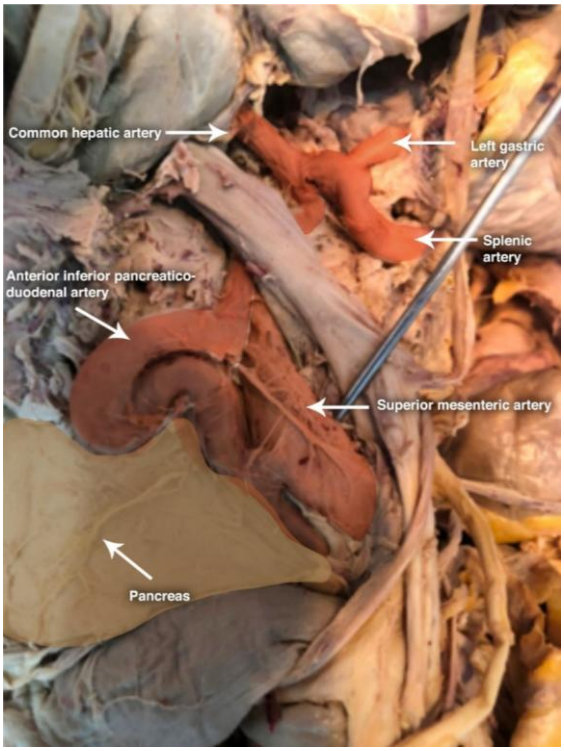

Figure 1: This figure shows the abnormal communication between the Celiac trunk (CT) and the Superior Mesenteric Artery (SMA), named the Anterior Inferior Pancreaticoduodenal Artery (AIPA)

This aberrant large vessel began at the Celiac Trunk and travelled inferiorly to communicate with the SMA at the region where the Inferior Pancreaticoduodenal artery branches off between the pancreas and the duodenum.

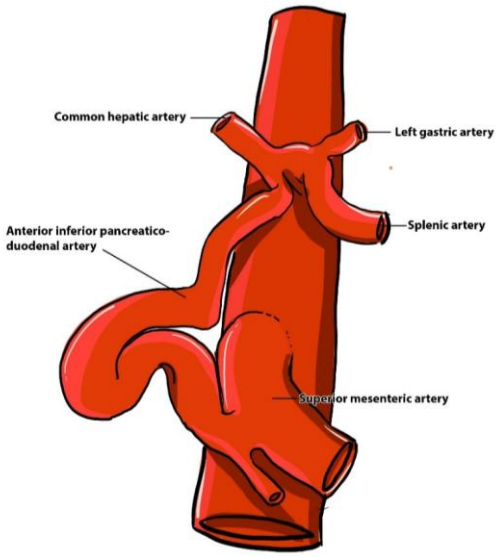

Figure 2: Schematic of the aberrant communicating Anterior Inferior Pancreaticoduodenal Artery (AIPA) with the Celiac trunk (CT)

Careful dissection revealed no other artery branching out within this region to supply the inferior part of the pancreas. With careful cleaning out of this aberrant communicating artery, we observed a bifurcation into anterior and posterior branches with the posterior branch running posteriorly to the pancreas to supply it. Thus, we concluded that the aberrant communicating artery was indeed the Anterior Inferior Pancreaticoduodenal artery (AIPA). Usually, the communication of the Inferior Pancreaticoduodenal artery is with the Superior Pancreaticoduodenal artery, a branch of the Gastroduodenal artery, which in turn is a branch of the Common Hepatic artery.

Discussion

The CT is the first of the three major branches of the abdominal aorta, which also includes the Superior Mesenteric and Inferior Mesenteric arteries, the CT branches off the Aorta at the T12/L1 level [11]. The CT is responsible for the blood supply to the lower part of the oesophagus, the stomach, the proximal duodenum, the liver and the pancreas, designated as the fore-gut [11].

The SMA bifurcates from the Abdominal Aorta at the level of L1 and is approximately 1 cm inferior to the celiac trunk. The SMA branches into the Inferior Pancreaticoduodenal, Intestinal, Middle colic, Right colic, and Ileocolic arteries. These arteries supply oxygenated blood to the jejunum and ileum [11].

The SMA is responsible for several looping and terminal branches in the middle abdominal area. These looping branches include the arterial arcades and vasa recta that supply the jejunum and ileum. The terminal branches include the Ileocolic artery that supplies the cecum and the Appendicular artery that supplies the appendix [11].

The development of both the CT and SMA are formed when the two dorsal aortas fuse and create one dorsal aorta by the middle of week five. The SMA is from the vitelline artery [12]. The lack of proper differentiation of the ventral segmental arteries that form the Celiac, Superior Mesenteric, and Inferior Mesenteric arteries most likely caused the abnormality observed in this cadaver.

CT abnormalities are quite common and have been categorized accordingly. Of the abnormalities that can occur in the celiac trunk, the least common is quadrification (8.33%) [13] of the artery, in which it has four branches.

Although there are many documented abnormal branches of the celiac artery as stated in the article by Alsharif et al., 2016 [14], there are very few documented cases of a connecting communicating artery between the Celiac and Superior Mesenteric arteries as seen in the gross dissection of our cadaver.

Our cadaver presented with a communicating artery between the CT and the SMA. As discussed in the case report by Santos et al., 2018, there are many different variations that can be seen within the CT. Although CT variations are rather common, it is important to understand the different possibilities [13]. This qualification variation of the CT has not been documented before in any literature. The abnormal communication believed to be the AIPA is normally a small branch coming off the SMA with the Posterior Inferior Pancreaticoduodenal artery and supplies the lower head of the pancreas and anastomoses with the Superior Pancreaticoduodenal artery, which is, in turn, a branch of the Gastroduodenal artery. This aberrant communication was, however, abnormally large as well as the other abdominal vessels. This could be ascribed to a background vascular developmental problem. The size of the AIPA could have provided a significant anastomosis between the CT and SMA. This anastomosis would have been able to allow a more than normal blood flow from the CT directly to the SMA, which would be beneficial in some cases and detrimental in others. This anastomosis could allow for adequate blood supply to the SMA and the rest of the abdominal aorta if there were ever an occlusion in between the CT and SMA. However, this increased diameter of the AIPA could cause some relative ischemia to the organs supplied by the CT as some of the blood flow could be sent down this large bored AIPA instead of organs of the foregut. According to Santos et al., 2018 [13], being aware of the CT variations is important for surgeons during many procedures involving the arteries of the CT. These procedures range from laparoscopic surgery, hepatic transplantation, and abdomen trauma or lesions. Abnormalities in the CT, especially one of this rarity, may increase both the difficulty and the risk of radical surgical procedures.

In conclusion, knowledge of CT structure variations is important to clinicians in planning for surgical procedures. The quadrification of the CT with a communicating branch considered to be the AIPA of the SMA is a novel anatomical malformation never reported in the literature. This anastomosis is a potential alternative blood source in the case of occlusion between the CT and SMA. Of importance is how this aberrant communicating branch might have affected blood flow distribution within the foregut and midgut of the body. Fluid dynamics suggests that there should be generally decreased blood output to the organs supplied by the CT, secondary to this split. However, in vivo measurement of arterial blood flow to individual organs is needed to confirm this. Consequently, further research could look deeper into a global study of CT variations and the resultant blood flow in vivo using ultrasonic or electromagnetic techniques.

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Case Report

Benign Lymphoepithelial Cyst: An Unusual Cause of Parotid Swelling in Two Immunocompetent Patients

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Keywords: Parotid gland; Lymph nodes; Differential diagnosis; HIV

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Abstract

BACKGROUND: Lymphoepithelial cysts, which are benign and slow-growing tumours, usually involve the head and neck regions. Benign lymphoepithelial cysts (BLECs) are the most common cause of parotid swelling in human immunodeficiency virus (HIV)-positive patients and are less common in immunocompetent patients.

CASE PRESENTATION: Here, we present two cases of immunocompetent patients with long-standing, progressively enlarging parotid swelling. Postoperative histopathological examination of these patients revealed features of BLEC.

CONCLUSION: Wide surgical excision is the gold standard for treatment and recurrences is rare. These cases are of particular interest because of the rarity of BLEC in HIV-negative patients and highlight an important differential diagnosis of parotid swelling.

Introduction

Benign lymphoepithelial cysts (BLECs) are benign, slow-growing, uninoculated or multiloculated lesions that are usually associated with the salivary glands in the head and neck regions. BLECs are more commonly observed in up to 60% – 80% of female patients [1]. They are pathognomonic for human immunodeficiency virus (HIV) infection and can occasionally manifest as the first symptoms of retroviral infection [2].

BLECs originate from epithelial remnants retained in the lymphoid tissue, which are trapped in the parotid gland during embryogenesis [3]. Here we report two cases of HIV-negative patients with BLEC of the parotid.

Case Reports

Case 1

A 45-year-old female presented to our clinic with a 1-year history of swelling in her left cheek, which was slowly increasing in size and causing discomfort and cosmetic impairment. She had no other comorbidities or family history of head and neck tumours. Physical examination revealed a soft non-tender mass, measuring 4 cm × 4 cm, over the left parotid gland. Intraoral examination was unremarkable, and facial nerves were intact. These findings were confirmed via computed tomography (CT), which revealed a solitary well-marginated rim-enhancing cystic lesion, measuring 3.2 cm × 3.3 cm, within the superficial lobe of the left parotid gland.

Although fine-needle aspiration cytology (FNAC) was performed, the sample obtained was inadequate and unsatisfactory. Left superficial parotidectomy was performed without complications, and there was no recurrence at 6-month follow-up.

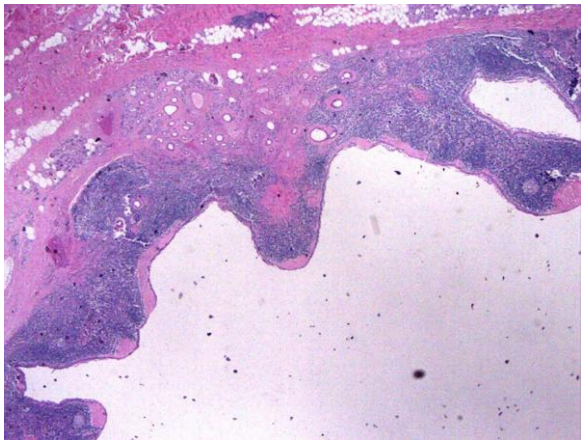

Figure 1: Haematoxylin and eosin staining of parotid cyst showing subepithelial stroma with reactive lymphoid follicles and germinal centres (Magnification x 20)

Gross specimen revealed a grey/ brown encapsulated cystic lesion, measuring 3.0 cm × 4.5 cm × 2.5 cm, filled with brownish mucinous fluid. Microscopic examination revealed subepithelial stroma with reactive lymphoid follicles and germinal centres. Adjacent normal salivary glands and fatty tissue appeared normal, and there was no oncocytic cell lining or neoplastic tissue (Figure 1 and 2). A final diagnosis of BLEC of the left parotid gland was made based on the radiological and histomorphological findings. HIV antibodies were analysed using ELISA, and a negative result was obtained.

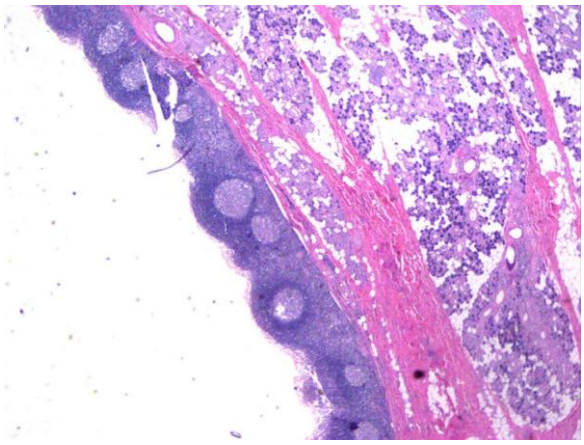

Figure 2: of parotid cyst showing reactive lymphoid follicles and germinal centres with adjacent normal salivary glands and fatty tissue (Magnification x 40)

Case 2

A 48-year-old female presented to our ear, nose, and throat outpatient clinic with a history of persistent swelling of the left cheek for the past 11 years, which had gradually progressed to the current size of 4 cm × 5 cm. The swelling was not associated with pain but was cosmetically disfiguring. Clinical examination revealed a swelling over the left parotid region, measuring 4 cm × 5 cm, which was mobile, soft, and not adherent to the underlying skin. There was no cervical lymphadenopathy, Stenson's duct was normal with no purulent discharge, and facial nerves were intact. CT revealed a non-enhancing, homogenous lesion involving the superficial lobe of the left parotid gland (Figure 3 and 4).

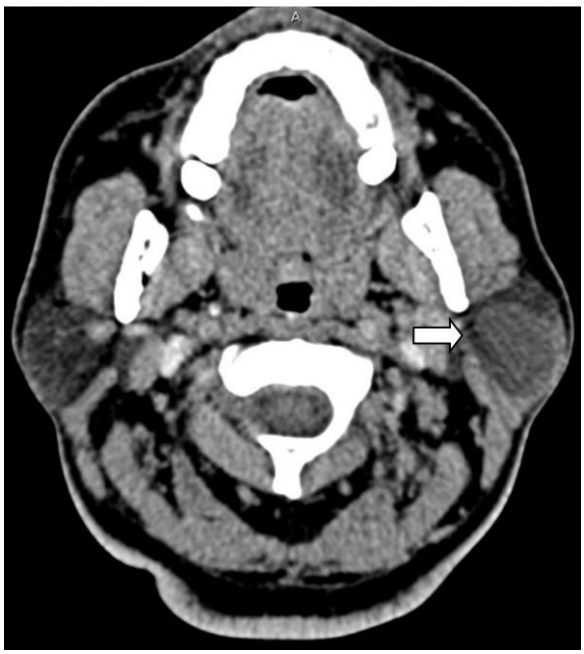

Figure 3: Preoperative CT scan neck, axial view. Cyst like lesion seen in the left parotid gland (Arrow)

FNAC was inconclusive. She underwent left superficial parotidectomy under general anaesthesia. Excised parotid mass measuring 45 mm x 25 mm x 20 mm.

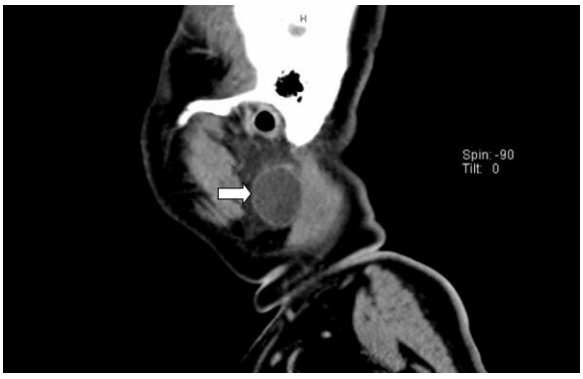

Figure 4: Preoperative CT scan neck sagittal view, Cyst like lesion of the left parotid gland (Arrow)

Cut surface shows a large cystic area containing clear fluid (Figure 5). Histopathological analysis revealed cystic structures comprising dense

polymorphous and polyclonal lymphoid tissue forming scattered reactive follicles, closely associated with the glandular lining epithelium of the cyst. A final diagnosis of BLEC was made. HIV antibodies were analysed using ELISA, and a negative result was obtained.

Figure 5: Post-operative specimen (left parotid cyst measuring 4.5 x 2.5 cm)

Discussion

BLECs are single or multiple cysts occurring in the lymph nodes within the salivary gland. They are usually slow-growing and are commonly observed in HIV-positive patients in whom cysts often bilaterally present and are accompanied by cervical lymphadenopathy [1]. The pathogenesis of BLECs is associated with the migration of HIV-infected cells into the lymphoid tissue of the salivary glands, leading to lymphoid hyperplasia and metaplasia in the salivary duct and ultimately resulting in ductal obstruction, dilatation, and cyst formation [1], [4]. BLECs are less common in immunocompetent patients and may be associated with Sjögren's syndrome [5]. Although the pathogenesis of BLEC in immunocompetent patients is unclear, it is postulated to have a similar process of lymphoid hyperplasia in viral infections other than HIV. Naidoo et al. reported a case of BLEC in an immunocompetent patient with chronic otitis media and concluded that a long-standing ear infection caused chronic lymphatic drainage into the intracarotid lymph nodes, leading to ductal obstruction and cyst formation [6].

BLECs are diagnosed based on history, physical examination, and FNAC. Histopathological findings, including the presence of proteinaceous background with mixed infiltration of lymphocytes, histiocytes, plasma cells, and metaplastic squamous cells, are usually suggestive of BLEC [7]. CT and magnetic resonance imaging typically show the bilateral and multicystic appearance of the lymph nodes in immunocompromised patients and unilateral involvement in immunocompetent patients [4]. Radiological examinations are useful tools to aid in the diagnosis of BLECs and access the borders of the mass to evaluate the involvement of the surrounding structures.

Although the definitive treatment is surgical excision, other treatment modalities include conservative observation, cyst aspiration, sclerotherapy, radiotherapy, and highly active antiretroviral therapy in immunocompromised patients [3]. Superficial parotidectomy was performed in both patients in the present case, and the 3- and 6-month follow-up revealed no signs of local recurrence of the tumour. Close monitoring is required in immunocompromised patients with BLECs because of the high risk of developing lymphomas [1].

In conclusion, it is important to be aware that lymphoepithelial lesions of the salivary glands can manifest as a cystic lesion in immunocompetent patients. Histopathological and radiological examinations are the gold standard for a definitive diagnosis of BLECs, and surgical excision is the first-line treatment in such cases, as demonstrated in our two cases.

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Declaration of Patient Consent

The authors certify that both the patients have signed the appropriate patient consent form. In the form, the patient has given his consent for his images and other clinical information to be reported in the journal. The patient and his parents understand that his name and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Case Report

Concealing Clothing Leading to Severe Vitamin D Deficiency, Osteomalacia and Muscle Weakness

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Keywords: Osteomalacia; Myopathy; Vitamin D deficiency

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Abstract

BACKGROUND: Vitamin D deficiency is the most common nutritional deficiency worldwide in all ages. Prolonged and severe vitamin D deficiency can result in secondary hyperparathyroidism and osteomalacia. Vitamin D deficiency can be caused by various factors included here institutionalisation, malabsorption, inadequate exposure to sunlight etc. Osteomalacia is a disorder of decreased mineralisation of newly formed osteoid at sited of bone turnover, which can be manifested with symptoms such as diffuse body aches and pain. Muscles weakness from vitamin D deficiency causes difficulty in walking, developing proximal myopathy. Nearly 30-50% of all age groups are Vitamin D deficient worldwide.

CASE PRESENTATION: We report a case of 51-years-old woman, with a religious garment, with slowly progressing weakness of the proximal limb muscles, extreme fatigue, chest and lower spine pain, paresthesia, depression, difficulties in walking and waddling gait. On whole-body bone scintigraphy diffuse metabolic changes were present, and in DXA osteoporosis was shown due to severe vitamin D deficiency and secondary hyperparathyroidism. Treatment with high doses of vitamin D and calcium replacement improved clinical manifestation of osteomalacia for few months. Absent of waddling gait with no pain was evident due to the better muscle and bone performance after the treatment.

CONCLUSION: Suspicious cases for osteomalacia in population wearing a religious garment and those that are not adequately exposed to the sunlight, laboratory evaluation should include measurement of 25 (OH) vitamin D, PTH, calcium, alkaline phosphatase and performing of DXA in order such cases do not get undiagnosed.

Introduction

Vitamin D deficiency is the most common nutritional deficiency worldwide in both children and adults. It has been defined and recently recommended by the Institute of Medicine (IOM) as a 25(OH) vitamin D less than 20 ng/mL. Nearly 30-50% of all age groups are Vitamin D deficient worldwide [1].

Osteomalacia is a disorder of decreased mineralisation of newly formed osteoid at sited of bone turnover, which can be manifested with symptoms such as diffuse body aches and pain. Prolonged and severe vitamin D deficiency can result in secondary hyperparathyroidism and osteomalacia.

Vitamin D deficiency can be caused by various factors as nutritional, inadequate exposure to sunlight or concealing clothing, malabsorption resulting from intestinal inflammation, celiac disease or gastric surgery, prolonged use of anticonvulsants and corticoids, at hospitalised persons, as well at institutionalised persons and others [2]. Vitamin D is crucial for calcium, phosphorus and bone metabolism. Low 25(OH), vitamin D status, leads to reduced efficiency in intestinal calcium and phosphorus absorption, and the body reacts by increasing the secretion of parathyroid hormone (PTH) [3]. To maintain serum calcium in the normal range, secondary hyperparathyroidism is activated mobilising calcium from the skeleton and increasing phosphorus wasting in the kidneys. High level of PTH increases osteoclastic activity resulting from the inadequate calcium-phosphorus product, causing mineralisation defect in the skeleton (local foci), which can contribute to a generalised decrease in bone mineral density (BMD), resulting in osteopenia and osteoporosis. Alkaline Phosphatase (ALP) levels are usually elevated in secondary hyperparathyroidism due to

osteomalacia.

Vitamin D deficiency and increased serum PTH concentration, in the children, can result with rickets, in adults when epiphysis plates are closed, this defect in mineralisation, bone turnover and bone loss, can result as osteomalacia. Muscles weakness from vitamin D deficiency causes difficulty in walking from the abnormal muscle contraction and increasing their risk of fracture. And decreased muscle strength is observed at the level below 30 nmol/l of 25(OH)Vitamin D.

Vitamin D receptor (VDR) is a member of the nuclear receptor, located in various tissues and cells in the body, revealing different biological effects based on different molecular pathways [4]. Based on the results of different studies, it was shown that vitamin D is implicated in the regulation of the immune system, the cardiovascular system, oncogenesis [5], and cognitive functions. More than 30 years ago, *Yoshikawa et al.,* showed in their study a direct link between hypovitaminosis and muscle function at the patients with rickets and developing proximal myopathy in osteomalacia [7].

Women who wear concealing clothing like hijab may be at greater risk for vitamin D deficiency and have poor bone status and also can adversely affect muscle function [6].

Case Presentation

We report the case of a 51 years old woman admitted in our Clinic on April 2014, with several years' history of extreme fatigue, pain in lower extremities, pelvis, lower spine and the chest, paresthesia, depression, weight loss and difficulties in walking (waddling gait). The patient has no history of metabolic diseases, married and 2 healthy children and 1 child dead from leukaemia. Before two years she was hospitalised and treated as fibromyalgia (with NSAIDs etc.), but with no improvement in her clinical manifestation. In physical examination, demineralisation of teeth, Trendelenburg sign positive (waddling gait) was detected. Twenty-five (OH) vitamin D3 was extremely low 3 ng/mL and parathyroid hormone level was found to be very high (PTH) 423 pg/mL (Table 1) which shows severe Vitamin D deficiency.

Table 1: Laboratory analyses before and after the treatment

	Baseline data	Data after the treatment	Reference values
Vitamin D (25-OH Vitmanin D)	3	30.1	< 9.9 ng/mL severe deficiency
iPTH	423.6	97.8	12.0-72.0 pg/mL
Total Calcium	2.0	2.1	2.15-2.57 mmol/L
Ionized Calcium	1.0	1.1	1.12-1.32 mmol/L
Alkaline phosphatase	200	324	53-128 U/L
Phosphorus	0.6	0.98	0.48-2.26 mmol/L

Inflammation markers and thyroid hormones were normal. Antibodies for celiac disease were negative, renal and hepatic functions were normal. In electrocardiography (ECG), no signs were evident for coronary insufficiency. On whole-body bone scintigraphy (Figure 1) diffuse metabolic changes were present (characteristic looser zones in chest ribs and knees bones). In dual-energy X-ray absorptiometry scan (DXA) were shown marked changes as low body mass density and severe osteoporosis (Table 2). Biopsy through gastroesophagoscopy-revealed-chronic gastroduodenitis – lymphoplasmacytic inflammatory infiltrates. Thyroid and parathyroid ultrasound were normal, and no signs and symptoms for cardiovascular diseases (CVD).

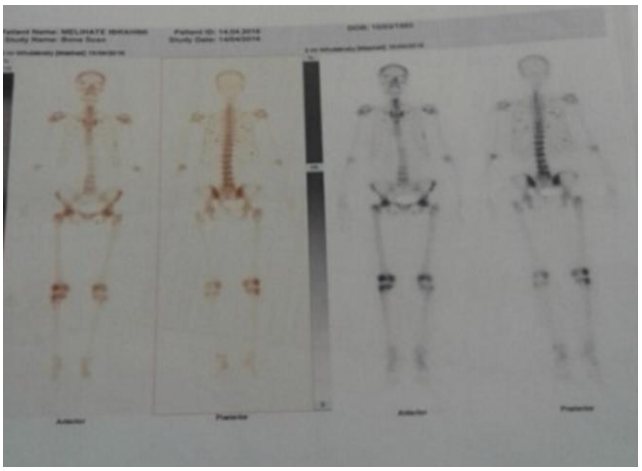

Figure 1: Whole-body bone scintigraphy before the treatment with characteristic chest and knees bone looser zones

The patient was wearing the religious garment (hijab) and had almost no exposure to the sunlight for years.

In the following are presented laboratory investigation that revealed a severe deficiency of 25(OH) vitamin D, high PTH, low calcium and high alkaline phosphatase before the treatment and improvement of these data six months after the treatment (Table 1). In the meantime, DXA revealed low BMD in all relevant part of both femur region and L-spine and an impressive improvement of these data after the treatment for the same period (Table 2).

Table 2: DXA* before and after the treatment

	Baseline data			Data after the treatment		
Region	BMD**	T-	Z-	BMD	T-	Z-
Right femur	0.521	-3.4	-2.9	0.787	-1.3	0.7
Left femur	0.586	-2.9	-2.4	0.819	-1.3	-0.6
L-spine	0.686	-3.3	-2.5	0.919	-1.2	-0.6

* DXA-dual-energy X-ray absorptiometry scan; ** BMD-bone mineral density.

In the lumbosacral radiography, there were revealed the sign of osteomalacia like biconcave vertebral bodies (fish vertebrae) and upper anterior fracture of the 5th lumbar vertebra (probably fracture of osteophyte (Figure 2).

Figure 2: Biconcave vertebral bodies (fish vertebrae) and upper anterior fracture of the 5th lumbar vertebra osteophyte

To avoid malabsorption of vitamin D due to gastrointestinal lymphoplasmacytic inflammatory infiltrates, it was initiated Cholecalciferol (vitamin D_3) i.m. 300 000 UI at once, followed by vitamin D_3 50 000 UI/per week oral solution and, calcium 1000 mg 1x1 daily together with dairy products for one month, then followed with 25 000 UI/per week oral solution for other two months. Appropriate sun exposure in daily bases was recommended and other necessary treatment. Last three months, appropriate doses of Vitamin D were ordered in oral tablets.

After 6 months of the treatment, she gains 3 kg and impressive improvement in walking were evident (no Trendelenburg sing and waddling gait), no pain and paraesthesia, and no signs and complaints of depression were observed. The last DXA revealed normal values of BMD and T score (Table 2).

Discussion

In this report, we described our female patient, which was admitted with muscles weakness, pain in a different part of the body and waddling gait due to osteomalacia. Latest evidence showed the very important role of vitamin D in muscle growth, strength and gait, confirming that hypovitaminosis D is always associated with a decrease in muscle function and performance and an increase in disability. Progressive difficulties in changing the body position, or rising from a chair followed with diffuse muscle pain are all symptoms of myopathy from osteomalacia [8]. Effect of active form of vitamin D, calcitriol or 1, 25-dihydroxy vitamin D_3 on the muscle tissue is based in two molecular mechanism by binding to the nuclear receptors (genomic effect) and protein synthesis through RNA messenger to drive cellular differentiation and proliferation [8], [9] and by binding to a membrane-bound receptors (non-genomic effect) which in turn lead to a rapid influx of calcium into the cell [9].

In cases where is not available DXA, PTH values can be considered for evaluation of cases with severe vitamin D deficiency, [10] and normal renal function. The effects on muscular fibres are based on hypocalcaemia that can impact on decreased inhibition of nerves and muscle fibres depolarisation [11]. In the meantime, high level of PTH has to be considered except on bone tissues, also on progressive muscular weakness. [12]

Vitamin D supplementation is needed for women wearing concealing clothes to reach or maintain a healthy vitamin D status [13] and to avoid osteomalacia.

Severe vitamin D deficiency, along with electrolyte abnormalities such as low calcium and associated elevated PTH would suggest severe osteomalacia which may be manifested as muscular and neurological symptoms such as proximal weakness and paresthesia. Suspicious cases for osteomalacia in population wearing a religious garment and those that are not adequately exposed to the sunlight, laboratory evaluation should include measurement of 25 (OH) vitamin D, PTH, calcium, alkaline phosphatase and performing of DXA in order such cases do not get undiagnosed. In these cases, treatment with high doses of vitamin D and calcium supplements is essential to treat osteomalacia and myopathy.

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Case Report

Large Bowel Occlusion: Clinical and Imaging Characteristics - Caecum Volvulus

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Abstract

BACKGROUND: Caecum volvulus (CV) is defined as an axial twisting that causes an inversion position of the caecum, ascending colon and terminal ileum. This anatomical finding is responsible for some clinical features. Obstruction and strangulation are the most important and life-threatening.

CASE PRESENTATION: We are presenting a 50 years old woman presented to the hospital with sudden acute severe abdominal pain and distension of about 24 hours associated with vomiting and no flatus.

CONCLUSION: Mortality in patients with CV can be kept near 10%-12% if operative intervention is accomplished before caecum strangulation. Once the cecum has become gangrenous, a death rate of 30 to 40% can be expected. Tailored made surgery on patients status is the strategy.

Introduction

Caecum volvulus (CV) is defined as the torsion of the caecum around its mesentery [1]. The incidence of CV account 1%-1.5% of intestinal obstruction with female predominance and account 25% of colonic volvulus with highly variable age presentation between 30 years to 60 years making it a difficult pathology to identify [1], [2]. That causes strange diagnosis and delayed decision making [3]. Caecum volvulus (CV) is a clear result of incomplete intestinal rotation during embryogenesis featured by an inadequate right colon fixation to retroperitoneal structures during the final clockwise step of colon embryogenesis [1], Figure 1.

Parietocolic ligaments laxity is the main cause of improve caecum motility up and down or right and left and axial rotation. Clinical reports suggest that 40% of caecum strangulation are found in patients with prior abdominal and pelvic surgery.

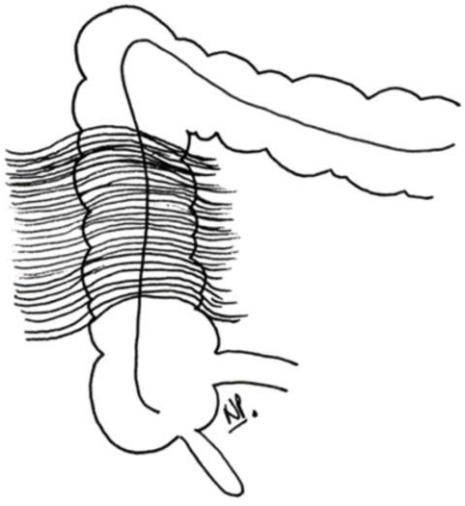

Figure 1: Normal Anatomy of Right Colon

Prior surgery causes adhesions and a new fulcrum of rotation [1]. Laxity of ligaments associated with pelvic surgery are the most important etiologies

of CV [3], [4], Figure 2.

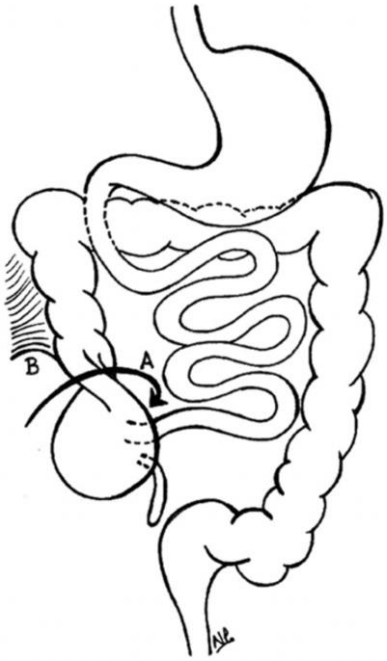

Figure 2: Mechanism of Cecal Volvulus

Case Report

A 50 years old woman presented to the hospital with sudden acute severe abdominal pain and distension of about 24 hours associated with vomiting and no flatus. In her clinical history, she has had a hysterectomy for myxomatosis. She denied other medical problems. On physical examination, we found altered vital signs and hemodynamic instability.

Figure 3: A 50-year woman CT scan: coffee bean sign

Abdominal examination noticed diffuse abdominal distension, tympanic sound and positive Blumberg sign. Laboratory studies showed WBC 20 cell/mm3 and elevated PCR. Rx abdomen showed intestinal distension suggestive of sigma volvulus. CT scan confirmed caecum volvulus with bird beak sign Figures 3, 4, 5.

Figure 4: A 50-year woman TC scan: Bird Beak Sign

So, we performed an urgent colonoscopy to view mucosa and possible volvulus untwisting. Colonoscopy showed normal mucosa and no volvulus Figure 6.

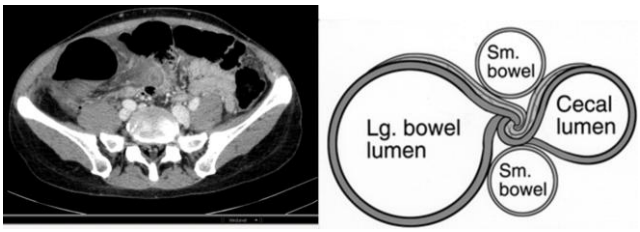

Figure 5: A 50-year woman ct scan: whirl sign

The patients underwent an urgent laparotomy with findings at the operation showing the axial rotation of the caecum and terminal ileum with ischemia extended from the terminal ileum to right colon flexure Figure 7 and Figure 8.

Figure 6: A 50-year woman: Colonoscopy

We performed a classical right colectomy with ileocecal mechanic side to side anastomosis for good patient status. The patient was discharged in seven postoperative days with no medical problems [3].

Figure 7: A 50-year-old woman: caecum volvulus

Discussion

The most urgent clinical features are acute obstruction and strangulation associated with sharp pain, no flatus, vomiting, abdominal distension.

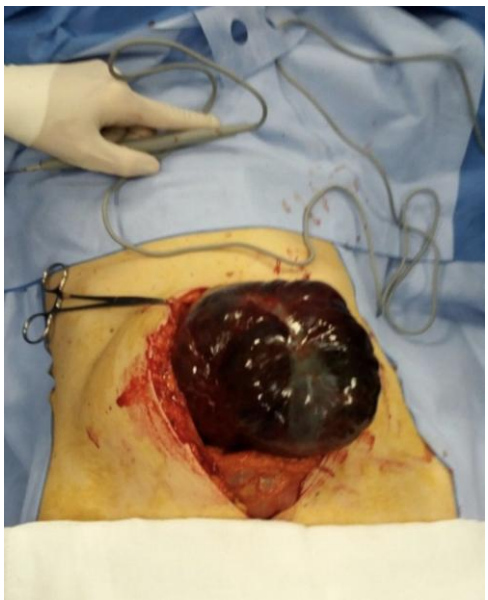

Figure 8: A 50-year-old woman: caecum volvulus

Differential diagnosis with left colon obstruction, small bowel obstruction, mesenteric ischemia, aortic aneurysm, acute pancreatitis makes the diagnosis difficult. Untreated volvulus may progress in strangulation- ischemia and perforation with peritoneal irritation and hemodynamic instability presentation [3]. It is recommended that these patients must undergo early laboratory and radiological evaluations. Laboratory findings are nonspecific. Advanced obstruction may show high WBC, high PCR and fluid or electrolytes changes. Abdominal X-ray shows intestinal dilatation in 100% of cases, but for the rarity of this pathology, many of the patients are erroneously given the diagnosis of small bowel obstruction or sigma volvulus [5]. CT scan has 80%-90% sensibility / specificity. There are three clinical findings: "Coffee beans", "bird beak" and "whirl" signs. Coffee beans sign demonstrated an axial view of the dilated cecum with air. The loop may resemble a coffee bean, which has an appearance similar to that of the well-known radiographic sign sigmoid volvulus Figure 3 [6].

Bird beaks sign shows bowel loops Figure 4. Whirl sign shows fat attenuation of a soft tissue mass Figure 5 [7].

Colonoscopy may exclude sigma volvulus Figure 6 [8]. Barium enema CT may confirm the colon site of occlusion with 88% of accuracy [2], [3]. Advances in knowledge show: two defined transition points are seen in the minority of cases of caecum volvulus; a single transition point is more sensitive for caecum volvulus. CT findings of a whirl, ileocecal twist, the X-marks-the-spot sign and the split-wall sign are specific for CV. The coffee bean sign and a distended cecum directed toward the left upper quadrant on CT images are insensitive but specific for CV. Distal colonic decompression on CT images is relatively sensitive and specific for CV [6], [9]. Treatment choice is surgery.

The most appropriate strategy depends on the patient's status, the timing of volvulus treatment (early or delayed) and surgical team expertise. The clinical review recommended: manual or laparoscopic untwisting with caecopexy in the viable intestine [2] — Caecostomy in the viable intestine but low-grade patient status. Laparoscopic or open right colectomy is recommended in the non-viable ischemic or perforated colon in good performance status patient [2], [3], [10]. Caecum volvulus (CV) is a rare colonic obstruction. It is caused by a bad rotation of bowel during embryogenesis with ligaments laxity. Diagnosis is STRANGE for the rarity and nonspecific presentation: acute severe abdominal pain is the main clinical features associated with abdominal distension. CT scan has more accuracy. COFFEE BEAN sign is specific. WBC and PCR alteration associated with peritoneal irritation are signs of strangulation, ischemia or perforation. Surgical Treatment is the best choice with adhesiolysis and caecopexy in viable colon and resection in the non-viable colon [11].

In conclusion, mortality in patients with CV

can be kept near 10%-12% if operative intervention is accomplished before caecum strangulation. Once the cecum has become gangrenous, a death rate of 30 to 40% can be expected [11], [12]. Tailored made surgery on patient's status is the strategy [13], [14].

Patient Consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Case Report

Fenestrated Vertebral Artery in A Routine Cadaveric Dissection

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Keywords: Vertebral artery; Transverse foramina; Fenestrated; Suboccipital triangle

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Abstract

BACKGROUND: Anomalies associated with the vertebral arteries are relatively rare. The vertebral arteries arise from the first part of the Subclavian artery and pass through the transverse foramina of C6 through C1.

CASE PRESENTATION: However, in this article, we describe a unique variation in the anatomical orientation of the right vertebral artery during a routine cadaveric neck dissection where the right vertebral artery gives an oblique branch from the extradural segment (C2) forming a fenestrated Vertebral artery.

CONCLUSION: Despite the lack of established clinical significance, multiple co-morbid vascular malformations are yet associated with the Vertebral artery fenestration with a possibility of iatrogenic injuries if not taking into cognisance.

Introduction

Vertebral artery (VA) is an important blood vessel in the craniocervical transition region because it contributes significantly to the posterior part of the brain's circulation. The VA is divided into four parts and two segments, extradural segment (including prevertebral -V1, cervical -V2, and Atlantic -V3 parts), and intradural segment, as its fourth, or intracranial part (V4) [1].

True anomalies of the origin of the VA are relatively rare. However, there are already documented anatomical variants of the VA such as asymmetry of the VA due to hypoplasia resulting in its absence or termination into the Posterior Inferior Cerebellar Artery (PICA), partial or complete duplication of the VA, fenestration of the VA, variable orientation of the ostium, and variable origins of the VA [2].

However, the most common anomaly is an origin from the aortic arch (4%), with the anomalous vertebral artery usually arising between the left common carotid and left subclavian arteries [3].

We present for the first time, to the best of knowledge, a fenestrated vertebral artery in routine student cadaveric dissection. A VA is said to be fenestrated when a single vessel lumen divides into two separate conduits that eventually rejoin distally to form the primary vessel [4]. This is opposed to VA duplication, where the vessel has two origins that distally fuse at varying locations in the cervical region [5].

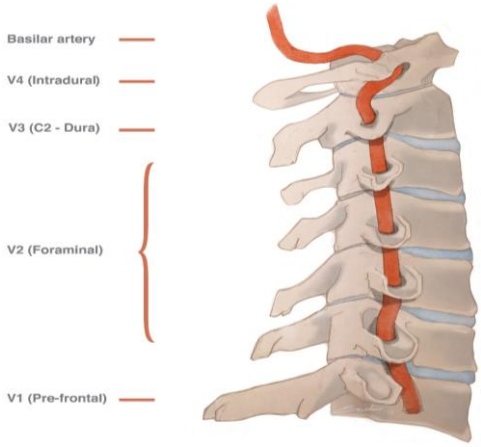

Figure 1: Schematic of the normal course of the VA

Case Report

During a routine cadaveric neck dissection by medical students of a 72-year-old man at the University of Medicine and Health Sciences, St. Kitts & Nevis, an unusual oblique branch coming off the right VA at the extradural segment (Atlantic – V3 part) specifically in the suboccipital triangle and lateral to the rectus capitis was observed. While exploring the neck, the greater occipital nerve was identified and followed deeply, the semispinalis capitis muscle was reflected and the muscles that bound the suboccipital triangle (Obliquus capitis inferior muscle, Rectus capitis posterior major muscle and Obliquus capitis superior muscle) where identified. While exploring the content of the suboccipital region (vertebral artery and suboccipital nerve) this unusual branch was found.

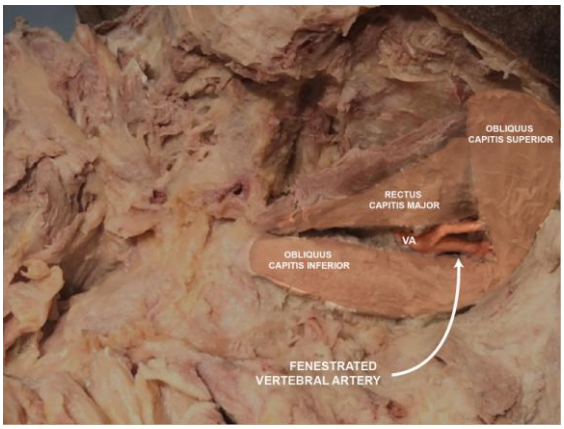

Figure 2: Fenestration of the right Vertebral Artery (V3 part) in the Suboccipital triangle

However, the branch could not be followed right away as it was a systematic dissection. The left vertebral artery did not present such anomaly, and the rest of the structures in the posterior neck on both sides were normal. Subsequently, upon dissection of the brain, the anastomosis of the vertebral arteries forming the basilar artery where normal depicting a reunion of the branches.

Figure 3: Angiogram of a fenestrated VA

Discussion

The pair of vertebral arteries arise as the first branches of the subclavian arteries. They rise through the neck and go through the transverse foramina of C6 through C1, they then pass through the dura and arachnoid space and pass through the foramen magnum. Both the vertebral arteries join and combine to create the basilar artery. Clinically Oriented Anatomy textbook by Moore refers to this as the “posterior circulation of the brain” [6].

The vertebral arteries will typically branch into four different segments and are named: V1 (pre-foraminal), V2 (foraminal), V3 (Atlantic, extradural or extraspinal) and V4 (intradural or intracranial). The V3 segment showed the variation in this dissection. The V3 segment passes through the transverse process of the axis and crosses laterally through the transverse foramen of the atlas. The VA then makes its way through dura and arachnoid to become V4 which continues to the pons. The path of the V3 is very long and tortuous, allowing for movement of the head and neck [2].

The vertebral arteries develop from longitudinal anastomosis, linking the embryonic first to seventh cervical intersegmental arteries. The seventh cervical intersegmental artery persists and develops into the base of the subclavian artery as well as the proximal part of the vertebral artery [7], the first to the sixth cervical intersegmental arteries regresses to give

rise to the remaining part of the vertebral artery [8].

A VA is said to be fenestrated when a single vessel lumen divides into two separate conduits that eventually rejoin distally to form the primary vessel, as shown in the angiogram in Figure 3 [4]. This is opposed to VA duplication, where the vessel has two origins that distally fuse at varying locations in the cervical region [5].

Several theories have been proposed as to the aetiology of the VA fenestration such as; failure of the second intersegmental artery to regress [7], and failure of the plexiform anastomosis to involute [9] resulting in extracranial fenestration. Whereas, the persistence of fetal anastomotic vessels has been thought to result in intracranial VA fenestrations [9], [10].

Autopsy and angiographic studies suggest that the incidence of vertebral artery fenestration is 0.23%-1.95% [6]. Although fenestration of the VA can occur either intra- or extracranially, the extracranial fenestration at the upper cervical level is more commonly reported [6]. Even though the clinical significance of the fenestrated VA is yet to be determined, several vascular malformations and embryological abnormalities have been associated with it, such as; saccular aneurysm formation [11], intracranial aneurysms [12], arteriovenous malformations and middle cerebral artery fenestrations [11], corpus callosum agenesis, trigeminal artery persistence, and epidermoid cyst [10], [11].

Given these associated variations, it will be imperative for vascular and Neurosurgeons to investigate patients radiologically for further associated vascular abnormalities should a fenestrated VA be observed intraoperatively or while working the patient up for surgery to prevent iatrogenic injuries.

In conclusion, the vertebral arteries are the major suppliers for the posterior circulation of the brain. They are rarely associated with variations. The authors herein present a case of anatomical variation in a 72-year-old male cadaver that demonstrated an oblique branch coming off the right VA at the extradural segment. This unusual branching of the VA at this level is referred to as a fenestration, which could be a possible cause of iatrogenic injuries during endovascular investigations or procedures.

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Dental Science

Influence of Thermomechanical Treatment on the Mechanical Behavior of Protaper Gold versus Protaper Universal (A Finite Element Study)

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Keywords: Finite element analysis; Mechanical behaviour; NiTi rotary file; Thermomechanical treatment

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Abstract

AIM: To compare and evaluate the influence of thermomechanical treatment of Protaper Gold file versus Protaper Universal file during testing of bending and torsion using finite-element analysis.

METHODS: Two nickel-titanium NiTi rotary files (ProTaper Gold and ProTaper Universal) were used in this study. The files were imaged using stereomicroscope to produce 3D models. The behaviour of the instrument during bending and torsion was numerically analysed in CAD/CAM software package.

RESULTS: Under bending, ProTaper, Gold showed higher flexibility and flexural resistance than ProTaper Universal. The highest stress was related at the cutting edge of both files. While during testing of torsion, the maximum amount of stresses was related to the base of the flutes in both files. ProTaper Gold showed higher torsional resistance than the ProTaper Universal file.

CONCLUSION: Thermomechanical treatment improved the mechanical response (bending and torsional resistance) of NiTi files.

Introduction

During root canal treatment, different types of rotary file systems are used for cleaning and shaping. The root canal system has complex anatomy with different angles of curvatures, so root canal preparation with rigid stainless-steel instruments may lead to more canal aberrations. Recently, nickel-titanium (NiTi) rotary files have been widely used due to the superelastic behaviour of nitinol base material [1].

However, the fracture of rotary files during cleaning and shaping remains the major concern in endodontic practice. The file could fracture at different levels of stress, even without any signs of plastic deformation near the fracture site; this is due to the residual stress in the file after being used. These residual stresses could affect the durability of the file if it were used repeatedly [2]. Thus, flexibility and torsional resistance are important properties that could affect the mechanical performance of NiTi files during root canal preparation [3]. NiTi files fracture during root canal preparation due to either flexural or torsional fatigues. When the file is moved in a curved canal, it will be subjected to repeated cycles of compression and tensile stresses so that flexural fatigue could occur, while when the files' tips were locked in the walls of the canal while the shank was in continuous rotation, torsional failure could occur [4].

The mechanical response of NiTi alloy under different clinical situations is determined by the

relative proportions and characteristics of the microstructure of NiTi alloy. Thus, the manufacturer improved the mechanical properties of NiTi instruments using new techniques [5]. Thermomechanical treatment is one of the recent approaches for changing the transition temperatures of NiTi alloys to improve the fatigue resistance of NiTi endodontic files [6].

ProTaper Gold file (PTG; Dentsply, Tulsa Dental Specialties, Tulsa, OK, USA) has similar geometry as ProTaper Universal file (PTU; Dentsply Maillefer, Ballaigues, Switzerland). Both files have a progressive taper and a convex triangular cross-section design. However, PTG instruments have been subjected to thermomechanical heat treatment that resulted in the production of a file with advanced metallurgy. PTG has a 2-stage transformation behaviour with high Af temperatures while PTU instruments have 1-stage transformation [7], [8].

Hence, this study aimed to compare the influence of the thermomechanical heat treatment on the mechanical behaviour of Protaper gold versus ProTaper Universal using Finite Element Analysis.

Methods

Real-size digitised models of two NiTi rotary instruments with same cross-sectional geometry but with different heat treatment were selected for this study: ProTaper Gold instruments (PTG; Dentsply, Tulsa Dental Specialties, Tulsa, OK, USA) and ProTaper Universal (PTU; Dentsply Maillefer, Ballaigues, Switzerland). The PTG and PTU files were of size 25, .08 variable taper.

The files were imaged and measured using stereomicroscope (Technical 2, Carl Zeiss JENA) at X 5, X 10 and X 16 magnifications to obtain a detailed shape to obtain an accurate measurement of the files (Figure 1).

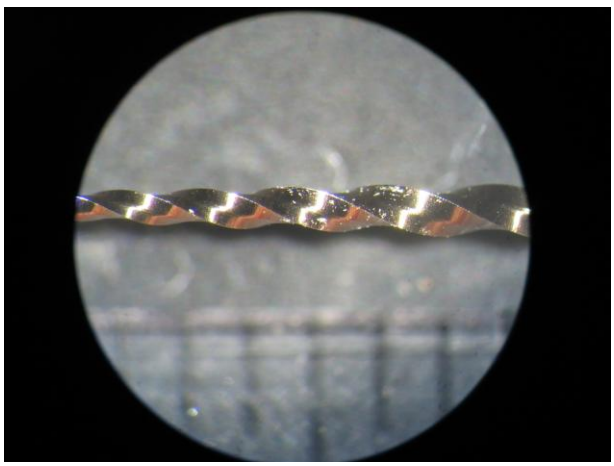

Figure 1: Stereomicroscope imaging for Protaper Gold

The file's cross-section (convex triangular) was drawn in 2D using Computer-Aided Design CAD programs SolidWorks 2012 (Dassault Systèmes SolidWorks Corporation, Waltham, MA, USA) then exported as Stereolithographic (.stl) file format. The building of 3D model in the form of sections was performed by MATLAB (MathWorks, Inc., Natick, Massachusetts, USA) using the following data: variable taper of the file and the change in pitch length (Figure 2 and Figure 3).

Figure 2: 3D model of the file

Using SolidWorks FE model was created and meshed using linear, six nodes trihedral elements. The final FE model of both files consisted of 1850 elements with 3610 nodes.

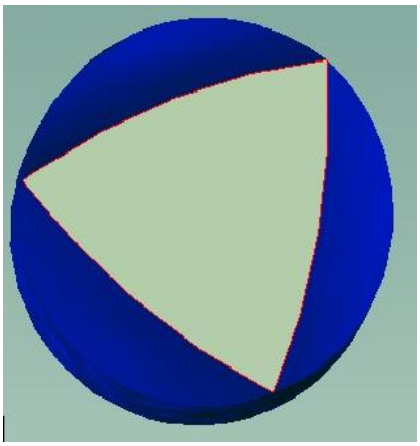

Figure 3: Convex triangular cross-section design of the file's models

The maximum element size was 0.526743 mm, while the minimum element size was 0.105349 mm. The stress-strain behaviour of PTU and PTG file's alloy was obtained from the literature and entered in the SolidWorks.

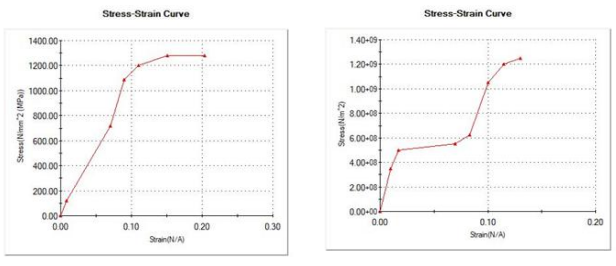

Figure 4: Stress-strain curve for (A); Protaper Gold (B) Protaper Universal files

The Young's modulus of the alloy was 36 GPa and the Poisson's ratio 0.3. (Figure 4 and Figure 5).

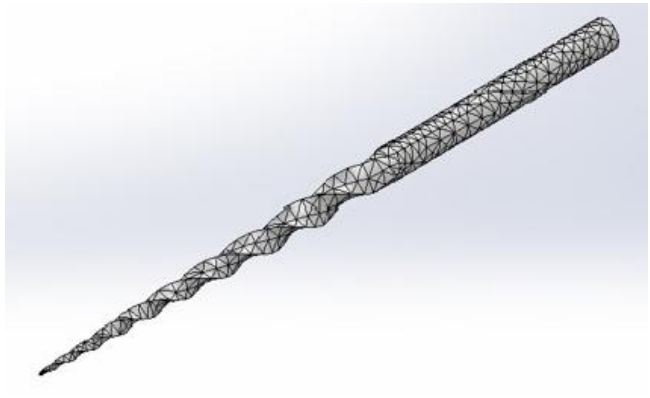

Figure 5: Meshing of the 3D models

Bending

Using a constant load, bending was simulated by applying a concentrated load of 1 N at the tip of the file with its shaft rigidly held in place. Both the vertical displacement and the von Mises stress distribution was evaluated [9].

Application of a shear moment (torsion)

A sheer moment 2.5 N mm of force was applied to the shaft in a clockwise direction while the last 4 mm of the tip was rigidly constrained. The stress distribution was evaluated [9].

Results

Bending

Applying load of 1 N at the file's tip, the end deflection for Protaper Gold was 8.6 mm, while ProTaper Universal 6.1 mm, indicating that PTG is more flexible than PTU. Stress analysis showed that the maximum von Mises stress of 453 MPa was related to PTG; while in PTU, the maximum von Mises stress recorded was 882.7 MPa.

Stress distribution showed that the maximum von Mises stress was related to the cutting edges both in Prptaper Gold and Protaper universal files. The maximum values of stress were recorded in Protaper universal at 4 mm from the tip, while in Protaper Gold it was recorded in the middle and in the last third of the file (Figure 6a, 6b, 6c and 6d).

Torsion

Applying 2.5 Nmm torque to the files showed higher values of von Mises stress in Protaper Universal file (893.9 MPa), while in Protaper Gold file it was 144.1 MPa.

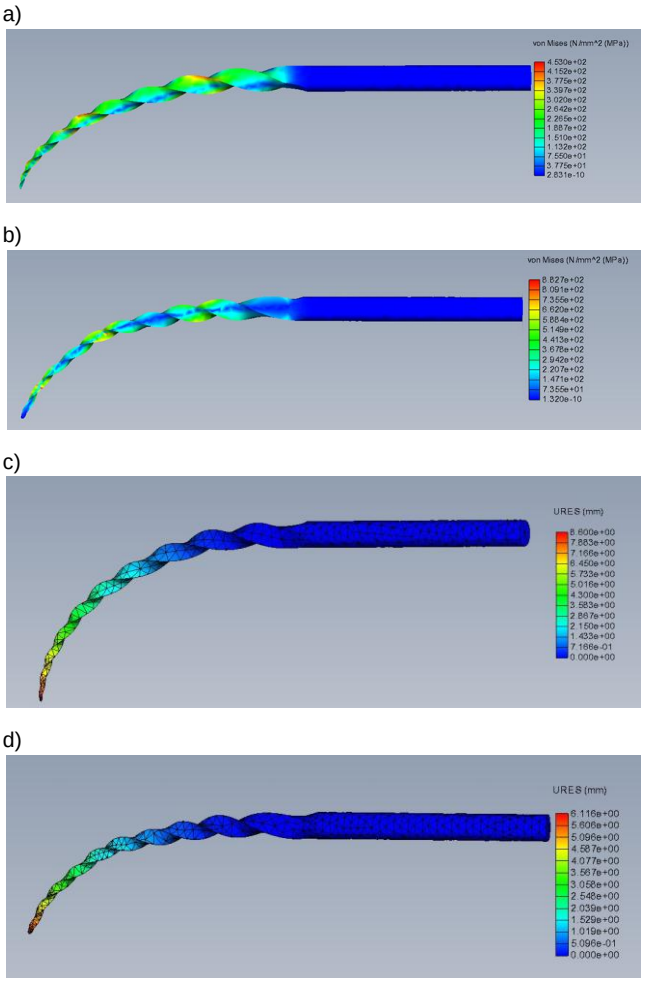

Figure 6: a) Stress distribution during bending of Protaper Gold; b) Stress distribution during bending of Proatper Universal; c) Deflection during bending of Protaper Gold; d) Deflection during bending of Protaper Universal

Stress distribution was related to the base of the flutes of both files (Figure 7a and Figure 7b).

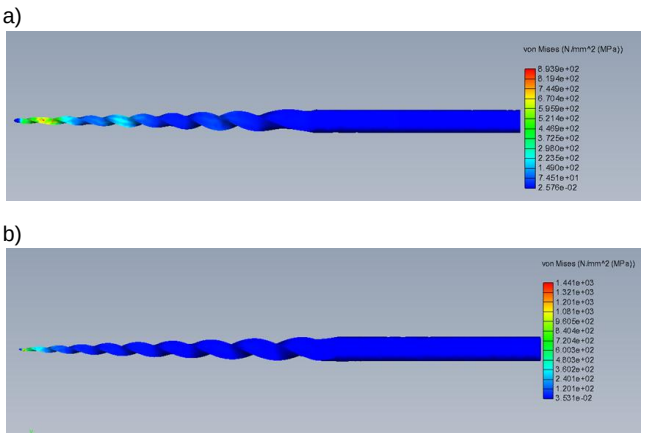

Figure 7: a) Stress distribution during torsional testing of Protaper Universal; b) Stress distribution during torsional testing of Protaper Gold

Discussion

During the last decades (NiTi) files have become widely used because of their superelasticity. However, during the preparation of root canals, NiTi files are subjected to bending and torsional stresses because of the curved anatomy of the root canals and the narrow lumen of the canals. Several factors could affect the stress distribution in NiTi instruments such as the design, alloy, and the heat treatment applied during manufacturing [10].

Deflection under bending reflects the instrument's flexibility, PTG had a greater deflection than PTU, indicating that PTG files possess a higher flexibility. When both files were subjected to 1 N bending, PTG showed more flexural resistance and a lower concentration of stress than PTU file. Under bending conditions, stress distribution in PTG and PTU was near the cutting edge of both files. This was related to the mechanics of bending a triangular cross-section design; this was in full agreement with Kim et al., [9]. As the geometry of both files is the same (from the same manufacturer), the difference in flexural resistance between them is related to the different metallurgy.

The torsional fatigue resulted from friction between the file and the narrow canal. Thus, we examined the torsional resistance under a similar amount of torque rather than a twist-angle [9]. ProTaper Gold has higher torsional resistance than Protaper Universal file. Thermomechanical treatment of Protaper Gold file resulted in increasing its torsional resistance. Stress distribution showed that concentrations of stress were related to the base of the flutes for both files. This stress distribution was related to the convex triangular cross-section design of both files.

Recently, A new era of thermomechanical treatments of the alloy has started to overcome the drawbacks of the traditional NiTi alloy. These new thermal treatments improved mechanical behaviour when compared with conventional superelastic NiTi instruments [11]. Thermomechanical treatment is one of the recent techniques for improving the mechanical performance of NiTi endodontic files [12], [13].

The NiTi alloy has two crystalline phases (austenite and martensite). They have different mechanical properties when the martensitic phase is subjected to heat; it converted to the austenite phase, while in the austenitic phase, the alloy will have shape memory and superelastic characteristics [14].

PTG files have identical design as PTU files, but it developed with thermomechanical treatment. PTG files have 2-stage specific transformation behaviour and high Af temperatures, whereas PTU instruments have 1-stage transformation [7], [15]. This transformation was responsible for the altered mechanical response of PTG instruments. The differences in the fatigue resistance between the PTG and PTU files were related to the presence of martensite, which due to the high transformation temperatures found in the PTG files.

In the present study, the results showed that PTG files have higher flexural and torsional resistance than PTU files. Also, PTG files showed greater flexibility than PTU files. This is due to the thermomechanical treatment of the alloy that altered the mechanical response of the alloy and increased its flexibility. This was in full agreement with Plotino et al., [15] and Hieawy et al., [16] and Aoun et al., [17] who compared the mechanical properties of both PTG and PTU.

In conclusion, the present study evaluated the stress distribution during bending and torsion using FE analysis for two NiTi files of the same design and different metallurgy. With thin the limitations of this study, It was concluded that the thermomechanical treatment improved the mechanical response (bending and torsional resistance) of NiTi files. Further investigations may be needed to investigate the effect of different temperatures on the mechanical behaviour of NiTi file to improve the life span of the files during clinical practice.

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Dental Science

Evaluation of Shear-Bond-Strength of Dental Self-Adhering Flowable Resin-Composite versus Total-Etch One to Enamel and Dentin Surfaces: An *In-Vitro* Study

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Abstract

AIM: This study aimed to assess the shear bond strength of a self-adhering flowable resin composite versus a total-etch one to different surfaces of permanent-molars.

MATERIAL AND METHODS: Thirty-six sound human permanent molars were used. The teeth were embedded in acrylic blocks, such that their buccal surfaces were shown. The teeth were divided into three groups: Group I: Uncut-Enamel, Group II: Cut-enamel-surfaces with minimal-grinding and Group III: dentin-surfaces. Half of the teeth in each group were used for bonding to a self-adhering flowable resin-composite (Dyad-flow, Kerr, USA). While the other half of each group was bonded to a total-etch flowable resin-composite (Filtek™Z350-XT,3M-ESPE, USA) which necessitate etching and bonding. Teflon-mold was used for constructing resin composite cylinders (3 × 3 mm) over the buccal surfaces. The Dyad-flow was applied in the central hole of the mould placed upon tooth-surface, and then light-cured for 20 seconds. The Filtek-Z350-XT was applied similarly after etching and bonding steps. The teeth were stored in 37°C distilled water for 24 hours. The strength was measured using a universal testing machine and statistically analysed. Modes of failure were studied using digital-microscope.

RESULTS: Mean values of shear bond strength for the Dyad and Filtek-Z350-XT in the uncut-enamel were 3.5 and 24.6MPa respectively, while that for cut-enamel were 4.5 and 12.7MPa respectively (Both highly statistically significant $P \leq 0.01$) and in dentin were 4.3 and 6.7MPa respectively (Statistically significant $P \leq 0.05$). The failure mode for Dyad was mainly adhesive (un-cut or cut-enamel 83.3% adhesive and 16.7% mixed, while in dentin 100% adhesive). While the modes of failure for Filtek-Z350-XT in enamel, either cut or un-cut, were 50% cohesive and 50% mixed, whereas in dentin 100% adhesive.

CONCLUSION: Bonding of self-etch ″Dyad-flow″ flowable resin-composite was lower than the total-etch one in enamel and dentin. Thus further material improvement may be required.

Introduction

Recently, flowable composite resins become very common in dental clinics [1]. The low viscosity of these materials allows their easier manipulation with more adaptation than conventional resin composites [2]. A new self-adhering flowable composite (Dyad™-flow, Kerr, USA) was recently introduced in the market [3]. Incorporation of an acidic adhesive-free composite may lead an interaction between the material and tooth structures, both chemically and micro mechanically [4].

The multi-step etch and rinse approach, contrary to the self-etch, involves a phosphoric acid-etch step which results in deep pits in enamel's hydroxyapatite. The resin tags incorporated within this acid-etched enamel lead to an optimal bond with the enamel surface effectively sealing the restorations' margins. The traditional etch and rinse adhesives are still regarded as 'gold-standard' [5], [6], [7], [8].

For a strong bond between the tooth structure

and a restoration, primary chemical interaction between resin and tooth structure could be a tremendous aid. Functional monomers, in particular like 10-MDP (10-methacryloyloxydecyl dihydrogen phosphate), have been proven to interact with hydroxyapatite through primary ionic binding which results in chemical bond in addition to micro-mechanical interlocking [9], [10]. However, this chemical bonding should also be stable in an aqueous environment. Chemical bonding promoted by 10-MDP was proven to be more stable in water than that provided by other functional monomers like 4-META (4-methacryloyloxyethyl trimellitic acid) and phenyl- P (2-methacryloyloxyethyl phenyl phosphoric acid) [10]. The 10-MDP is present in some commercial bonding agents applied before resin-composite restorations.

On the other hand, the self-adhering flowable composite "Dyad-flow" contains an adhesive monomer called glycerol phosphate dimethacrylate "GPDM" having two functional groups; the first is an acidic phosphate for both tooth etching and chemical bonding with its calcium content, while the other is a methacrylate group for polymerisation [3]. Contrary, it was reported that GPDM "etches" instead of "bonds" to hydroxyapatite [10].

This study aimed to assess the shear bond strength of self-adhering flowable resin-composite "Dyad-flow" compared to the total-etch one (preceded by etching and bonding steps) to un-cut and cut enamel as well as dentin of permanent molars.

Methods

Specimens' preparation and grouping

Thirty-six permanent molars were embedded in acrylic blocks (19 mm diameter X 16 mm height), such that their buccal surfaces were shown and aligned with the acrylic surfaces (Cold cure acrylic resin, Acrostone, Egypt). The sample size was calculated using G-power with effect size 0.7, Power 80% and alpha error 5% for a final total sample size 36 molars. The teeth were randomly divided into three groups: Group I: Enamel surfaces without any intervention (Uncut Enamel), Figure 1a, Group II: Enamel surfaces with minimal grinding (Cut Enamel), Figure 1b and Group III: Dentin surfaces, Figure 1c. In groups II and III, the buccal surfaces of the teeth were ground using a grinding machine (Redwing, Handler, USA) under water coolant. Half of the teeth in each group were used for bonding to a self-adhering flowable resin-composite (Dyad™-flow, Kerr, USA). While the other half of each group was bonded to a total-etch flowable resin-composite (Filtek™ Z350-XT, 3M-ESPE, USA) which necessitate etching and bonding.

Figure 1: A photograph showing the different teeth surfaces: A) Uncut Enamel); B) Cut Enamel; C) Dentin

Bonding Procedure

A specially designed holed-split Teflon mould was used for constructing composite-resin cylinders from the previous flowable composites (3 × 3 mm) over the buccal surfaces of the mounted teeth.

Self-adhering flowable resin-composite

The Dyad™-flow was applied in a central hole of the mould upon each tooth surface, and then light-cured for 20 seconds using a light-curing unit (Satelec, Acteon, France). The teeth with the bonded resin cylinders were stored in distilled water at 37°C for 24 hours.

Total-etch flowable composite

Before the application of Filtek™ Z350-XT flowable composite, etching and bonding steps were performed. A phosphoric acid etching gel 37% (Eco-Etch, Ivoclar Vivadent, Liechtenstein) was applied for 15 seconds, then rinsed and air-dried. A bonding agent containing 10-MDP (Universal Single Bond, 3M ESPE, Germany) was applied for the teeth in a rubbing motion for 20 seconds. Then, a gentle air stream was applied over the bonding agent for 5 seconds, then light-cured for 10 seconds. Then, the flowable composite was applied through the mould and cured as previous. The teeth were stored in distilled water at 37°C for 24 hours.

Shear bond strength test

Each acrylic block was secured with tightening screws to the lower fixed compartment of a universal testing machine (Model LRX-plus; Lloyd Instruments Ltd., Fareham, UK), with a load cell of 5 kN. A shearing load with the compressive mode of force by mono-bevel-chisel was applied via materials testing machine at a crosshead speed of 0.5 mm/min. The load required for de-bonding was recorded in Newton. The data were recorded automatically using computer software (Nexygen-MT Lloyd Instruments).

Shear bond strength was calculated as the load at failure divided by bonding area to express the bond strength in MPa: $\tau = P/\pi r^2$

Where; τ = bond strength (in MPa), P = load at failure (in N), π = 3.14, r = radius of cylinder (in

mm). The strength was recorded blindly by a different assessor, and the data were statistically analysed. Modes of failure were studied using a digital microscope (Scope Capture Digital Microscope, Guangdong, China), and recorded as cohesive, adhesive or mixed failure by a different assessor blindly.

Statistical Analysis

Statistical analysis was performed for all data using the statistical package for social science IBM®, SPSS® statistics for windows computer software version 20 {IBM® (IBM corporation, NY, USA) and SPSS® (SPSS Inc., an IBM company, USA)}. Analysis of variance (ANOVA) was used for determining the statistical significance of the mean shear bond strength values between the groups. All graphs were made using Excel Microsoft windows in 2010. The *p*-values were considered statistically significant if less than or equal 0.05 and highly statistically significant if less than or equal 0.01, while not statistically significant if greater than 0.05.

Results

Mean values of shear bond strength for the Dyad and Filtek-Z350-XT in the uncut-enamel were 3.5 ± 1.6 and 24.6 ± 6.2MPa respectively, while that for cut-enamel were 4.5 ± 2.7 and 12.7 ± 4.5MPa respectively (Both highly statistically significant $P \leq 0.01$) and in dentin were 4.3 ± 1.6 and 6.7 ± 1.7MPa for Dyad and Filtek-Z350-XT respectively (Statistically significant $P \leq 0.05$), as shown in Table 1.

Table 1: Mean Shear Bond Strength Values for the different groups

	Uncut Enamel*Dyad	Uncut Enamel*FiltekZ350-XT	Cut Enamel*Dyad	Cut Enamel*FiltekZ350-XT	Dentin*Dyad	Dentin*FiltekZ350-XT
Mean	3.5	24.6	4.5	12.7	4.3	6.7
SD	1.6	6.2	2.7	4.5	1.6	1.7
P-value	0.000224		0.006954		0.0319845	
	Highly statistically significant $P \leq 0.01$		Highly statistically significant $P \leq 0.01$		Statistically significant $P \leq 0.05$	

The failure mode for Dyad was mainly adhesive either in un-cut enamel or cut-enamel (both were 83.3% adhesive and 16.7% mixed), while in dentin was 100% adhesive, Figure 2.

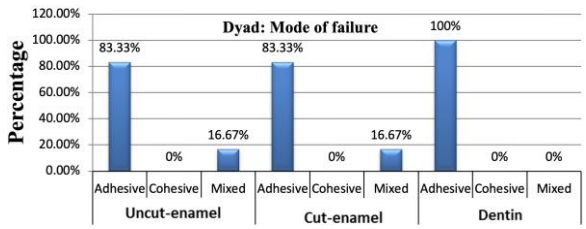

Figure 2: Histogram showing the mode of failure for Dyad in uncut, cut enamel and dentin

While the modes of failure for Filtek-Z350-XT in enamel, either cut or un-cut, were 50 % cohesive and 50 % mixed, whereas in dentin 100% adhesive, Figure 3.

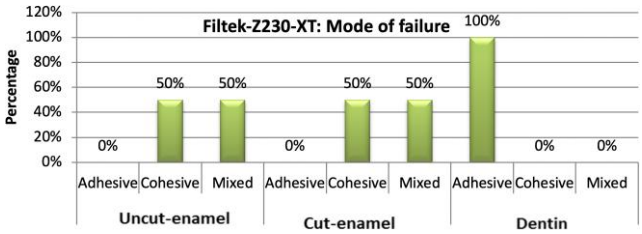

Figure 3: Histogram showing the mode of failure for Filtek-Z350-XT in uncut, cut enamel and dentin

Discussion

For this study, two types of flowable resin-composite were tested: self-adhering one (Dyad™-flow) versus flowable composite (Filtek-Z350-XT) proceeded by etching, rinse and bonding agent (Universal Single Bond). Both flowable composites were applied to uncut, cut enamel and dentin surfaces of permanent molars. In the three different surfaces of teeth, the multistep Filtek-Z350-XT showed significantly higher bond strength than the self-adhering Dyad-flow. This may be attributed to the etching and bonding steps before its application. The multi-step etch and rinse approach, contrary to the self-etch "Dyad-Flow", involved a phosphoric acid-etch step which resulted in deep pits in the hydroxyapatite-rich substrate of enamel. The mechanical interlocking of the resin tags of the bonding agent with the acid-etched enamel leads to the best bond to the enamel which effectively seals the restorations' margins on the long term [5], [6], [7], [8]. While, in the dentin the phosphoric acid demineralised the smear layer, exposing the collagen fibres of the superficially demineralised dentin. These may also increase the micromechanical interlocking of the bonding agent within the dentin surface [11], [12], [13].

Furthermore, the multi-step approach in this study utilised the single universal bond which may aid in increasing the bond strength. This may be attributed to the strong chemical bonding to the tooth structure by phosphate monomer group 10-methacryloyloxydecyl dihydrogen phosphate monomer (10-MDP) in their composition [14], [15], [16]. It was shown that an effective chemical interaction occurs between hydroxyapatite and MDP, resulting in the formation of a stable nano-layer that could form a stronger phase at the adhesive interface increasing the bond strength [17], [18]. Also, stable "MDP-Ca" salt deposition accompanied by nano-layering may clarify the high bond stability which has

been proven previously in the laboratory and clinical researches [16], [19], [20]. Moreover, the ethanol solvent of single universal Bond, due to its high vapour pressure, competed with moisture, replacing it. This promoted the infiltration of monomer through the nano-spaces of the exposed collagen network. This served as a framework for the formation of a resin-demineralised dentin hybrid layer [13].

On the other hand, the Dyad-flow contains an adhesive monomer called glycerol phosphate dimethacrylate "GPDM" having an acidic phosphate functional group which could etch the teeth and claiming that it could also bond chemically with their calcium content [3]. However, it was reported previously that the chemical bonding potential of GPDM was not available [10]. This might explain the lower bond strength of self-adhering flowable resin-composite "Dyad-flow" to different teeth surfaces compared to other resin-composite (Filtek-Z350-XT) preceded by 10-MDP application in a universal single bond which chemically bond to tooth structure [14], [15], [16]. It was noticed that in both types of flowable resin-composite, dentin recorded lower shear bond strength value in comparison to uncut and cut enamel and the mode of failure was 100% adhesive in dentin regardless of the bonding technique used. This might be attributed to the higher organic content of the dentin, its heterogeneous composition and the poor wettability of its collagen fibrils by the adhesive material [4], [21], [22], [23], [24], [25]. Thus, bonding to dentin is still representing a challenge in many adhesive systems.

In conclusion, bonding of self-adhering ″Dyad-flow″ flowable resin-composite was lower than the total-etch one in enamel and dentin. Thus further material improvement may be required.

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Evaluation of Bond Strength of Aesthetic Type of Posts at Different Regions of Root Canal after Application of Adhesive Resin Cement

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Abstract

AIM: This study aimed to evaluate the bond strength between esthetic posts and dentin at different regions of the root canal in passive mode or push-out active mode.

METHODS: Twenty extracted human bicuspid single canal teeth were used in this study. Teeth were sectioned below the cement-enamel junction. The roots of teeth were endodontically treated. Glass fibre posts (Glassix plus, Harald Nordin SA, Switzerland) and zirconia posts (Zirix, Harald Nordin) were then adhesively luted with total-etch (Variolink N, Ivoclar Vivadent, Schaan, Liechtenstein) and self-adhesive (Multilink Speed, Ivoclar Vivadent) resin cement. The roots were divided into two main groups of 10 samples each, according to the type of post used. Each group subdivided into 2 subgroups of 5 samples each, according to the type of cement used. The specimens were transversally sectioned into three slices of 2 mm thickness to perform the push-out test. The push-out tests were performed at crosshead-speed, 0.5 mm/min). Failure modes were evaluated using a scanning electron microscope at magnification (x 150).

RESULTS: The results revealed that push-out bond strengths were significantly affected by the type of luting agent and the type of post ($P \leq 0.05$). The mean push-out bond strength values for fibre post were significantly higher than those for zirconia post independent of the luting strategy used. The score values of total-etch adhesive resin cement were higher than those for self-adhesive resin cement irrespective of other variables. Regarding the effect of the root segment on push-out bond strength, results revealed that bond strength decreased from the coronal to the apical section. The cement-dentin interface found to be the weakest part of the root-cement-post unit.

CONCLUSION: Glass fibre posts revealed better results in all root third when they adhesively luted with total-etch or self-etch adhesive resin cement and provided significantly increased bond strength compared to the zirconia posts.

Introduction

It is known that teeth that have been undergone endodontic treatment are weak, the structure is less tolerable to the external stresses, mechanical shocks and less withstand the masticatory forces. This become clearer if the remaining walls of those teeth are not enough to provide the resistant form against fracture and breakage. Therefore, the proper reconstruction of such teeth is essential before the placement of the final restorative materials or crowns. The use of post and core is one of a successful application that leads to the supporting and strengthening of endodontically treated teeth, but the choice of the most suitable type of each case needs proper diagnosis and the ability of the dentist to perform these procedures in a high skilled manner [1]. Several in-vitro studies have been conducted to assess the different aspects of post and core. These studies have indicated the importance of using a complex combination of materials (dentin, metal

posts, cement, and core materials) with varying degrees of rigidity and stiffness. With the increasing demands for aesthetic procedures, aesthetic posts have become more used by dentists than ever before, specifically, the usage of metal-free post-and-core systems, e.g. zirconia and fibre posts [2]. Selection of a suitable adhesive and luting procedures for bonding posts to root dentin is a challenge. The actual bond strength at the post cement-root interface is affected by many factors including the degree of dehydration of the root canal dentin, the type of conditioning agent, type of cement used; the unfavorable cavity configuration of the root canal; the use of eugenol-containing sealers and the anatomic differences in density and orientation of the dentinal tubules at different levels of the root canal areas.

Furthermore, the difficulty of moisture control and the lack of direct vision into the root canal adversely affect the bonding procedures [3], [4], [5]. The push-out tests have been used to measure post retention in different regions of post space. This method was shown to have fewer premature specimen failures and a lower data distribution variability compared to conventional shear test. This may be due to the nature of fracture occurred in the push-out test that takes place parallel to the dentin bonding interface, which may make it a true shear test [6], [7].

Material and Methods

Twenty single-rooted human mandibular first premolar teeth were collected from middle-aged male patients (35-45 years old). Teeth selected to be used in this study were of an average similarity in size, shape and root morphology. The roots were inspected under proper lighting with a magnifying lens to ensure the absence of caries, cracks or fracture. The teeth were cleaned from loose debris, hard and soft attachments using scalar (Martin, Germany). The selected teeth were then placed in a 0.9% standardized saline solution and stored at room temperature until use.

Specimen preparation

The crown of each tooth was sectioned horizontally 2 mm coronal to the cement-enamel junction, on a plane perpendicular to the long axis of the tooth. A fissure carbide bur (Komet-Brasseler GmbH, Lemgo, Germany) mounted on a high-speed handpiece was used with copious air-water spray, to obtain a root length of 15 ± 1 mm. The cut surfaces were smoothened using a fine diamond disc (Komet-Brasseler) The coronal access opening was sealed with eugenol-free temporary filling Coltosol F (colténe AG, Altstätten, Switzerland) and the teeth stored in normal saline solution at room temperature till endodontic treatment. The Canal patency was determined by a file size 15 K-file. The roots were endodontically instrumented at a working length of 1mm from the apex using a 35-master apical file. All root canals were instrumented by the same operator, and the step-back technique was used for cleaning and shaping of root canals, which were enlarged to size 50 H-file. Irrigation was performed with 5.25% sodium hypochlorite solution (NaOCl) using a plastic syringe after each change in the size of file throughout the shaping process. Then, the root canals were rinsed with distilled water, dried with paper points (Dentsply-Maillefer) size 35, being ready for obturation.

The master cone was tried to fit the prepared canal and to reach the full working length with a tug-back action. The prepared canals were filled with calcium hydroxide sealer (Apex plus, Ivoclar Vivadent) with the aid of intra-canal tips, Guttapercha points (Dentsply-Maillefer) were coated with the sealer and placed in the root canal. A finger spreader was inserted into the root canals to a level approximately 1 mm short of the working length; then it was removed by rotating it back and withdrawn. Accessory points were selected and applied. The process was repeated until the canal was filled. Excess gutta-percha was removed, and the coronal mass was compacted with a plugger. Finally, the canal openings were filled with Coltosol F temporary filling material. The roots were embedded in auto-polymerising acrylic resin (Acrostone Dental Factory, England) surrounded by a custom-made hollow plastic cylinder having the 25 mm external diameter and 30 mm height. To ensure an accurate vertical and centralised position of each root and its canal in the acrylic resin cylinder, a specially designed centralising device was constructed for this purpose.

Post luting procedures

The length of the samples was determined to be 15 ± 1 mm. By leaving 4 mm of gutta-percha in the canal space as an apical seal, the post space length was determined to be 11 mm. The gutta-percha was removed up to the appropriate depth using pilot reamer (Harald Nordin), a rubber stopper was inserted in its shaft and adjusted to the desired post length. The root canal of each specimen was then enlarged with a low-speed drill provided by the manufacturer of the post system, using the corresponding drill of the selected post**.** The canals were flushed using sterile water then dried with paper points. The prepared roots were randomly divided into 2 main groups of 10 samples each, according to the type of post used (Glass fibre and Zirconia) posts, 5 samples each, according to the type of cement used (total-etch and self-adhesive) resin cement.

Post cementation with total-etch resin cement

The canal was etched with 37% phosphoric acid (Total Etch, Ivoclar Vivadent). The etching gel was introduced into the canal with a needle, and after 15 seconds, the post space was rinsed with distilled water using a plastic syringe and dried with paper points. Using micro-brush, the (ExciTE F DSC, Ivoclar Vivadent) dual-cure single component adhesive system was applied, excess adhesive solution was absorbed with paper points, gently air-dried and exposed to light for 10 seconds from a coronal direction. An equal amount of Vriolink N (Ivoclar Vivadent) base and catalyst of low viscosity was mixed on the mixing bad for 10 seconds using cement spatula and applied into the canal with a lentulo spiral (Dentsply-Maillefer) using a low-speed handpiece, in addition, the external surface of each post was coated with cement prior to placement into the prepared canal, the post firmly and carefully placed into the canal within 1-1.5 minutes from initial mixing, finger pressure was maintained and excess cement was removed with a micro-brush. Samples were then light-cured (LED curing light, Guangzhou, China) through the cervical portion of the root for 40 seconds to accelerate the curing process.

Post cementation with self-adhesive resin cement

The auto-mixing syringe of the Multilink Speed (Ivoclar Vivadent) resin cement was prepared for use. This was done by examining the level of cement base and catalyst in the two orifices of the syringe to ensure even flow of both base and catalyst. The mixing tip with intra-oral tip was then attached to the syringe. The cement was gently dispensed with the help of intra-oral tip inserted into the canal. The post was then placed into the canal, finger pressure was maintained, and the excess cement was removed with a micro-brush. Samples were then light-cured through the cervical portion of the root for 20 seconds to accelerate the curing process. All posts were cemented by a single operator adhering strictly to each manufacturer's instructions. To standardise the load application during cementation procedure, each post was maintained under a load of 1 kg for 2 minutes; the cement was then light-cured. A specially designed loading device was fabricated for this purpose. After cementation, all samples underwent 1500 thermal cycles in a thermo-cycling device (MPM Instruments, Bernareggio MI, Italy) between 5°C and 55°C, with a 30 seconds dwell time in each bath and 10 seconds transfer between temperature baths [43]. The temperatures were checked every 15 minutes using a thermometer in each bath and time checked by using a stopwatch. The samples were then stored in a distilled water at room temperature for one week.

Preparation of the samples for testing

The prepared roots of the tested teeth were sectioned horizontally, and perpendicular to the long axis of the root into 2 mm sections using a diamond saw underwater irrigation for the push-out test. The apical section was cut 4 mm away from the root apex to avoid cutting through obturating materials. The first coronal 2 mm together with the most apical 4 mm of the root was discarded. Three sections of the 2 mm thickness (cervical, middle, and apical) were obtained (Figure 1). Each section was marked on its coronal side with an indelible marker, and the thickness of each section was measured with a digital calliper.

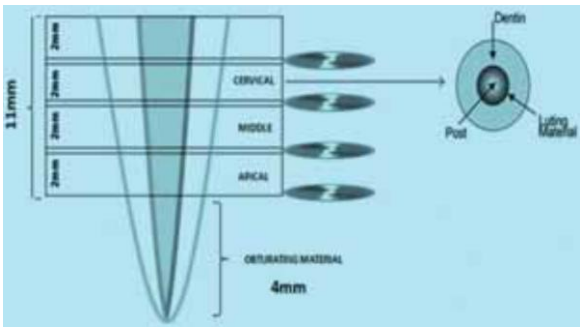

Figure 1: Schematic view of specimen preparation for the push-out test

Push-out bond strength test

Each section was subjected to compressive loading via a computer-controlled material testing machine (Model LRX-plus; Lloyd Instruments Ltd., Fareham, UK) with a load cell of 5kN. Data were recorded using computer software (Nexygen-MT; Lloyd Instruments). Each sample loaded by 3 plungers of different diameters (1, 0.75 & 0.5 mm). The plunger centred on the post segment provided that, contact with the surrounding dentin surface was avoided. Loads were applied in an apical-to-cervical direction (Figure 2).

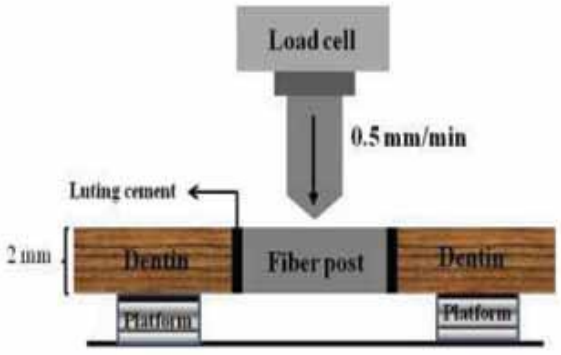

Figure 2: Schematic representation of the push-out test procedure. Consequently, shear bond strength MPa

The force in Newton (N) required to debond the post was recorded for all posts. To express the bond strength in MPa, the load at failure recorded in

Newtons was divided by the computed surface area as calculated by the following formula:

Bond = F/A A = πh (r1+r2)

where: A; area of the post/dentin interface, π; is the constant 3.14, r1; coronal radius, r2; apical radius and h; is the thickness of the slice in mm, standardised at 2 mm.

Scanning electron microscope

Representative samples for sectioned roots were gold-sputtered and used to evaluate the distribution of the luting material in the canal and to assess the resin dentin inter-diffusion zone, the hybrid zone, at magnification (X1000).

Results

Data were presented as mean and standard deviation (SD) values. Regression analysis using repeated measures Analysis of Variance (ANOVA) was used for studying the effect of post type, cement type, root segment and their interactions on mean push-out bond strength. Tukey's post-hoc test was used for pair-wise comparison between the mean values when the ANOVA test is significant. Detailed comparisons between the two post types as well as between the two cement types were performed using Student's t-test. The significance level was set at p ≤ 0.05. Statistical analysis was performed with IBM SPSS Statistics Version 20 for Windows.

Push-out test

The push-out bond strengths were significantly affected by the type of luting agent and the type of post (*P ≤ 0*.05). The mean push-out bond strength values for fibre post (10.1 MPa) were significantly higher than those for zirconia post (6.5 MPa) at *P ≤ 0.05* independent of the luting strategy used. The total-etch adhesive resin cement (13.8 MPa) score values higher than those for self-adhesive resin cement (2.8 MPa) at *P ≤ 0.05* irrespective of other variables.

Results also revealed that the coronal segment showed statistically significant highest mean push-out bond strength (10.9 MPa), followed by middle segment (7.9 MPa), while the apical segment showed the statistically significant lowest mean push-out bond strength (6.1 MPa) at P ≤ 0.05, regardless of other variables. The effects of different variables interactions on push-out bond strength are presented in Table 1.

Table 1: The mean, standard deviation (SD) values and results of the comparison between the different interactions

Post type x Cement x Root segment	Mean	SD	*P*-value
Fiber x Variolink x Coronal	20.7 a	1.1	
Fiber x Variolink x Middle	16.3 b	1.6	
Fiber x Variolink x Apical	13.3 c	0.6	
Fiber x Multilink x Coronal	6 f	0.3	
Fiber x Multilink x Middle	2.7 h	0.3	
Fiber x Multilink x Apical	1.8 i	0.2	< 0.001*
Zirconia x Variolink x Coronal	13.4 c	0.2	
Zirconia x Variolink x Middle	10.7 d	0.5	
Zirconia x Variolink x Apical	8.4 e	0.8	
Zirconia x Multilink x Coronal	3.6 g	0.3	
Zirconia x Multilink x Middle	1.7 i	0.2	
Zirconia x Multilink x Apical	1 j	0.1	

*: Significant at P ≤ 0.05, Different letters are statistically significantly different

Microscopic observation

The hybrid zone between radicular dentin and total-etch (Variolink N) resin cement at the ***cervical*** section revealed that: The hybrid layer is well defined with abundant resin tag formation, and tight interfacial seal, the extension of resin tags into the dentin surface can be observed. They appear to belong, thin and arranged in a relatively parallel pattern (Figure 3).

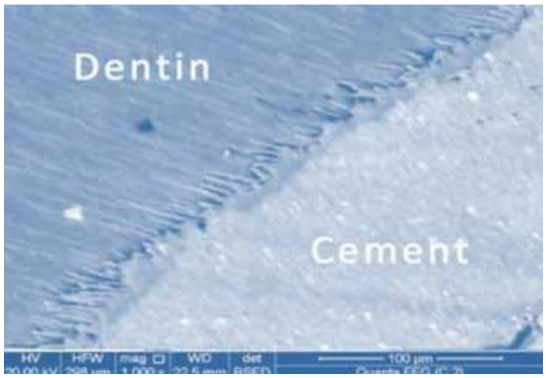

Figure 3: SEM photomicrograph of the hybrid zone for the segment of a Variolink Nresin cement sample cervical at (X1000)

In comparison, the hybrid zone between radicular dentin and self-adhesive (Multilink speed) resin cement at the cervical section revealed that: The hybrid layer is ill-defined. The resin tags appear to be irregular and intermingling with the dentin in some areas. The interfacial gap can be seen along the whole length of the specimen (Figure 4).

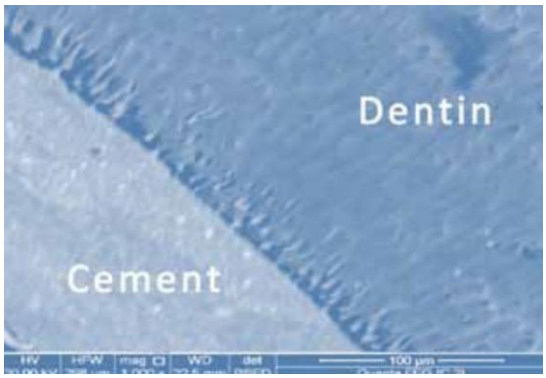

Figure 4: SEM photomicrograph of the hybrid zone for the cervical segment of a Multilink speed resin cement sample at (X 1000)

Discussion

In the present study, the push-out bond strengths of two different post types with two different luting cement at three different root regions were measured. The statistical significant difference in bond strength results between glass fiber and zirconia posts suggested that the elastic modulus of is approximately similar to that of both the fiber post and dentin, so the resultant homogeneous biomechanical unit allows a more uniform stress distribution, which better preserves the weakened tooth structure and reduces microleakage at the dentin-cement interface, secondary caries and consequently increase the bond strength [8]. Also The fibre posts are capable to bond chemically with the adhesive resin cement, indicating a good bond between the resin matrix of the fibre post and resin luting agent [9], [10]. On the other hand, establishing a reliable bond to zirconia-based materials has proven to be difficult, which is the major limitation against fabricating adhesive zirconia restorations. Its surface stability resulted in the problem of establishing a durable chemical or a mechanical bond which has proven to be a difficult task [11]. In spite of the creation of micro retention on zirconia posts, the adhesion between the post and resin luting agent was not uniform, thus indicating that the nature of post material was responsible for the bonding of the post to the tooth structure [12]. Unfortunately, H3PO4 and HF cannot be used effectively on non-silica-based ceramics, like ZrO2, making it difficult to roughen the surface for micromechanical retention. The lack of silica also removes the chemical bonding between silica – silane necessary for silanization [13]. Also, the ceramic posts present modulus of elasticity higher than those of dentin and resin cement. This difference in stiffness resulting in stress concentration on the tooth during masticatory function, thus, when a load is applied, it is transmitted to the softest material, the resin cement, in this case, to compromise the bond [14].

Further, the total-etch resin cement showed a significantly higher mean bond strength than self-adhesive resin cement, irrespective of the type of post used. This could be attributed to the separate phosphoric acid etch step which removes the thick surface smear layer on root canal dentin and the smear plugs in dentinal tubules formed during post space preparation, to allow more effective micromechanical retention of resin-based cement [15], [16], [17]. On the other hand, the methacrylate phosphoric esters in the self-adhesive resin cement cannot penetrate adequately through the retained partly dissolved smear layer on the root bond strengths [17], [18]. These findings were confirmed by the SEM evaluation in figures (Figure 3 and 4). Higher bond strength in the coronal section of the root canal is most commonly explained by the higher density of dentinal tubules and the longer resin tags formed in this area [4], [19], [20]. Accordingly, there would be an unequal response to mechanical testing of each portion and subsequent variation in retention properties. Another point that may shed light on the lower bond strength values recorded for the apical region is the limited ability of the light to diffuse across the entire length of the resin cement thus compromising the polymerisation of the cement in the most apical regions. Moreover, it is increasingly more difficult to control moisture and adhesive application towards the apical region of the canal [21].

In conclusion: 1. post type has a significant effect on push-out shear bond strength, which was superior for fiber post compared to zirconia post; 2. total-etch adhesive resin cement provided higher push-out shear bond strength compared to self-etch adhesive resin cement; 3. the coronal root canal region was significantly more retentive than the apical root canal region; and 4. the cement-dentin interface is the weakest part of the root-cement-post unit.

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Effect of High Light Intensity Bleaching Protocol versus Descending Light Intensities Bleaching Protocol on Post Bleaching Teeth Sensitivity: A Randomized Clinical Trial

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Keywords: Teeth bleaching; High light intensity protocol; Descending light intensity protocol; Teeth sensitivity; Teeth shade

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Abstract

AIM: The study aimed to compare teeth sensitivity and shade after bleaching protocol with descending different light intensities versus bleaching protocol with the same high light intensity.

MATERIAL AND METHODS: Sample size was twenty-four patients. Each group consisted of twelve patients. Group I patients received bleaching protocol of descending different light intensities. Group II patients received bleaching protocol with the same high light intensity; both groups used the same home bleaching gel kit for seven days according to manufacturer instructions and protocol. Baseline records were digital photographs, teeth sensitivity and teeth shade for 12 anterior teeth. Teeth sensitivity was assessed using five points verbal rating scale and Standardized 100 mm Visual analogue scale after 1 day, after 2 days and after 1 week. Teeth shades for twelve anterior teeth were recorded by VITA Easy Shade V (VITA Zahnfabrik H. Rauter GmbH & Co. KG, Germany) after 1 week by VITA Easy Shade V. Mann-Whitney test (non-parametric test, 2 independent samples) was used to compare teeth sensitivity between both bleaching protocols at each period. A paired t-test (parametric test, 2 related samples) was performed to compare the colour change in shade guide units (SGU) and ΔE values within high light intensity bleaching protocol. While Wilcoxon Signed-Rank test (non-parametric test, 2 related samples) was used to compare colour change light intensities bleaching protocol. Comparison of bleaching effectiveness (ΔSGU and ΔEdiff) between both bleaching protocols was performed by the Mann-Whitney test.

RESULTS: Descending light intensities protocol showed a lower teeth sensitivity than high light intensity protocol after 1 and 2 days. There was no teeth sensitivity reported at 1-week post-bleaching. Regarding the teeth shade, descending light intensities protocol had a little higher effect on colour change in shade guide units (SGU) than high light intensity protocol effect. Both bleaching protocols showed there was no significant difference in ΔSGU recorded after bleaching between high and descending light intensities protocols.

CONCLUSION: Descending different light intensities protocol showed a lower teeth sensitivity than high same light intensity protocol. Descending light intensities protocol had a little higher effect on colour change in shade guide units (SGU) than high light intensity protocol effect.

Introduction

Teeth discolourations are usually various, multifaceted and have been classified as extrinsic, intrinsic, and internalised discolourations. Adult teeth get darker due to changes in enamel and dentin properties, losing their natural white shade [1]. Tooth shade is one of the most important factors determining satisfaction with dental appearance [2]. Self-satisfaction decreases with increasing severity of discolourations. White teeth look been positively correlated with high social competence, intellectual ability, psychological adjustment and relationship status [2].

Nowadays, vital tooth bleaching is one of the most requested cosmetic dental procedures asked by patients for a more pleasing smile [3]. The American Dental Association (ADA) has advised patients to ask the dentists to select the most suitable bleaching treatment, especially for those complaining of teeth sensitivity, dental restorations, extremely dark discolourations, and single dark teeth [4]. Success of

teeth bleaching is affected by many factors: stains type, age, bleaching activation systems, the concentration of the bleaching agents, and duration of application as well as home bleaching application [5].

Teeth sensitivity (TS) is the most common clinical side effect of vital teeth bleaching; more than 60% of the patients undergo it. It can cause severe discomfort that is the main obstacle to patients completes the bleaching treatment [6].

Activation of the bleaching agents by heat or light may hurt pulpal tissue. It was reported that the use of intense lights elevated bleach temperature and resulted in an increased intra pulpal temperature, which may further impact patient sensitivity and pulpal health [7].

Accordingly, a randomised clinical trial (RCT) on the effect of high light intensity bleaching protocol versus descending light intensities bleaching protocol on post bleaching teeth sensitivity would be of value.

Material and Methods

Trial Approval and Registration

The trial was designed following the SPIRIT 2013 Statement (Standard Protocol Items: Recommendations for Interventional Trials). The protocol was approved by Evidence-Based Dentistry Committee (EBD) – Faculty of Dentistry, Cairo University-Egypt. This study protocol was registered on ClinicalTrial.gov website; It's ClinicalTrials.gov identifier number: NCT02888847.

Sample size calculation

The sample size was 24 patients, based on the previous data [8], it indicated that the probability of no sensitivity among controls is 0.09. If the true probability of exposure among intervention was 0.6, then it was needed to study 12 patients in each group to be able to reject the null hypothesis, so that both groups were equal with probability (power) 0.8. The Type I error probability associated with this test of this null hypothesis was 0.05. An uncorrected chi-square statistic was used to evaluate this null hypothesis.

Participants' recruitment

The principal investigator screened patients in Outpatient clinics of departments of Conservative Dentistry and Restorative Dentistry in Faculty of Dentistry of Cairo University and British University in Egypt respectively, who require teeth bleaching to participate in this trial. Medical and dental histories were carefully assessed. Thorough extra- and intra-oral examinations of the participants were performed and recorded in the diagnostic chart to fulfil the eligibility criteria of the trial which were recognised from previous studies [9], [10], [11]. The inclusion and exclusion criteria are listed in Table 1.

Table 1: Eligibility criteria of the trial

Inclusion Criteria for participants:	Exclusion criteria for participants:
1. Patients included in this clinical trial were at least18 years old. 2. Patients were free from any systemic disease that may affect normal healing and predictable outcome. 3. Patients agreed to the informed consent and committed to follow-up period. 4. Refrained from tobacco products and any coloured liquids or food (e.g. coffee, tea, tomato sauce, etc.) during the active study period.	1. Patients with any systemic disease that may affect normal healing. 2. Patient with bad oral hygiene. 3. Pregnant females. 4. Patients who could not participate in the follow-up period. 5. Untreated, periodontal diseases were not allowed. 6. Active caries or defective Restorations in six anterior teeth. 7. Sensitive Teeth. 8. Bleaching history. 9. Patients are participating in more than 1 dental study.

Participants' grouping

All participants fulfilling the eligibility criteria were randomly assigned using computer-generated randomisation www.random.org to either intervention (descending light intensity bleaching protocol) or control (high light intensity bleaching protocol) groups.

Study setting

The study was conducted in the outpatient clinic of the Department of Restorative Dentistry - British University in Egypt.

Pre-Bleaching Teeth Sensitivity Assessment

Teeth sensitivity records: through five points verbal rating scale [10], [12] and visual analogue scale (0-100 mm) [13], [14] presented in Figure 1 and Figure 2 respectively. Any patients were complaining of sensitive teeth were disregard during recruitment according to eligibility criteria.

0 = none

1 = mild,

2 = moderate

3 = considerable

4 = severe

Figure 1: Five Verbal Points

Upper and lower alginate impressions were taken to fabricate customised fit bleaching trays. Two pairs of bleaching trays were fabricated for the patient. One pair was used as a positioning guide in recording teeth shade. The second pair was used with post-care gel and home bleaching gel. Teeth were scaled using ultrasonic scaler (SUPRASSON P5, ACTEON, France) followed by teeth polishing utilising white Nylon Bowl Polishing Polisher prophy Brushes and prophylaxis paste (Quartz Prophylaxis Paste, medium

grit by Dharma, USA).

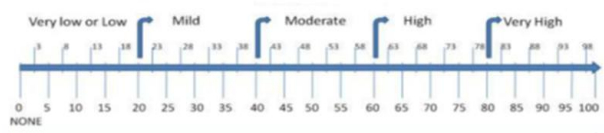

Figure 2: Visual Analogue Scale

Pre-bleaching Base Line Shade Assessment

Pre-bleaching digital images of the patient were taken. Baseline teeth shades for 12 anterior teeth (6 maxillary anterior teeth and 6 mandibular anterior teeth) were recorded by VITA Easyshade V (VITA Zahnfabrik H. Rauter GmbH & Co. KG, Germany), through punching the middle one third of the labial surface of the 6 anterior teeth in maxillary and mandibular bleaching trays [14]. The measurement area of interest for shade matching was the middle third of the facial surface of anterior maxillary teeth, according to the American Dental Association (ADA) guidelines [15].

VITA Easyshade V shade-matched teeth with value-ordered VITA® Classical Shade Guide into the following order presented in Figure 3 [14]. The arrangement from B1 (lightest colour) to C4 (darkest colour), corresponding to a grade of whitening from 1 to 16, in which a smaller number means the tooth was lighter. Although this scale is not linear in the truest sense, the changes were treated as though they represented a continuous and approximately linear ranking for analysis.

Arrangement of Vita Classic Shade Guide (Adapted From Browning and Others)

Vita Shade Guide															
B1	A1	B2	D2	A2	C1	C2	D4	A3	D3	B3	A3.5	B4	C3	A4	C4
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Brightest shade												Darkest shade			

Figure 3: Arrangement of Vita Classic Shade Guide

Shade parameters that were recorded: shade guide units (SGU) and its relative ΔE directly through spectrophotometer measurements of VITA Easyshade V.

Δ E: The overall shade deviation closest value-ordered VITA® Classical Shade Guide in the colour space.

In-office Bleaching Phase

Three cycles were carried on according to manufacture instructions on ten anterior maxillary teeth and ten mandibular anterior teeth for two study groups.

Group I: Patients received Bleaching Protocol with same high light intensity. It represented the control group. *(Philips Zoom!™ Advanced Power whitening gel and lamp, Discus Dental, Inc., Culver City, CA, USA.). It's light source was* Ultra Violet – A band. Its average wavelength of 365 nm. It had one high light intensity mode.

Group II: Patients received Bleaching Protocol with descending (high followed by moderate then low) light intensities. *(Philips Zoom!™ WhiteSpeed in-office whitening gel and lamp, Discus Dental, Inc., Culver City, CA, USA.)*. It represented the intervention group. It's light source was Blue Light Emitting Diode (LED). Its wavelength was 465 nm. It had three light intensities high, medium and low modes.

Only one in-office bleaching gel kit was used utilising by two different light-activated bleaching devices with different light intensities. Also, one home bleaching gel kit was utilised in this study. The materials used as well as their composition, manufacturers' details and lot numbers were listed in Table 2.

Table 2: Specifications of materials, composition, manufacturers and batch numbers Material

	Specifications	Composition	Manufacturer	Batch number
Philips ZOOM!	In-office (chairside) light-activated bleaching gel	25% Hydrogen Peroxide and Ferrous Gluconate photo-catalyst	Discus Dental, LLC., Ontario, CA, 91761 USA	16243005
Philips Zoom nite white	Home chemical activated the bleaching gel Professional take-home whitening system	22% Carbamide Peroxide Formulated with ACP (amorphous calcium phosphate), potassium nitrate and fluoride.	Discus Dental, LLC., Ontario, CA, 91761 USA	423502124491
Relief® ACP Oral Care Gel	Desensitizer included into Philips ZOOM! Kit in-office (chair-side) light-activated bleaching gel	Water, Poloxamer 338, Potassium Nitrate, Mentha Piperita, Calcium Nitrate, Sodium Saccharin, Sodium Fluoride, Disodium Phosphate and Monosodium Phosphate.	Discus Dental, LLC., Ontario, CA, 91761 USA	16243005

Immediate Patient Post Care

Relief® ACP Oral Care Gel included in Philips ZOOM! The kit has been shown to decrease sensitivity and remineralise teeth. It was placed into trays and seated on patient teeth. Normal wear time was 30 minutes according to manufacturer instructions. The patients were asked not to drink or eat for 30 minutes after application according to manufacturer instructions. Digital imaging and videos were taken during the whole clinical procedures.

Instructions were given to the patients after ending in-office bleaching sessions

Patients were advised not to consume coffee, tea, tobacco, alcoholic drinks, and soft drinks for 2 hours after Zoom, until pellicle reforms.

Also, they were asked to decrease their intake of any stainable food or drinks and to drink using a straw.

Home bleaching phase

They were asked to wear the tray with the gel Philips Zoom NiteWhite (*Discus Dental, LLC., Ontario, CA, 91761 USA*) on daily routine at night before bedtime. Starting from the next day of bleaching sessions for seven successive days. Wear time was two hours per night.

1. Post-Bleaching Teeth Sensitivity Evaluation:

Teeth sensitivity records through five points' verbal rating scale and visual analogue scale (0-100 mm) after 1-day, after 2-days and after 1-week.

1.a. Five points verbal rating scale Evaluation:

Participants were asked to mark their sensitivity experience after in-office bleaching by 1-day, 2-days and 1-week, using the following points: 0 = none, 1 = mild, 2 = moderate, 3 = considerable, and 4 = severe.

1.b. Visual Analogue Scale (0-100 mm):

The patients were asked to mark a standardised 100 mm visual analogue scale (VAS) ranging from 0 to 100. One end was labelled 'no discomfort' whereas the other end was labelled 'severe discomfort'. After the in-office bleaching treatment by 1-day, 2-days and 1-week, the patients were asked to mark the position that best indicated their current opinion.

2. Post-Bleaching Shade Evaluation:

Digital imaging of the patient was taken after one week (7 days) from the bleaching session. Also, post bleaching teeth shades for 12 anterior teeth (6 maxillary anterior teeth and 6 mandibular anterior teeth) were recorded by VITA Easy Shade V (VITA Zahnfabrik H. Rauter GmbH & Co. KG, Germany) after one week (7 days) through wearing punched bleaching trays used in baseline records by VITA Easy Shade V.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics Version 2.1 for Windows. Data were presented as mean and standard deviation (SD). The significance level was set at $P \leq 0.05$. Kolmogorov-Smirnov and Shapiro-Wilk tests were used to assess data normality.

Kruskal-Wallis (non-parametric test, repeated comparisons) followed by Bonferroni-corrected Wilcoxon Signed-Rank test was conducted to compare post-bleaching teeth sensitivity (TS) assessed by both pain scales at different periods within each bleaching protocol. Mann-Whitney test (non-parametric test, 2 independent samples) was used to compare teeth sensitivity between both bleaching protocols at each period.

A paired t-test (parametric test, 2 related samples) was performed to compare the colour change in shade guide units (SGU) and ΔE values within high light intensity bleaching protocol. While Wilcoxon Signed-Rank test (non-parametric test, 2 related samples) was used to compare colour change light intensities bleaching protocol. Comparison of bleaching effectiveness (ΔSGU and ΔE_{diff}) between both bleaching protocols was performed by the Mann-Whitney test.

Results

In this study, a total of twenty-four patients received teeth bleaching (3 cycles) re-evaluated after 1-day, 2-days and 1-week.

1. Post-Bleaching tooth sensitivity (TS):

1.a. Effect of different periods on post-bleaching teeth sensitivity within each bleaching protocol:

Mean, Standard deviation (SD) of VRS and VAS and P-value for the effect of different periods on post-bleaching teeth sensitivity within each bleaching protocol are presented in Table 3, Figure 4 and Figure 5.

Table 3: Statistical analysis for mean ± St. deviation of VRS and VAS and P-value for the effect of different periods on post-bleaching teeth sensitivity within each bleaching protocol

	High light intensity		Descending light intensities	
	VRS	VAS	VRS	VAS
Baseline	0 [a]	0 [a]	0 [a]	0 [a]
1-day post-bleaching	2.1 ± 0.7 [b]	42.1 ± 7.1 [b]	1.3 ± 0.4 [b]	28.3 ± 15.5 [b]
2-days post-bleaching	0.5 ± 0.5 [c]	10.5 ± 12.4 [c]	0.3 ± 0.4 [c]	8.3 ± 12.3 [c]
1-week post-bleaching	0 [a]	0 [a]	0 [a]	0 [a]
P-value	< 0.001*	< 0.001*	< 0.001*	< 0.001*

*Different superscript letters within the same row indicates a statistically significant difference at p ≤ 0.05; *: significant (p ≤ 0.05); NS: non-significant (p > 0.05).*

In both high and descending light intensities protocols, the significantly highest VRS and VAS values were recorded 1-day post- bleaching, followed by those recorded 2-days post- bleaching. Then 1-week post- bleaches, there was no teeth sensitivity in both protocols.

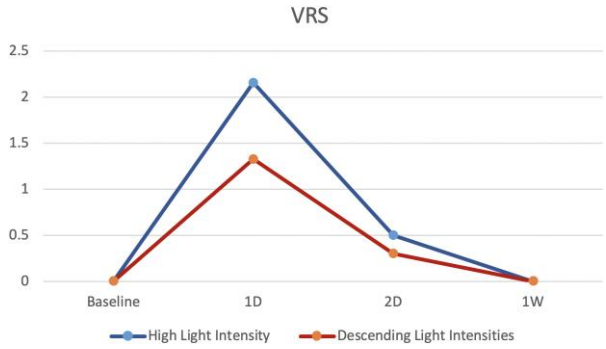

Figure 4: Line graph chart showing VRS at different periods within each bleaching protocol

In high light intensity protocol, values recorded 1-day post- bleaching by VRS (VRS Verbal Rating Scale), and VAS (Visual Analogue Scale) were (2.1 ± 0.7) and (42.1 ± 7.1) respectively. While descending light intensities protocol, values recorded 1-day post- bleaching by VRS and VAS were (1.3 ± 0.4) and (28.3 ± 15.5) respectively.

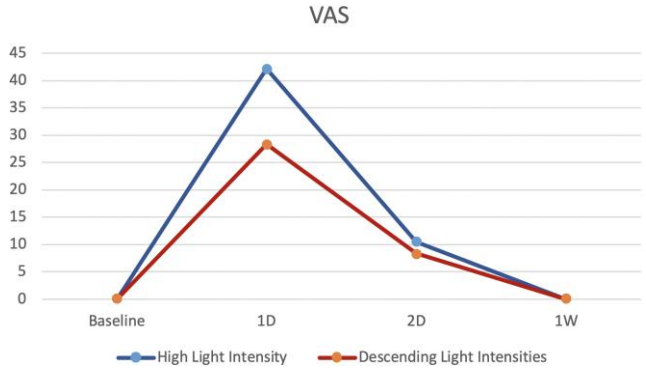

Figure 5: Line graph chart showing VAS at different periods within each bleaching protocol

In high light intensity protocol, values recorded 2-days post- bleaching by VRS and VAS were (0.5 ± 0.5) and (10.5 ± 12.4) respectively. While descending light intensities protocol, values recorded 2-days post- bleaching by VRS and VAS were (0.3 ± 0.4) and (8.3 ± 12.3) respectively.

1.b. Effect of bleaching protocol on post-bleaching teeth sensitivity at each period:

Mean, Standard deviation (SD) of VRS and VAS and P-value for the effect of bleaching protocol on post-bleaching teeth sensitivity at each period are presented in Table 4, Figure 6 and Figure 7.

Table 4: Statistical analysis for mean ± St. Deviation of VRS and VAS and P-value for the effect of bleaching protocol on post-bleaching teeth sensitivity at each period

		Baseline	1-day post-bleaching	2-days post-bleaching	1-week post-bleaching
VRS	High light intensity	0	2.1 ± 0.7	0.5 ± 0.5	0
	Descending light intensities	0	1.3 ± 0.4	0.3 ± 0.4	0
	P-value	---	0.006*	0.418NS	---
VAS	High light intensity	0	42.1 ± 7.1	10.5 ± 12.4	0
	Descending light intensities	0	28.3 ± 15.5	8.3 ± 12.3	0
	P-value	---	0.019*	0.435NS	---

**: significant (p ≤ 0.05); NS: non-significant (p > 0.05).*

At 1-day post bleaching, descending light intensities recorded lower sensitivity values than high light intensities at both scales. Mann-Whitney test showed that there was a statistically significant difference between both high and descending light intensities protocols after 1-day in VRS and VAS values (p = 0.006 and p = 0.019, respectively).

While on 2-days post bleaching, descending light intensities recorded lower sensitivity values than high light intensity at both scales. But there was no statistically significant difference between both protocols in VRS and VAS values on 2-days post bleaching (p = 0.418 and p = 0.435, respectively).

Table 4: Statistical analysis for mean ± St. Deviation of VRS and VAS and P-value for the effect of bleaching protocol on post-bleaching teeth sensitivity at each period

		Baseline	1-day post-bleaching	2-days post-bleaching	1-week post-bleaching
VRS	High light intensity	0	2.1 ± 0.7	0.5 ± 0.5	0
	Descending light intensities	0	1.3 ± 0.4	0.3 ± 0.4	0
	P-value	---	0.006*	0.418NS	---
VAS	High light intensity	0	42.1 ± 7.1	10.5 ± 12.4	0
	Descending light intensities	0	28.3 ± 15.5	8.3 ± 12.3	0
	P-value	---	0.019*	0.435NS	---

**: significant (p ≤ 0.05); NS: non-significant (p > 0.05).*

On 1-week post bleaching, descending light intensities and high light intensity protocols recorded zero sensitivity values at both scales.

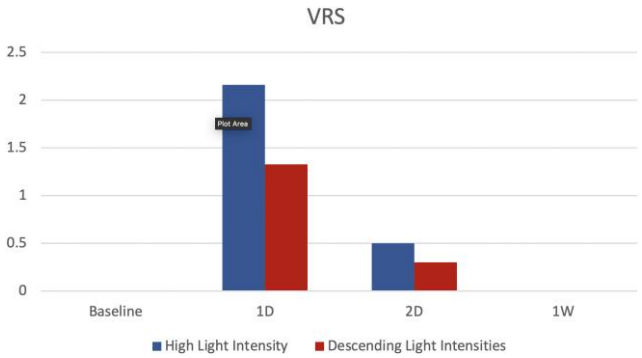

Figure 6: Bar chart showing VRS of both bleaching protocols at each period

Therefore, descending light intensities protocol scored lower sensitivity values than high light intensity protocol over the whole period time of investigation (1-day post, 2-days post and 1-week post).

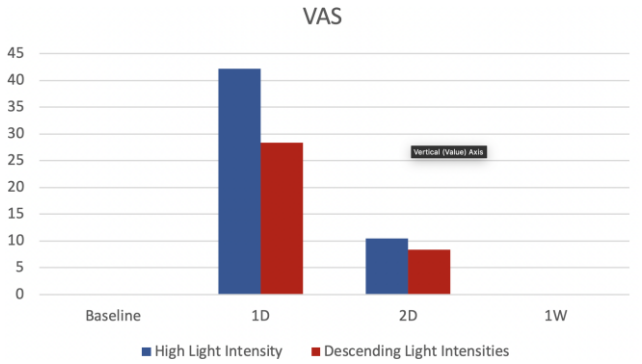

Figure 7: Bar chart showing VAS of both bleaching protocols at each period

2. Colour change:

2.a. Effect of bleaching protocol on colour change in shade guide units (SGU):

Mean, and Standard deviation (SD) for the effect of bleaching protocol on colour change in shade guide units (SGU) are presented in Table 5 and

Figure 8.

Table 5: Statistical analysis for mean ± St. deviation for the effect of bleaching protocol on colour change in shade guide units (SGU)

	Before bleaching	Post-bleaching	P-value
High light intensity	5.9 ± 3.1	2.8 ± 1.5	0.001*
Descending light intensities	6.6 ± 1.3	2.9 ± 1.6	0.002*

**: significant (p ≤ 0.05); NS: non-significant (p > 0.05).*

High light intensity protocol yielded significant change in SGU [from 5.9 ± 3.1 to 2.8 ± 1.5] at (p = 0.001). Descending light intensities protocol led to significant change in SGU [from 6.6 ± 1.3 to 2.9 ± 1.6] at (p = 0.002). Thus, descending light intensities protocol had a slightly higher effect on colour change in shade guide units than high light intensity protocol effect.

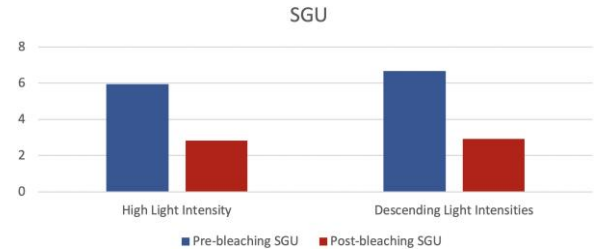

Figure 8: Bar chart showing a colour change in SGU within each bleaching protocol

2.b. Effect of each bleaching protocol on colour change in ΔE:

Mean, and Standard deviation for the effect of bleaching protocol on colour change in ΔE are presented in Table 6 and Figure 9.

Table 6: Statistical analysis for mean ± St. Deviation for the effect of bleaching protocol on colour change in ΔE

	Before bleaching	Post-bleaching	P-value
High intensity	5.6 ± 2.1	4.3 ± 2.8	0.267NS
Descending intensities	5.6 ± 1.7	5.3 ± 1.8	0.875NS

**: significant (p ≤ 0.05); NS: non-significant (p > 0.05).*

High light intensity protocol yielded no significant change in ΔE (p = 0.267). Descending light intensities protocol also yielded no significant change in ΔE (p = 0.875).

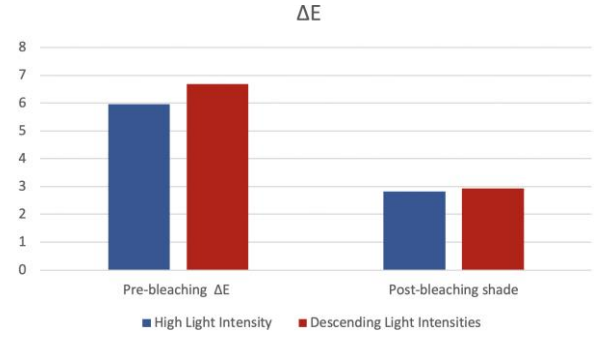

Figure 9: Bar chart showing a colour change in ΔE within each bleaching protocol

2.c. Comparison of bleaching effectiveness (ΔSGU) between both bleaching protocols:

Mean, and Standard deviation (SD) for the effect of bleaching effectiveness (ΔSGU) between both bleaching protocols are presented in Table 7 and Figure 10.

Table 7: Statistical analysis for mean ± St. deviation for the effect of bleaching effectiveness (ΔSGU) between both bleaching protocols

	High intensity	Descending intensities	P-value
ΔSGU	3.1 ± 2.3	3.7 ± 2.7	0.726 NS

**: significant (p ≤ 0.05); NS: non-significant (p > 0.05).*

There was no significant difference in ΔSGU recorded after teeth bleaching between high and descending light intensities protocols (p = 0.726).

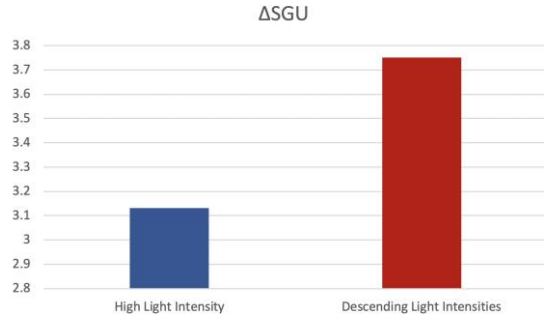

Figure 10: Bar chart showing ΔSGU of each bleaching protocol

2.d. Comparison of bleaching effectiveness (difference in ΔE) between both bleaching protocols:

Mean, and Standard deviation (SD) for the effect of bleaching effectiveness (difference in ΔE) between both bleaching protocols are presented in Table 8 and Figure 11.

Table 8: Statistical analysis for mean ± St. deviation for the effect of bleaching effectiveness (ΔE_{diff}) between both bleaching protocols

	High intensity	Descending intensities	P-value
Difference in ΔE	1.3 ± 3.9	0.25 ± 2.2	0.165NS

**: significant (p ≤ 0.05); NS: non-significant (p > 0.05).*

Mann-Whitney test showed that there was no significant difference in ΔE_{diff} measured after teeth bleaching between high and descending light intensities protocols (p = 0.165).

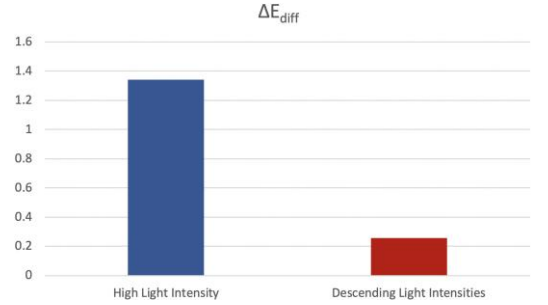

Figure 11: Bar chart showing the difference in ΔE of each bleaching protocol

Discussion

Tooth discolouration is caused by the presence of large organic compounds called chromogens, present within enamel and dentine that have conjugated double bonds in their chemical structure. Teeth bleaching works via the release of hydrogen peroxide (H_2O_2) either delivered directly as a concentrated bleaching agent or via the breakdown of carbamide peroxide upon contact with water [17]. H_2O_2 diffuses through enamel and dentine then breaks down into reactive free radicals that oxidise the chromogenic molecules into smaller molecules by breaking their double bonds [18].

This study was designed to assess teeth sensitivity using the bleaching protocol with *descending different light intensities* versus bleaching protocol with the *same high light intensity*. It was followed by one home bleaching kit in both groups.

All patients received dental prophylaxis before beginning the study, using a prophylaxis paste to remove extrinsic stains. The dental prophylaxis was performed before the teeth bleaching. It removes both saliva and polyphenols (such as tannins) on the tooth surface, which can inactivate reactive oxygen species [19].

It was evidenced that the in-office bleaching technique proved to be less efficient than home bleaching for removing stains deposited in dentin [20]. That's why both in-office and home bleaching are recommended. Therefore, a bleaching protocol of in-office bleaching followed by home bleaching is advocated [21]. Concerning post bleaching teeth sensitivity, it is reported that teeth sensitivity is higher during the first 24 hours' post-treatment [1]. This side effect extends for 5 days [1]. Thus, explain the duration one-week of assessing post bleaching sensitivity in this randomised clinical trial.

Different light sources have been used to activate bleaching agents, including halogen curing lights, ultraviolet and infrared lamps, plasma arcs, light-emitting diodes (LEDs), and lasers (CO2, argon, and diode lasers). Experimental studies and reviews showed controversial results regarding the effect of different forms of physical activation of in-office bleaching in colour enhancement of bleaching compared to non-activated bleaching [16], [22], [23]. The oxygen released from the hydrogen peroxide breaks down conjugated bonds in protein chains associated with stain into a single bond. This may result in more absorption of colour wavelengths and result in a reflection of colour (i.e., Lightening effect) [16], [24].

Descending light intensities protocol recorded lower sensitivity values than high light intensity protocol at both scales [13]. Due to the LED source produces the least thermal insult during the light activation process. And also, Blue LED light is optimal for photobleaching since it is not absorbed by water or hydroxyapatite. Thus, diffuse well through the tooth structure where it can remove chromophores that absorb blue/green light [19].

While UV (Ultra Violet) has very good photobleaching properties but does not diffuse well through teeth and causes pulp heating [19], thus, it confirms the high sensitivity values resulted from high light intensity bleaching protocol.

Contrarily, it was confirmed that the main success of the bleaching agents depends mainly on the nascent oxygen produced from the hydrogen peroxide, during the oxidation reaction and not on the light source, either LED lamps with the different intensities or UV lamp used [25].

It explained that descending light intensities protocol after day 1 and day 2 were lower than same high light intensity protocol. Due to lower rate production of free radicals, thus, allowing a rapid and large amount of inflammatory cells to react [26].

It was reported the increased release of substance P, a neuropeptide whose functions are linked to inflammation. It is evidenced after in-office bleaching associated with light/heat. While in the home bleaching, no increase in the release of SP and only slight histological changes were shown [9]. Therefore, sensitivity values were higher after both lights activated in-office bleaching protocols than home bleaching. Regarding the sensitivity results of this RCT, comes in agreement with El Hoshy et al., a study [27].

It embraced the high variability of the individual's pain response among the population. Because of, positive emotional deposition which activate the central pain inhibitory system, and release endorphins that decrease painful sensations [28].

It was concluded that the LED lamp could increase the bleaching of HP by 0.4 units SGU [29]. The shade results were in agreement with *previous studies* [10], [27], [30], [31] and [32] that found that light application has markedly enhanced the lightening efficacy of bleaching materials.

While there was disagreement regarding shade, with other studied [33], [34] that reported no difference in efficacy between teeth bleached with or without different irradiation. They concluded that chemicals added to the bleaching gels acted as catalysts in the bleaching process and were only responsible for activation, whereas lights did not affect.

It has become obvious that blue LED light-activated teeth bleaching plays an important role in the generation of radicals and subsequent break down of stains. Contrarily, the bleaching process through UV is not advocated since it leads to thermal running through exothermic decomposition into water and oxygen.

Under the limitations of the current study, the following conclusions could be derived:

1. Descending different light intensities protocol showed a lower teeth sensitivity than high same light intensity protocol.

2. Descending light intensities protocol had a little higher effect on colour change in shade guide units (SGU) than high light intensity protocol effect.

3. A combination of in-office tooth bleaching with home bleaching with 22% carbamide peroxide for one week revealed a superior bleaching effect.

Further recommendations for this study:

1. Comparison between both investigated bleaching protocols in split-mouth study design.

2. Unify the baseline teeth shade into one shade.

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Public Health

Stress Associated Alterations in Dietary Behaviours of Undergraduate Students of Qassim University, Saudi Arabia

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Abstract

BACKGROUND: Psychological stress associated eating habits among public health have now become a global concern.

AIM: This study was undertaken to investigate the levels of psychological stress among undergraduate students of Qassim University and to explore the stress associated alterations in their eating habits.

METHODS: This is a cross-sectional survey conducted on 614 undergraduate students of Qassim University, Saudi Arabia. A self-administered questionnaire was used, which included questions on socio-demography and eating habits. Level of stress was measured by a standardised questionnaire highlights the levels of non-chronic stimulation through difficulty relaxing, nervous arousal and being easily upset/agitated, irritable / over-reactive and impatient.

RESULTS: Our results show that 28.2% of total participants suffered from some extent of stress. Among stressed participants, 17.3%, 49.1%, 24.8% and 8.7% of participants suffered from mild, moderate, severe and extremely severe stress, respectively. Stressed participants were more preferred to eat junk foods such as fast foods, snacks and beverages as compared with unstressed participants ($p < 0.05$) and the junk food preference was increased with the increase of stress levels. Moreover, non-stressed participants preferred more healthy foods such as vegetarian food, fresh fruits as compared with stressed participants ($p < 0.05$). Taste and easy to access were the main reasons for the preference of junk foods by the stressed participants.

CONCLUSIONS: This is the first comprehensive study from Saudi Arabia to show stress associated dietary alterations in undergraduates of Qassim University. Data concluded that most of the young adults followed a healthy eating pattern, but a significant number from them were affected by stress. Therefore, specific intervention programs are strongly recommended for the reduction of stress and to improve their quality of life.

Introduction

Unhealthy eating habit is always a major public health issue in young adults, especially in those who experienced the transition into university life [1], [2]. During university life, young adults are usually exposed to psychological stress and lack of time, which generally alter their eating behaviours towards intake of junk foods [3], [4]. Stress alters the overall behaviour of eating which may be in either way, over- or under-eating, but continuous stress has directly or indirectly had a linked with intake of junk food preference, and now scientific evidence clearly conclude that prolonged life stress has directly linked with almost all human disorders [5], [6], [7]. Although these stress associated alterations in the eating behaviour are assumed to be temporary at this stage when persistent to older life, this might have led to several health problems [7], [8], [9]. In our previous studies, we pointed out that stress induces wrong choices of food, which is one of the main factors responsible for the onset of number of serious health problems including serious neurological and cardiac disorders, gastric ulcers, asthma, headaches, obesity, ageing, diabetes, and also premature death [9], [10], [11].

In young adults such as university students, fast psychosocial development and physical growth occur, which make them exposed to unhealthy eating that fails them to meet daily dietary requirements [12]. Skipping of routine food intake, intake of junk food such as pizza, burgers, chicken nuggets, sausage, hot dogs, French fries, chips, cake, brownies, cookies, chocolates, muffins, doughnut, pastries, ice cream, milkshake, excessive drinking of tea, coffee, soft drinks, etc. are common poor eating patterns among

university students [1], [2], [3], [4]. Not only have these, outside factors such as jaunting in shopping malls, vending machines, fast food outlets and general stores are also making them more susceptible for taking unhealthy food [1], [13]. Now it is well documented that young adults, including university undergraduates, were unsuccessful to follow the WHO recommended health food intakes [14]. It is important for us to point out that studies also revealed that undergraduate students demonstrated early risk factors for the onset of chronic disorders just because of unhealthy poor eating behaviour [15]. In recent years, several studies have pointed that that social and psychological factors are responsible for affecting eating habits among university students [1], [16] and stress was found to be associated with poor eating habits which lead to induce serious health problems [16], [17], [18].

Therefore, the present study was hypothesised to determine the levels of stress among undergraduate students of Qassim University and to investigate whether stress associated alterations affect their eating habits.

Methods

Study design and studied subjects

This is a cross-sectional survey performed on undergraduate students of Qassim University from November 2018 to March 2019. Six colleges were selected, and data were obtained by using simple random sampling technique. A total of 614 students participated in the study, among them, 394 were males, and 220 participants were females. Inclusion criteria of the participants were that all participants must be undergraduate students and the participants having any chronic disorders such as diabetes, hypertension, etc. were excluded from the study. The study was carried out by the Code of Ethics of the World Medical Association (Declaration of Helsinki as revised in Tokyo 2004) for humans and was approved by the Ethical Committee of Qassim University. Written informed consent from all participants was taken before the data collection.

Collection of data

The data were collected by the distribution of questionnaire among undergraduate students of Qassim University. The distributed questionnaires have already been validated successfully among different populations, as described previously [1], [4], [19]. The questionnaire has three main sections: (section 1) social and demographic section, (section 2) assessment of stress section measures, and (section 3) behavioural habits for food selection as described previously [1], [4]. Briefly, the social and demographic information was collected from the section of the questionnaire comprised questions on personal information such age, marital status, smoking status, parent's education level, family monthly income, residential details, general health and daily physical activities. Whereas, the levels of stress among participants were assessed by the self-report Depression Anxiety Stress Scales (DASS). The DASS was a well-validated method and was proved by many researchers among different populations [19]. In this study, we used only one section of this scale, which was 'the stress scale section' which comprised 14 questions deal with difficulty, relaxing, nervous arousal, and being easily upset/agitated, irritable/over-reactive and impatient as described previously [4], [19]. The pattern of food intake was assessed using a food frequency questionnaire (FFQ) as described previously [1], [4] with some modifications. Briefly, assessment of dietary pattern among the participants, different types of food and beverages were used to identify the dietary pattern among stress and non-stress undergraduate students. To determine whether stress-induced triggering of unhealthy food items, such as fast food, snacks, etc. the selected junk foods were further divided into three groups: fast foods (such as burgers, pizza, chicken nuggets, sausage, hot dogs, fries, etc.); snacks (such as chips, cakes, brownies, cookies, chocolates, muffins, doughnuts, pastries, ice cream, milkshake, etc.); and beverages (fruit juice, tea, coffee, soft drink, and energy drinks). Moreover, we also determine whether stress affects healthy eating behaviour, fruits and vegetables; the FFQ was designed to collect data on junk foods based on the participant's selection. Furthermore, the study also determined whether the pattern of food intake was the same or different before and after university joining.

Statistical analysis

The frequencies of distributed proportions were calculated by Statistical Data Analysis Software (SPSS, IBM, and Houston, TX, USA) using a chi-square test. And Graph Pad Prism-5 software (San Diego, CA, USA) was also used for the preparation of graphs.

Results

Social and demographic details of studied subjects

Out of 614 undergraduate students of Qassim University, 59.8% were aged between 18-20 years, whereas 40.2% were above or equal to 21 years old.

Majority of participants were unmarried (86.2%) and were non-smokers (91.4%). The complete details of social and demographic, including the status of participants' parent's education and their living and residential conditions are summarised in Table 1.

Table 1: Social and demographic details of studied subjects

Characteristics	N	Percentage
Gender		
Male	394	64.2
Female	220	35.8
Age (years)		
18-20	367	59.8
≥ 21	247	40.2
Marital status		
Single	529	86.2
Married	85	13.8
Smoking		
Smokers	53	08.6
Non-smokers	561	91.4
Father's education		
Primary or less	195	31.8
Secondary/Senior secondary	252	41.0
University or above	167	27.2
Mother's education		
Primary or less	263	42.8
Secondary/Senior secondary	246	40.0
University or above	105	17.1
Living standard		
Poor- Monthly Income < 5000 SAR	105	17.1
Average-Monthly Income 5000-15000 SAR	258	42.0
Good-Monthly Income > 15000 SAR	251	40.9
Residential details		
Day scholars (Lives / Stay with their Parents)	410	66.8
Hostlers (Lives/Stay Alone or with roommates / friends)	204	33.2

Out of all participants, our data showed that only 29.3% of participants were in excellent health, whereas 66.6% showed good health in general, and 4.1% were in extremely poor health. The calculated body mass index (BMI) results showed that 22.3% of participants were underweight, whereas 39.2% found to be obese. Not only have these, but our data also showed that 11.7% performed exercises daily, whereas 11.2% of them never performed exercises, but 50.8% also performed exercises occasionally. The complete status of their general health, calculated BMI and their routine physical activities are summarised in Table 2.

Table 2: General health, body mass index and physical activity of studied subjects

General health		
Poor	25	04.1
Good	409	66.6
Very Good/Excellent	180	29.3
Body Mass Index (BMI)		
Underweight (< 18.5)	137	22.3
Normal (18.5-22.9)	236	38.4
Obese (> 23.0)	241	39.2
Physical Activity / Exercise		
Once a day	72	11.7
Every alternate day	161	26.2
Occasionally	312	50.8
Never	69	11.2

Stressed levels in studied subjects

Applying DASS measurements, out of 614 participants, stress was found in 28.2% (Figure 1). Among stressed participants, 17.3%, 49.1%, 24.8% and 8.7% of participants suffered from mild, moderate, severe and extremely severe stress, respectively. The complete stress levels with the number of participants under varying levels of stress are summarised in Table 3.

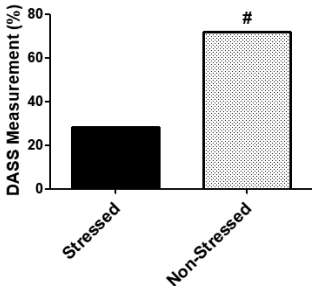

Figure 1: Stress measurements in undergraduate students (n = 614) of Qassim University. The data are shown in percentage of DASS scores; #p = 0.000 versus stressed participants

The regularity of food intake by stressed and non-stressed participants before and after university admission

Out of 173 stressed participants, 97 (56.1%) were followed regular meals pattern as they were taken regular breakfast, lunch and dinner before college admission, however, after college admission, this number was significantly reduced to 30 (17.3%) (p = 0.000).

Table 3: Stress levels among stressed participants

Stress levels	N	Percentage
Mild stressed[@]	30	17.3
Moderate stressed[#]	85	49.1
Severe stressed[$]	43	24.8
Extremely severe stressed[*]	15	08.7

[@]p = 0.000 versus moderate stressed; [#]p = 0.000 versus severe stressed; [$]p = 0.000 versus extremely severe stressed.

Whereas, in non-stressed participants, regular meal intake before and after college admission remains be the same (p = 0.665). As 56.6% of participants showed regular follow of the meal before college admission and 54.4% of participants showed regular meal follow up after college admission. Figure 2 summaries the complete detail of stressed and non-stressed participants before and after university admission.

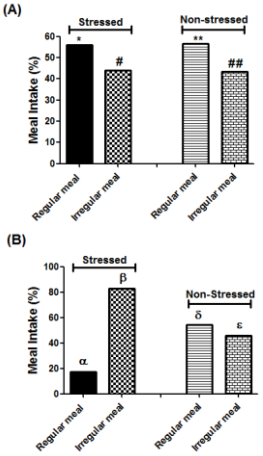

*Figure 2: Behavioral habits of meal intake of participants before (A) and after (B) joining of college; *p = 0.000 versus α; **p = 0.665 versus δ; *p = 0.886 versus **; #p = 0.771 versus ##; αp = 0.000 versus δ; βp = 0.00 versus ε*

Pattern of food intake by stressed and non-stressed participants

We analysed the pattern of food intake by stressed and non-stressed participants. The percentage of meal pattern once, twice, thrice and more than thrice a day by stressed participants were 12.2%, 14.3%, 54.9% and 18.6%, respectively and by non-stressed participants were 12.7%, 60.7%, 17.3% and 9.2%, respectively (Figure 3). Our novel data pointed out that the pattern of main food intake once a day was almost same in stressed and in non-stressed participants (p = 0.873) but this per day main meal was significantly different in twice (p=0.000), thrice (p = 0.000) or more than thrice (p = 0.004) a day. Figure 3 summaries the complete main meal pattern per day by stress and non-stress participants.

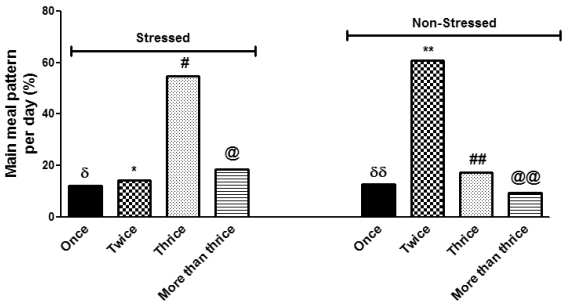

*Figure 3: Main meal pattern per day by stressed (n = 441) and non-stressed (n = 173) participants; δp = 0.873 versus δδ; *p = 0.000 versus **; #p = 0.000 versus ##; @p = 0.004 versus @@*

Food preferences by stressed and non-stressed participants

The consumption of vegetarian items, fresh fruits, preferred fast food, snacks and beverages by the stressed and non-stressed participants are summarised in Table 4.

Table 4: Behaviour of food preferences by stressed and non-stressed studied subjects

Characteristics	Stressed (n = 173) N	%	Non-Stressed (n = 441) N	%	*p values*
Consumption of VEG. items per day					
Once	11	6.3	199	45.1	0.000
Twice or more	10	5.8	204	46.2	0.000
Occasionally	150	86.7	31	7.0	0.000
Never	02	1.2	07	1.7	0.689
Consumption of FRESH fruit per day					
Once	14	8.0	92	20.9	0.000
Twice or more	35	20.2	300	68.0	0.000
Occasionally	110	63.5	30	6.8	0.000
Never	14	8.1	19	4.3	0.061
Consumption of TINNED OR FROZEN food per week					
1-2 times	40	23.1	38	8.7	0.000
More than 2 times	120	69.4	71	16.2	0.000
Occasionally	08	4.6	299	67.8	0.000
Never	05	2.8	33	7.5	0.033
Preferred FAST food					
Burger/Pizza	38	22.2	59	13.4	0.009
Chicken Nuggets/Sausage/Hot dogs	44	25.4	103	23.3	0.587
French Fries	72	41.6	150	34.0	0.078
None	19	11.0	129	29.2	0.000
Preferred SNACKS food					
Chips	30	17.3	33	7.5	0.002
Cake/Brownies/Cookies/Chocolate	47	27.0	84	19.0	0.027
Muffins/Doughnuts/Pastries	57	33.0	66	15.0	0.000
Ice-cream/milk shake	38	22.0	63	14.3	0.021
None	01	0.58	195	44.2	0.000
Preferred BEVERAGES					
Tea / Coffee	46	26.5	74	16.8	0.006
Soft drink	53	31.6	66	15.0	0.000
Energy drink	52	30.0	43	09.5	0.000
None	22	12.7	258	58.5	0.000

Abbreviation: n, the total number of participants tested; N, number of participants responded.

The data pointed out that the stressed participants were more preferred to eat junk foods such as fast foods, snacks and beverages as compared with unstressed participants (p < 0.05). Whereas, non-stressed participants preferred more healthy foods such as vegetarian food, fresh fruits as compared with stressed participants (p < 0.05). In summary, the behaviour of healthy or junk food preferences by stressed and non-stressed participants are shown in Figure 4. Our data showed that out of 173 total stressed participants, only 37.2% preferred healthy food, and the rest 62.8% preferred junk food (p = 0.000). Interestingly, the non-stressed participants showed almost reversed data, as shown by the stressed participants. The majority of non-stressed participants (72.8%) preferred healthy food and only 27.2% of them preferred junk food (p = 0.000). The data clearly showed that the majority of stressed participants preferred junk food, whereas non-stressed participants preferred healthy food.

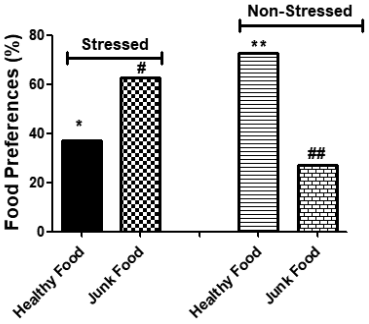

*Figure 4: Food preferences by stressed (n = 441) and non-stressed (n = 173) participants. *p = 0.000 versus **; #p = 0.000 versus ##; *p = 0.000 versus #; **p = 0.000 versus ##*

Preference of healthy and junk food by mild, moderate and severe stressed participants

As shown in Figure 5, mildly stressed participants preferred healthy food significantly higher as compared with junk food (p = 0.009), whereas the participants with moderate stressed they preferred more junk food as compared with healthy food, but this difference in junk food preference was significantly higher in severely stressed participants (p = 0.000).

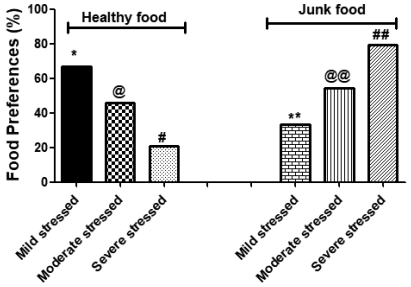

*Figure 5: Food preferences by mild (n = 30), moderate (n = 85) and severe (n = 58) stressed participants. *p = 0.009 versus **; @p = 0.283 versus @@; #p = 0.000 versus ##; **p = 0.000 versus ##*

The data showed in Figure 5, clearly indicated that the preference for junk food was increased with the increase in stress levels, whereas the healthy food preference was decreased with the increase in stress levels.

Reason for junk food preferences by stressed and non-stressed participants

To find out the reason why the studied participants preferred unhealthy junk food, out of all studied stressed participants, 37.6% responded to the option of taste, whereas 17.9%, 19.6% and 24.8% replied for easy to assess, relaxation and influenced by others, respectively. Whereas 20.9% of non-stressed participants responded to the option of taste, and 44.4%, 14.3% and 20.4% of them replied for easy to assess, relaxation and influenced by others, respectively (Table 5). The data pointed out that taste and easy to access were the main reasons for the preference of junk foods by the stressed participants.

Table 5: Reason for junk food preferences by stressed and non-stressed studied subjects

Characteristics	Stressed (n = 173)		Non-Stressed (n = 441)		*p-values*
	N	%	N	%	
Reason for junk food preferences					
Taste	65	37.6	92	20.9	0.000
Easy access	31	17.9	196	44.4	0.000
Relaxation	34	19.6	63	14.3	0.101
Influence by others	43	24.8	90	20.4	0.229

Discussion

This is the first comprehensive study from Saudi Arabia to show the association of stress and dietary behaviours among undergraduate students of Qassim University. It is now well established that as long as humans have been around, there must be stress [20]. It is now scientifically proved that stress plays a vital role in the onset of almost all major depressive disorders [9], [10]. In our previous studies, we proved on an animal model that stress is of two types: Acute and chronic stress and which also has been well validated by various other investigators [21], [22]. Acute stress is generated from specific situations which may keep humans in a poor sense of control, but sometimes this stress can be good also as it keeps individuals alert, motivated and primed to respond [6], [9], [12]. This happens due to the secretion of stress-associated hormones, which help humans to control the situation [9], [11], [23]. Now it is well documented that up to 70% of seriously diseased populations are believed to be affected by chronic stress [9], [10]. In case of young adults including university students, we believed that they experienced both types of stress, when they are having in a situation of acute stress, which we might think that this is good for them to make them alert, motivated and make them ready to respond against any bad situations. However, when this stress becomes prolonged, then it becomes chronic for them and has been associated with several disorders [9], [10]. In the present study, we demonstrated that a significant number of undergraduates were having some levels of stress. These results were fully supported by number of studies performed in various regions of the globe [24], [25], [26], [27], [28], [29], [30], [31]. A study performed at Kuwait University undergraduate students showed that more than 40% of the young adults suffered from some level of stress [4]. In another study performed on students of Malaysian University showed stress on 36% of the participants [24].

Furthermore, another study demonstrated 43% stress in the students of first-year tertiary education in Hong Kong [25]. Importantly, a much higher stress level was reported in students from western countries and other Middle Eastern countries such as 84% in Australia [26], 61% in Iran [27] and 70% in Jordan [28]. Moreover, studies have also reported that the first-year students were at an increased risk of poor mental health [25] with the prevalence of stress decreasing as students progressed to higher years [29]. Not only have these, but the results of this study were also supported by the various other studies performed on different other universities students across the globe [18], [30], [31] and thus the data obtained from this study further provide additional evidence on the prevalence of stress among university students. It is also important to point out that the differences in the occurrence of stress observed among university students in different countries may be due to the differences in the methodology used to determine stress. Another reason may be the socio-cultural characteristics of the participated young adults.

After demonstrated stress among the undergraduates of Qassim University, we investigated the regularity of food intake by stressed and non-stressed students before and after joining the university. Out of 173 stressed students, 56.1% were followed regular meals pattern as they were taken regular breakfast, lunch and dinner before college admission, however, after college admission, this number was significantly reduced to 17.3%. Whereas in non-stressed students, regular meal intake before and after admission remains be the same. These data pointed out that after joining university, the regularity of food intake among stressed students was disturbed, suggesting that the occurrence of stress among students during university life affect their food intake. Moreover, we also analysed the pattern of food intake in stressed and non-stressed students. Our novel data pointed out that the pattern of main food intake once a day was almost same in stressed and in non-stressed participants but this per day main meal was noticeable differ in twice, thrice or more than

thrice a day. They are increased of main meal twice a day in non-stressed students as compared with stressed students, indicating that non-stressed students followed the normal pattern of taking the main meal. However, main meal intake more than twice a day by stressed students, suggesting that the abnormal pattern of main meal taking by stressed students. These findings have further been supported by other studies showing an abnormal pattern of food intake in various other universities students [32], [33]. Arsiwalla *et al.* were examined interactions between stress and eating regulation in the prediction of weight-related outcomes and body fat among young adults [32]. Whereas, Nastaskin *et al.* in another study provided evidence that diet self-efficacy and perceived stress levels relate to nutrient intake in young adult, and that increasing diet self-efficacy and reducing perceived stress in a young adult may lead to the healthier eating habits [33].

Furthermore, the present study also determined that the stressed undergraduates preferred to eat junk foods such as fast foods, snacks and beverages. Whereas, non-stressed students preferred healthy foods such as vegetarian food, fresh fruits. Specifically, the study pointed out that out of 173 total stressed students, only 37.2% preferred healthy food, and the rest 62.8% preferred junk food. Interestingly, the non-stressed students showed almost reversed data, as shown by the stressed students. The 72.8% of non-stressed students preferred healthy food and only 27.2% of them preferred junk food. The data clearly showed that the majority of stressed students preferred junk food, whereas non-stressed students preferred healthy food. Not only have these, we further characterized the preference of junk food by mild, moderate and severe stressed students, our data showed that students with mild stressed preferred healthy food as compared with junk food, whereas the students with moderate stressed preferred more junk food as compared with healthy food, but this difference of junk food preference was remarkably higher in students with severe stressed. These data are suggesting that the preference for junk food was positively associated with increased levels of stress. To determine the reason why stressed students preferred junk food, out of all studied stressed students, 37.6% responded to the option of taste, whereas 17.9%, 19.6% and 24.8% replied for easy to assess, relaxation and influenced by others, respectively. These data are indicating that taste and easy to access were the main reasons for the preference of junk foods by the stressed students. These findings have also been supported by other studies that showed an association between stress and poor dietary pattern among young adults [4], [34]. Therefore, these findings are very important for the development of specific programs to decrease the levels of stress and to improve the food pattern, especially for young adults. Although some evidence exists indicating an educational intervention to increase dietary knowledge might be useful [34], but at the same time, environmental interventions should also be needed.

In conclusion, to the best of our knowledge, this is the first comprehensive study from Saudi Arabia to show stress associated dietary alterations among undergraduates of Qassim University. In general, most of the students studied in this study followed a healthy eating pattern, but still, stress was significantly associated with a large number of participants. Results show a clear difference in the food selection by the stressed and non-stressed students, indicating that stress has a direct association with the dietary pattern among studied young adults. These results strongly recommend specific intervention programs to reduce stress and to improve their food choices.

Authors' contributions

EA participated in study design, coordination and data collection, AMA, BHA and AFA data collection and data interpretation, NR has consulted for data interpretation and manuscript drafting. All authors have read and approved the final manuscript.

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Alarming Eating Behaviours among Adolescents in Egypt

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Keywords: Eating behaviours; Adolescents; BMI; Obese; Non-obese

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Abstract

BACKGROUND: Adolescence is a phase of rapid growth and increased nutritional needs. It includes the stressful burden of pubertal changes, both physically and psychologically. Moreover, it is associated with the utmost need for independence and identity formation. An adolescent finds a great way to practice taking their own decisions by making personal food choices. But unfortunately, wrong dietary choices lead to unsatisfactory nutritional status.

AIM: To investigate the prevalence of six eating behaviours among adolescents.

SUBJECTS AND METHODS: A case-control study was conducted on ninety Egyptian adolescents from 10 to 18 years old. Anthropometric measurements were taken. Body Mass Index (BMI) was calculated. The cases were forty-five children with body mass index ≥ 85th percentile. The control group involved forty-five of matched peers with body mass index < 85th centile. A questionnaire form was constructed according to local customs in Egypt.

RESULTS: Two unhealthy behaviours were mostly found in our study group. The first and the predominant one was multitasking while eating practised by 92.1% of candidates and showing the equal distribution in both groups. The second was skipping breakfast and was adopted by 51.7% of the study group with a significantly higher distribution in the < 85th centile group.

CONCLUSION: Faulty eating is a behaviour encountered in adolescence irrespective to BMI category. Thus, a normal BMI does not reflect healthy dietary behaviours.

Introduction

As a result of globalisation, heterogeneous western food habits were intruded in developing countries. This went in parallel with an increment in the frequency of chronic non-transmissible diseases. Thus, it became crucial to reassess the eating patterns in these countries and to evaluate their possible contribution as risk factors for these diseases [1].

Chronic non-transmissible diseases include overweight and obesity. The pervasiveness of high body mass index in the Middle East has been ranked the second worldwide, next to North America. Obesity is considered a serious health hazard, universally [2].

A sedentary lifestyle and faulty eating habits are two key threats that predispose to chronic non-transmissible diseases. Fortunately, these two risk factors are adjustable [3].

The World Health Organization (WHO) announced that almost 66.7% of early deaths, and 33.3% of grown-up illnesses, are consequent to undesirable behaviours that started in youth [4].

Thus the negative behaviours in adolescence are real challenges to face and overcome as early as possible before jeopardising lifetime health status [5]. That is why we should do this research.

Subjects and methods

The current study was conducted in the Nutrition Immunotherapy Clinic at the Medical Research Centre of Excellence (MRCE), National Research Centre (NRC). As a part of the in-house project entitled "Early Renal injury markers in obese adolescents".

Ninety Egyptian adolescents of both sexes were enrolled in this case-control study. The cases were forty-five children with BMI above or equal to 85th centile. The control group involved forty-five of matched peers with BMI below the 85th centile.

Inclusion criteria: adolescents of both sexes who are 10 to 18 years old.

Exclusion criteria: syndromic (e.g. Prader–Willi syndrome) and endocrinal (e.g. hypothyroidism) causes of obesity

Anthropometric measures: were assessed with a record of the height and the weight of each candidate. The height was measured to the nearest 0.5 cm on a Holtain portable anthropometer. The weight was determined to the nearest 0.1 kg on a Seca scale Balance with the subject dressed minimum clothes and no shoes. BMI was calculated as Weight (kg)/Height (m^2). Data were plotted on WHO curves through the software AnthroCalc v1.66 Home

A questionnaire form was constructed in the light of literature and modified according to local customs in Egypt. A "yes", or "no" or "sometimes" answers were obtained for each question by a personal interview with every child. The six questions were about: breakfast meal, fast food, multitask eating, dinner meal content, dinner meal timing and intake of sweetened beverages.

Ethical approval

The protocol of the study was approved by the “Ethical Committee” of the NRC. Written informed consent was signed by the legal guardian of each participant before enrollment in the study by the code of ethics of the world medical association (Declaration of Helsinki) approval number 16130.

Statistical analysis

Analysis of data was performed by using Statistical Package for the Social Science SPSS version 16.G. Data were presented as a mean and standard deviation. Chi-square test was conducted for the variables and was used to detect the significant difference in the distribution between groups at P - value < 0.05 (SPSS version 16.G.)

Results

The study group comprised of ninety adolescents. They were 31 (34.4%) males and 59 (65.6%) females. Their mean age was (12.62 ± 2.6) and (13.05 ± 2.61) for case and control groups respectively. According to BMI percentiles, we stratified the candidates into two equal groups of 45 children each. The cutoff BMI for the case group was ≥ 85th centile. While the cutoff BMI for the control group was < 85th centile, this was done according to the WHO growth charts. Detailed anthropometric data are presented as mean and standard deviation in Table 1.

Table 1: Anthropometric data

Variable	Case group (n = 45)	Control group (n = 45)
	Mean ± SD	
WT	73.41 ± 18.26	37.39 ± 10.68
WT Centile	96.26 ± 4.80	27.43 ± 25.05
HT	154.16 ± 10.65	146.05 ± 13.04
HT Centile	51.50 ± 28.89	32.60 ± 28.74
BMI	30.55 ± 5.61	17.22 ± 2.71
BMI Centile	98.30 ± 2.68	34.40 ± 28.16

Every child was personally interviewed to answer a form of questionnaires. This form was composed of six questions to monitor one desirable and five undesirable behaviours. The desirable one was tackled in question (Q1) and was about commitment to the breakfast meal. More than half of the participants (51.7%) skipped their breakfast either daily or sometimes with a per cent of 34.8% and 16.9% respectively.

The most prominent undesirable behaviour found was multitask eating (eating + watching TV) in Q3, it was adopted by a vast majority of 92.1% participants whether daily versus sometimes with a per cent of 91% and 1.1% respectively.

Otherwise, less than half of the study group members practised the four-remaining unhealthy behaviours, either daily or sometimes, as follows in a decrement order:

Ingestion of fast food > 2 times\week (Q2) and eating a heavy meal at dinner (Q4) were both encountered in a comparable per cent of participants (46.1%).

To a lesser extent, unhealthy intake of sugary beverages (Q6) was found in a per cent of 38.2%. A slightly lower per cent of 35.9% of candidates slept post-dinner by less than two hours (Q5), as illustrated in Table 2.

Table 2: Questions and per cent of different answers in the whole study group

Nine questions	Per cent		
	Yes	No	Some-times
Q1. A daily commitment to breakfast intake	48.3	34.8	16.9
Q2. Eat ready-made fast food more than twice a week	27.0	53.9	19.1
Q3. Eating in front of the television	91.0	7.9	1.1
Q4. Eat a heavy meal at dinner	28.1	53.9	18.0
Q5. Sleep after dinner by less than two hours	25.8	64.0	10.1
Q6. Three sugary drinks per day (soft drink/juices reserved / warm drinks + 3 teaspoonful extra sugar per cup)	32.6	61.8	5.6

Then a comparison between the two groups was made as regards the frequency of each behaviour (as shown in Table 3).

A significantly higher number among obese

adolescents consumed breakfast everyday (n = 27) compared to non-obese group (n = 16) (P-value = 0.042).

The fast-food consumption was more frequent in obese (13 daily + 12 sometimes) than non-obese (11 daily+ 5 sometimes) adolescents but with insignificant P-value.

The majority of candidates in both groups were eating while watching TV (93.3% of the obese and 88.6% of the non-obese).

Almost similar per cent of candidates from the two groups was drinking three sugary beverages daily (33.3% of the obese and 31.8% of the non-obese).

Unexpectedly, the two unhealthy behaviours of ingesting a heavy dinner and late dinner time were both encountered in a greater number of the non-obese (n = 15 and n = 14) versus the obese ones (n = 10 and n = 9).

Table 3: Comparison of the dietary habit's responses of the two groups

Parameters	Groups		Obese	Non-Obese	Chi-Square	P-value
Q1. Regular Breakfast intake	Yes	N	27	16	6.361	0.042*
		%	60.00%	36.40%		
	No	N	14	17		
		%	31.10%	38.60%		
	Some-times	N	4	11		
		%	8.90%	25.00%		
Q2. Fast Food > twice\week	Yes	N	13	11	4.372	0.112
		%	28.90%	25.00%		
	No	N	20	28		
		%	44.40%	63.60%		
	Some-times	N	12	5		
		%	26.70%	11.40%		
Q3. Eating in front of the TV	Yes	N	42	39	1.243	0.537
		%	93.30%	88.60%		
	No	N	3	4		
		%	6.70%	9.10%		
	Some-times	N	0	1		
		%	0.00%	2.30%		
Q4. Heavy Dinner meal	Yes	N	10	15	1.739	0.419
		%	22.20%	34.10%		
	No	N	27	21		
		%	60.00%	47.70%		
	Some-times	N	8	8		
		%	17.80%	18.20%		
Q5. < 2 hours dinner\bed-time	Yes	N	9	14	2.047	0.359
		%	20.00%	31.80%		
	No	N	32	25		
		%	71.10%	56.80%		
	Some-times	N	4	5		
		%	8.90%	11.40%		
Q6. Three Sugary Beverages\day	Yes	N	15	14	0.241	0.886
		%	33.30%	31.80%		
	No	N	27	28		
		%	60.00%	63.60%		
	Some-times	N	3	2		
		%	6.70%	4.50%		

*P-value < 0.05 considered significant.

Discussion

The WHO described adolescence as a highly vulnerable stage of life. Because, children at this stage, have a deceiving grown-up a physique that hides an immature psychosocial aspect. Therefore, the concern of the current study was directed towards children at this critical age. Same age group was also chosen to investigate dietary habits in Syria, Sudan, Arabian Gulf countries, China, India, Britain and many other countries [6], [7], [5], [8], [9], and [10].

The participants were grouped according to BMI percentiles. As in numerous studies and various fields of research, the BMI categorisation was the criteria of stratifying the candidates into case and control groups. This "BMI-based selection" in Egyptian studies was adopted by many authors [11], [12], [13], [14].

We classified the studied population into a case group with a BMI ≥ 85th centile and a control group with a BMI < 85th centile. The same cutoff for BMI percentile was chosen by bin Zaal and co-workers in their study about adolescents' dietary habits [15].

A personal face-to-face interviewing was chosen to ensure complete data collection by avoiding questions' misunderstanding and missing answers. The structured questionnaire used was quick, easy, reproducible, coded and interpreted. Six straightforward, closed-ended questions were the tool to retrieve information. These were questions with a limited fixed set of responses (yes\no\sometimes). In other studies, the self-administered questionnaires were used instead of interviewing technique. For example, the Arab Teens Lifestyle Study (ATLS) in which the large study group rendered personal interview impossible. Also, in the ATLS survey, a set of scaled questions was used [1].

In the current study, Q1 monitored the healthy behaviour of regular breakfast intake. More than half of the participants (51.7%) were breakfast skippers either daily or sometimes. Comparably, in the ATLS survey, 52% to 82% of Arab adolescents did not have breakfast every day [16]. Smaller per cent was reported in a Sudanese study where only 244 out of 945 adolescents (25.8%) were skipping breakfast meal [16]. While, in the survey done by Li et al., in China, the majority (93%) of the adolescents were having breakfast regularly (1661 from a total of 1774) [8].

A significantly higher number of breakfast consumers was noticed among the case group compared to the control group (P-value 0.042). On the contrary, several studies linked omitting breakfast with high BMI in childhood and adolescence [17], [18], [19], [20], [21]. Although Albertson et al., and Berkey et al., and recently Casazza et al., and Dhurandhar et al., did not support this "breakfast skippers\high BMI link" in their researches [22], [23], [24], [25]. This contradictory is due to the variability of meal content. If the consumed meal were rich in fibres and micronutrients, then it would have been an addition to the healthy eating index score. Also, it would have contributed to a lower intake of sweets and Trans-fat. But if the consumed meal was of poor quality high in fat and sugar, then skipping, it would not be harmful [26] and [27].

According to responses to Q2, candidates

with BMI < 85th centiles were consuming less fast food than those with BMI ≥ 85th centiles. This finding matched with the conclusion deduced by Bhadoran et al. They stated that junk food intake more than once a week was linked to a higher risk of obesity while eating fast food more than twice a week was associated with a higher risk of metabolic syndrome [28]. Similarly, Bhattacharjee et al. found a positive correlation between the frequency of junk food intake and high BMI among Indian adolescents [9].

As regards the answers to Q3, multitask eating was the most prominent undesirable behaviour found in the study group. It was practised daily by 91% of participants irrespective to BMI categories. This bad habit results in overeating, improper chewing; distraction of the brain from appropriate processing of eating and deprive the child of family interaction during mealtime. In an Egyptian study conducted in Cairo, a comparable per cent of 87.5% of adolescents were taking snacks while watching TV [29]. In Canadian research, eating in front of the TV was positively associated with unhealthy food choices [30].

Through the unpredictable answers to Q4 and Q5, it was noticed that the combined two unhealthy behaviours of late and heavy dinner meal were commoners in non-obese candidates and not their counterparts. The reverse was detected by Ong et al., who noted an association between high BMI and ingesting calorie-dense food late at night [31]. This unexpected finding may be due to the widespread Egyptian habit of skipping lunch and getting the main meal at dinner time. Thus, the total caloric intake may be adequate or even below the requirement of the day. Also, awareness of the overweight/obesity problem may be the reason for those with high BMI to get a light early dinner meal as a way of caloric restriction.

The unhealthy behaviour of drinking three sugary beverages per day, in Q6, showed an equivocal distribution in both groups (33.3% of the obese and 31.8% of the non-obese). This comparable per cent may be due to adolescents' tendency to imitate their friends in drinking soft drinks and preserved juices. Moreover, in Egypt, dark sweetened tea is quite popular, and it is considered the everyday beverage in most Egyptian homes. So, it is an Egyptian society issue rather than a high BMI related one. In an Egyptian study conducted in both rural and urban sectors, by Abdel-Hady et al., eighty per cent of adolescents had more than 3 small glasses of sweetened black tea daily [32]. In Oman and the United Arab Emirates, more than 50% of the teenagers' males and about 50% of the teenagers' females consumed ≥ 1 soft drink/day in the month before the study. While in Qatar, 60%/65% of adolescent's boys/girls respectively drank soft drinks every day [5].

In conclusion, many undesirable dietary behaviours are acquired in adolescence. Such behaviours are encountered in all BMI categories. Thus, a normal BMI does not always reflect healthful dietary intake. The rectification of faulty eating "behaviours" in youth is mandatory before becoming "habits" in adulthood.

Recommendations; The most powerful influencers on eating behaviours in adolescence are family, friends and media. Thus, good parental support and understanding have a major imprint on reforming such harmful conduct. Also, scholastic healthy eating programs are extremely important as a source of easy access to widespread information. The college's canteen must be an ideal exemplar of how healthy eating should be. The advertisements about food products should be under strict supervision. Further research studies are needed to explore more about eating behaviors on a larger number of adolescents and in different countries.

Limitations: The study was limited by the small number of participants.

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Advanced Stage Cancer Patients Experience in Seeking Treatment in Medan, Indonesia

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Keywords: Cancer; Seeking treatment; Complementary therapy; Alternative therapies

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Abstract

BACKGROUND: Proper treatment of cancer can make it easier for the healing process. Delay in patients seeking treatment is a problem that often occurs today. Handling of complementary and alternative therapies, which is not appropriate is one of the causes of delay in seeking treatment, which worsens the patient's condition. Patients usually come to health services already diagnosed with advanced cancer.

AIM: The purpose of this study is to explore the experience of advanced cancer patients in seeking treatment.

METHODS: This study is qualitative research with descriptive phenomenology approach. Participants in this study were 15 advanced cancer patients who were treated in the chemotherapy room at Adam Malik General Hospital Medan and Murni Teguh Hospital using purposive sampling technique. Data collection was done with in-depth interviews. All interviews were first recorded and then transcribed. This study was analysed using the Colaizzi approach method.

RESULTS: The six main themes indicated that advanced-stage cancer patients for seeking treatment. Themes such as 1) choose the complementary and alternative treatment rather than medical treatment, 2) seek medical treatment as a final decision, 3) integrating complementary treatment as supportive therapy in addition to medical treatment, 4) external sources influence patient decisions, 5) reduce negative feelings by surrendering to God, and 6) lack of involvement of basic health services.

CONCLUSION: Advanced cancer patients have experience using complementary and alternative therapies, which are not appropriate before using a medical treatment on the grounds of distrust of medical treatment and advice from the environment around patients. The importance of nurses in providing appropriate education related to complementary and alternative treatment to cancer patients according to the stage of the disease.

Introduction

Cancer is a cause of morbidity and mortality throughout the world with an increase in incidence each year based on the results of Basic Health Research in 2013; cancer was found to be the third cause of death in Indonesia. Sumatera Utara Province is the province with the highest estimate of the absolute number of cancer events in all Sumatra regions, namely 13,391 people [1]. The proper cancer diagnosis is very important for effective treatment because each type of cancer requires a specific treatment regimen that includes one or more modalities such as surgery, radiotherapy, and chemotherapy so that health services must be integrated. The main goal of this therapy regimen is to choose the most effective treatment that will eliminate or slow down cancer growth while ensuring the highest level of physical and emotional well-being during and after treatment [2].

According to the Hospital Information System (SIRS) in Indonesia, the handling of cancer is facing various obstacles that cause almost 70% of sufferers to be found in an advanced stage [3]. Based on medical records from Adam Malik General Hospital in Medan, the number of patients who came to the hospital with advanced cancer was recorded as many as 120 people from July to December 2013, out of 120 people who were treated at an advanced stage there were 10 people who died. Meanwhile, according to the results of research conducted at Adam Malik

General Hospital on April 2014, where it was found about 24 people from 30 breast cancer patients came to the hospital for the first time in an advanced stage [4].

Health-seeking behaviour is behaviour that is preceded by a decision-making process then regulated by the individual himself and family habits, norms that exist in society, and expectations and characteristics and availability of health services [5]. The behaviour of seeking treatment in cancer patients is how cancer patients seek treatment, both medical treatment and complementary and alternative treatments after the patient feels signs and symptoms.

The attitude and behaviour of cancer patients in choosing health assistance are strongly influenced by the socio-economic level and cultural background. Another thing that supports is fear of side effects in treatment, fear of surgery in cancer patients in general and medical expenses is the reason for the delay in cancer patients in seeking treatment [6]. In North Sumatra Province, the number of households using traditional health services is 26.3% using 38.8% ingredients, using 6.0% tools, without 79.5% tools and with 2.0% thoughts [*1].*

There are so many traditional or alternative medical practices not listed in Sumatera Utara, especially in Medan. The Sumatera Utara Provincial Health Office does not yet have exact data regarding the place of practice of traditional/alternative medicine. This shows the many developments in traditional and alternative medicine in remote areas of Sumatera Utara which are not recorded.

The delay seeking treatment will affect the condition and success rate of patients who seek treatment. Many patients, after undergoing traditional medicine or complementary and alternative medicine come to the hospital with increasingly worse conditions so that the prognosis of survival becomes worse. The poor prognosis of cancer will affect the quality of life of patients [7], financial conditions [8], the role and function of patients and families and even death.

Methods

This research is qualitative research with descriptive phenomenology approach. This research was conducted by taking preliminary data from the chemotherapy room at Adam Malik General Hospital and chemotherapy room at Murni Teguh Hospital, Medan. The research was conducted in July-November 2018.

Data collection was carried out by in-depth interviews. Participants were selected by purposive sampling with criteria: 1) willing to be participants, 2) stage IIIB, IIIC and stage IV cancer patients, 3) able to communicate verbally clearly, 4) ages 18 years and over, and 5) has undergone medical therapy and complementary and alternative therapies or traditional medicine. The number of participants was 15 people and had reached the data saturation of the 14 participants. Before conducting the research, the research protocol had passed the ethical test from the Research Ethics Committee of the Faculty of Nursing; the Universitas Sumatera Utara and every cancer patient who was a participant had agreed and signed informed consent.

The preliminary data in this study were taken in the chemotherapy room at Adam Malik General Hospital and Murni Teguh Hospital, Medan. Further, the researcher looks for participants according to the existing criteria.

After obtaining approval from the participants, the researchers conducted prolonged engagement 2 times at home before conducting in-depth interviews. In-depth interviews in this study were conducted 1-2 times with 30-55 minutes and using SONY ICD-PX470 voice recorders, field notes, and open interview guides as many as 8 questions. This interview guide has been tested for validity with 3 experts in the qualitative and cancer fields by obtaining the CVI value = 0.87.

Table 1: Demographic Data of Participants (n = 15)

Characteristics	f	%
Gender		
Male	2	13:33
Female	13	86.67
Age		
17- 25 years	1	6.67
36- 45 years	5	33.33
46- 55 years	6	40.00
56-65 years	2	13:33
65 - to the top	1	6.67
Ethnic		
Banjarnesse	1	6.67
Bataknese	4	26.67
Chinesse	1	6.67
Javanese	7	46.67
Niasnesse	1	6.67
Tapanulinesse	1	6.67
Cancer types		
Ca. Cervix	5	33.33
Ca. Colon	2	13:33
Ca. Mammary	6	40.00
Ca. Ovary	1	6.67
Melanoma	1	6.67
Duration of illness		
< 1 year	2	13:33
1-2 years	6	40.00
2-3 years	3	20.00
4 years and above	4	26.67
Stage of cancer		
IIIB	6	40.00
IIIC	4	26.67
IV	5	33.33
Occupational		
< 2.9 million rupiah	11	20.00
> 2.9 million rupiah	4	40.00

The results of the interviews were made in the transcript and carried out data analysis with the Colaizzi approach. This research uses the help of QDA Miner Lite software. The researcher obtained permission from the Research Ethics Committee of the Faculty of Nursing, Universitas Sumatera Utara. This research was funded by the researchers themselves and did not impose any costs on the participants of this study.

Results

Patients Experience in Seeking Treatment

The results of this study illustrate several themes based on the experience of participants who experience advanced cancer in seeking treatment.

Table 2: Themes Extracted from an In-Depth Data Interview

Themes	Sub-themes
Choose complementary and alternative treatment rather than medical treatment.	Choose complementary and alternative reasons for wanting to get well
	Roles and responsibilities in the family
	Do not trust medical treatment.
	Financial reasons.
	Complementary and alternative forms and processing.
	Choosing alternative/traditional treatments are not appropriate.
Seek medical treatment as a final decision.	I am stopping the use of CAM adverse.
	Strive for a series of medical treatments, both domestically and abroad.
Integrating complementary treatment as supportive therapy in addition to medical treatment..	Overcoming the side effects of medical treatment
	Increase stamina and the immune system
External sources influence patient decisions.	Family and friends.
	Cancer survivor
	Social Media
	Involvement of health workers.
	Treatment culture in the community.
Reduce negative feelings by surrendering to God	Negative feelings that arise.
	Get Closer to God
Lack of involvement of basic health services	Health centre only as a referral centre.
	Rejection of cancer patients in the clinic.

The thematic analysis process in this study found 6 themes, namely: 1) choose the complementary and alternative treatment rather than medical treatment, 2) seek medical treatment as a final decision, 3) integrating complementary treatment as supportive therapy in addition to medical treatment, 4) external sources influence patient decisions, 5) reduce negative feelings by surrendering to God, and 6) lack of involvement of basic health services.

Theme 1: Choose complementary and alternative treatment rather than medical treatment

Choose complementary and alternative reasons for wanting to get well. When patients diagnosed with cancer generally experience rejection, sadness, and disbelief in a medical diagnosis so that patients feel they want to get well soon. The patient's desire to recover makes patients choose to use alternative therapies that are not rational and even mystical.

"... Yes, the term is if the harsh words of people say shamans like that. The problem is that people say that there is someone who has a disease like you are here, and he is cured. We want to get well, so I try it, but the doctor stays. Over time there is no change. I am lazy again; I have already used up a lot. The shaman asked for a goat, asked the chicken for the media ... "(P7).

"... At that time, what did I do? Because I also want to get well, so I lose my mind. I was recommended by my sister. Flowers bathed me and the dukun knew that I had stage 3 cancer... " (P13).

Roles and responsibilities in the family. The patient feels the signs and symptoms of cancer, but the patient does not care about it because there is no time to go to the hospital, afraid that no one will take care of other family members. If they are hospitalized, their role and function in the family cannot be done properly.

"... Then I left my little boy, right? ... still, there was no school; no one was on guard. If you are admitted to the hospital, you will be treated later; it will not be treated for one day. Let's say it's just alternative treatment while on the go... " (P10).

"... Besides that, my children will not take care of it later. If my husband is already there, he rarely goes home. My second child is what I think about. There is no one who will take care of him later if his brother is used to wandering around.... " (P11).

Do not trust medical treatment. Some participants have undergone medical treatment by checking themselves into health services but the illness they suffer from cannot be diagnosed appropriately. This condition makes participants and families give up and choose alternative medicine in addition to medical treatment.

"... but how come huh? the initial harm was not detected by medical treatment. Do not know what the disease is. So, we are looking for another alternative ... " (P1).

"... I am afraid because people say they will be bald, this is black and black (pointing to the fingernails and toes). Make the family can't. "I just drank the herbs," he said. Ya… I obey him to drink herbal medicine.... " (P3).

Financial reasons. The financial condition of cancer patients is very influential when patients seek treatment. Some patients with conditions that lower economic class and does not have health coverage prefer to use handling of complementary and alternative (CAM).

"... I thought yesterday; there would be a lot of costs if the conditions of the long distance to the hospital, in addition to eating it too..." (P11).

"... Just the doctor said it was mandatory to operate, and after all that time I didn't have the cost. How do you want surgery, right? Yes, I also sold land, sold a house, but it was not enough for operating costs. Yesterday the sale of land was around 35 million for medical treatment ... " (P14).

Complementary and alternative forms and processing. The handling of complementary and alternative therapies used by participants in overcoming cancer is generally herbal therapy, listening to the Quran, kusuk or sequence. *"... At this halfway house, you can often read the verses of the Koran by the guard in charge. Let our hearts cool. Sometimes there are watching lectures; sometimes there are direct recitations, just our little ones. Later*

we will also read Yasin or read together ... " (P11).

"... Boiled then drank the water. Hmhm ... make 5 glasses of boiled water until the mixture becomes 3 cups of water. Just wait for cold to drink. Then that's it, Sis, until 7 times changed place... " (P15).

Choosing alternative/traditional treatments are not appropriate. Participants who carry out complementary and alternative therapies generally attend traditional types of treatment that do not have permission from the local public health officials. Participants said if they did not know whether the practice had permission or not.

"... Using the sign is the schedule; there is a schedule. I don't know about the permit; the important thing is that in front of the house there is a signpost and the schedule is that Friday night he closes.... " (P10).

"... I don't know, sis. I don't think there is one who will come to my house. I don't know where the treatment is. My husband knows. But it seems like it doesn't exist. Because the problem is that he's not coming anymore, he's surrendered, he can't bite anymore... " (P11).

Theme 2: seek medical treatment as a final decision

Stopping the use of CAM adverse. Complementary and alternative therapies can have a bad effect if the user is not done properly. Generally, the condition that occurs in participants is broken cancer, cancer cells are growing and severe, or do not give any effect at all. Therefore, patients decide to choose medical actions that after being carried out by participants are felt to be quite beneficial.

"... No Sis, I haven't used it anymore, it's an alternative method, there's no effect. Just to medical, I have improved my condition... " (P10).

"... Because I have given up, already tired. I was tired of alternative treatments; now I just go to medical treatment. Medically told what the treatment is like. Surrender me. Follow medical treatment only. ..."

Strive for a series of medical treatments, both domestically and abroad. Participants took part in a series of medical treatments to treat cancer both in the form of initial examinations, seeking clarity of medical diagnosis, carrying out therapy and other supporting examinations in many referral hospitals. Surely the search for treatment was not limited to domestic hospitals only, 2 participants even sought treatment abroad, namely in Penang Malaysia.

"... In short, I went to Penang. in Penang I do it again, I bring all the results again, they feel less accurate. So, we carry on the same examination again from the beginning, blood test, CT Scan. X-ray... " (P13).

"... the doctor asked me if I was a cancer patient? I justify it. "Yes, doc" I replied. I was dealt with first, put on oxygen, and the doctor said "Sis. instead, we don't want to serve you, but you have to be referred to another hospital, "he said." If we were forced to serve you, we would be disqualified with the health department because you are a cancer patient, so you should be treated at hospital class A not C class hospital, that's how it is". So, it was explained by the doctor that I must be treated at the HAM Hospital. Ha "to be able not to be treated here". "Then I should be treated where is the doc?" I said. "At the HAM hospital. Hospital class A. ha. From there, continue to HAM until now. Hmm. Almost 3 years.... " (P14).

Theme 3: Integrating complementary treatment as supportive therapy in addition to medical treatment

Overcoming the side effects of medical treatment. Patients use some complementary therapies such as the consumption of external drugs and nutritious foods such as juice, milk to deal with the side effects of medical treatment.

"... Just treat it using Pikangsuang (herbal ointment), ointment so that it doesn't itch, hmm ... we bought it ourselves without using a prescription — just own initiative.... " (P1).

"... Yes, if this skin, I diligently use lotion. It's already gotten before it was worse. If nausea vomiting, there is a medicine from the doctor, and I also drink milk E. If the hair, hmm... I just cover it, use this (pointing my head) ... " (P5).

Increase stamina and the immune system. Cancer patients usually consume herbal products that are sold freely to increase endurance and increase stamina.

"... The trick: beet fruit as an addition to blood and guava. So, it's not bitter, add the red guava, it's fragrant. That is pretty beet. In the beet fruit, there is this weight gain; I am the most victorious, here my friend is down on his Hb. All down.... " (P9).

"... Actually, no. That's all my family recommends mixed, medical as well, but for endurance, take this kind of vitamin right. The cancer is in the womb, so don't clash with medical, don't clash with chemical, there's no effect it says it's just been drunk. But sometimes I forget to drink it, that's the HPAI ... " (P12).

Theme 4: external sources influence patient decisions

The decision to choose the kind of treatment in patients with advanced cancer can be affected by external sources. These external sources who played

a major role in the right decision made by cancer patients because it affects the delay to be handled correctly.

Family and friends. Participants considered the family, especially husband/wife, children, siblings, parents, friends, and neighbours, to influence the accuracy of their seeking cancer treatment. The influence given is information and support in the form of financial support, assisting participants, and influencing participant decisions.

"... my brother. He was the one who helped with all the costs, the cost of my life and the children at the expense of medical treatment, he helped... " (P7).

"... my child. That's what gives enthusiasm too. Get well soon, huh, don't get sick. If I pray, I pray for you, so that the mother will recover quickly. Yes, I will be excited to hear children like that, right, right, Sis, because of children..." (P10).

Cancer survivor. The experiences experienced by cancer survivors are told and become a source of motivation for other cancer survivors. Participants said the support provided by cancer survivors was more on moral support, sharing experiences in running appropriate treatments so that they could influence participants' decisions, especially as cancer survivors, in seeking appropriate treatment.

"... Sis, spirit, Sis, if we are in surgery, it doesn't hurt, I say that. This is mine (breast), it's been operated on, it's already over, but it's just a spirit, don't be afraid the operation won't hurt. Later we will sleep, be sedated; then the doctor invites the story, I say that. After a long time, we sleep. Yes, why can't I do anything, I want to be healthy, "he said. Look like me. Eee, we must be enthusiastic. The name wants to be healthy; I say to people who are barriers, the same people like us a lot... " (P10).

"... Right now, there are many friends in the hospital who are passionate, mam. Every chemo, every radiotherapy, there is always a friend who encourages. We often exchange ideas, mam. Those who have been sick for a long time, I keep on encouraging them, I salute them. From there, I learned to be thankful even though this illness has a family that supports me, meets with friends in the hospital who support it too. Exchange our telephone number.... " (P15).

Social media. The progress of science and technology also affects participants in seeking treatment. The existence of the internet and easy access to health sites, the use and dissemination of information through Whatsapp, Facebook and radio can help participants find knowledge about cancer and treat it.

"... There was one and a half months ago with WA. from WA; I tried, I drank, yes even though I once opened on Google, what is that ...? the effect is soursop leaves he said. But I don't know I just drink. I think now, my chemotherapy does and I also drink the cooking water... " (P5).

"... I heard from the radio. I heard it when advertising on the radio, but it seems like my heart said, ah…, I want to drink this because I listen to it on the radio, not from people.... " (P8).

Involvement of health workers. Generally, doctors and nurses forbid the use of alternative medicine other than juice because it does not have a good effect on the therapy that will be undertaken. Medical personnel, especially nurses, also explain the side effects of medical treatment and provide medical treatment options that patients can make.

"... The doctor said you used to take medication using an alternative, sir? Yaa... I said Doctors prohibit using alternative therapies, but if the juice is okay. The nurse said that beat fruit juice was good.... " (P1).

However, there were participants who stated that there were health workers who suggested using alternative medicine before medical treatment.

"... Hmmm ... today if you want to say that alternative medicine can be said to be inappropriate. But there are health workers who recommend using alternative medicine before doing surgery, Sis! They suggested that. Even though he works at a health centre but my friend is in Perwiritan.... " (P14).

Treatment culture in the community. Culture includes the habits of treatment carried out for generations. Participants said that when the initial symptoms appeared and resolved the symptoms, the participants chose the treatment carried out from generation to generation by their families the existence of certain tribal habits is also an option to overcome the symptoms that arise from this cancer.

"... I just know myself, usually when boils, that is. So I thought maybe this is a boil too. Our parents used it when we were kids... " (P2).

"... The herbs that come with that bike. I usually drink turmeric, mix kencur rice, mix betel leaves. If the Javanese believe betel leaves are many benefits, one of them is for vaginal discharge. Only I rarely drink it. Not often or every day, just once... " (P9).

Theme 5: Reduce negative feelings by surrendering to God

Negative feelings that arise. The patient says in seeking treatment and carrying out this therapy, the feelings that arise are in the form of fatigue, sadness, boredom, despair, and feeling useless with the current situation. Some participants also said regret, shocked and resigned.

"... Regret, mam! Why not from the first ...

Just what is it.? at the beginning of my treatment he said it was just an irritation, but it didn't heal. There are several my neighbours using a shaman, and using herbal medicine ... His name is also the business he wants to recover; I try. I mean, while I have a way to do it, I go ahead. I drink too, mam even though it tastes bitter, yea! But yeah ... finally like this ... maybe it's fate ... " (P11).

"... Yes, frankly, bro, actually, sometimes I'm tired of being tired, yes, where are humans, too, is tired of all this. Sometimes my heart is crying, there is still a cure here and there is no cure, but yes when it also looks at the face of the husband, it becomes sad, he is excited, too tired to bring me there to here but don't give up, so get up again " (P15).

Get closer to God. Participants said that with the current conditions, they were more willing to surrender to God.

"... Yes ... I still try to get treatment, try my best. Whatever the outcome will be, in the future, even if it's possible that my uterus will be removed later, I leave it to God, Sis. This is a trial. My family and I tried, then it was up to God... " (P4).

Theme 6: Lack of involvement of basic health services

According to participant, Puskesmas only as a referral centre. Participants made visits to the healthcentre to take referrals for treatment to the hospital. Generally, cancer in participants is diagnosed not at the clinic but the clinic or already in the hospital.

"... No, hmm ... there is no health centre staff (health centre) coming to my house giving counselling. We just ask for referrals yesterday.... " (P9).

Rejection of cancer patients in the clinic. A large number of cancer patients makes the length of service provided, so the hospital recommends doing some basic actions in basic health services. But one participant received a rejection because there was no service for cancer, both drugs, and other facilities.

"... I will drop it again, right? Even though sitting all day was difficult, that chemo time. So, it's said, if it's just changing bandages, just at the health centre. So, it was 1 person who guarded the pharmacy at the health centre; he objected when I was treated at the health centre. I mean that cancer should be treated in a hospital, not here ... " (P14).

Discussion

In this study, the majority of patients were women (86.67%) and men (13.33%). Based on the results of the research conducted, it was found that men were more likely to be late in seeking a diagnosis of cancer symptoms and late in treating cancer compared to women. This is because men are more masculine, so they tend to be less likely to seek help and less exposed to the health system [9], [10]. While women more often utilise health services related to pregnancy and role [11].

In this study, most types of cancer found were breast cancer (40%), then cervical cancer (33.33%) and colon cancer (13.33%). Colon cancer in this study entirely occurs in men. This is related to the research conducted by Iyer et al., the type of cancer that often affects women, such as cervical cancer and breast cancer [11]. According to research conducted by the type of cancer that often experiences delays is a type of cancer experienced by men compared to the type of cancer associated with women. This relates to women, in general, tend to act as role models for other people living with cancer and are more often exposed to health services [10].

The study found that participants who were advanced cancer patients chose complementary treatment and alternative therapies rather than medical treatment. At the beginning of the signs and symptoms appear, some participants chose the medical treatment as an initial examination and then diagnosed with early-stage cancer, but participants were afraid of medical treatment both medical measures themselves and medical treatment side effects. Also, the detection of diseases experienced by participants in medical services made sense of disbelief in medical treatment appears, so participants switched to using an alternative, complementary therapies. Participants choose complementary and alternative treatments with reasons for wanting to recover. Participants choose traditional medical practices such as sensei, "smart people" or shamans. The practice of this sensei treatment generally uses herbal medicines from plants but costs a very expensive one without any guarantee of recovery.

On the other hand, participants also visit shamans or smart people for reasons of wanting to recover. This practice calls for participation in surrendering chickens, goats, and other animals under the pretext of being dowry or media transfer. Other participants were also asked to take a bath using the prayed flower water. This is certainly not rational but is still carried out by participants. In this study, it was found that participants used an unlicensed alternative treatment site. In the Regulation of the Minister of Health of the Republic of Indonesia No. 61 of 2016 on empirical traditional health services states that every community who has a job as a traditional healer must have a permit/registered traditional healer (SIPT / STPT) issued from the local health office. Also, the Minister of Health Regulation also requires that each traditional practice place make a sign for a healthy home

according to the stipulated conditions. This is certainly contrary to the experience of cancer patients [12].

Delay in cancer patients seeking treatment due to the role and responsibility of patients in the family. The patient acts as a husband/wife with duties and responsibilities as the head of the family and takes care of other family members making the participants ignore the signs of symptoms that arise. This is different from research showing that cancer patients with married status are more quickly seeking treatment for cancer that is suffered compared to single or divorced [13], this is due to the support of other family members to treat cancer.

The financial condition of participants also influences the choice of using complementary and alternative therapies. Most of the participants have a family income less than 2 million per month, and some patients at the beginning of being diagnosed with cancer do not have health insurance or insurance, so they are looking for complementary and alternative treatments. Cancer patients did not consult medical staff due to financial reasons [14]. However, this finding contradicts the research, which states that as the socio-economic increase of cancer patients increases, it allows patients to seek treatment that tends to decline rapidly [13].

Complementary therapy used by participants is generally herbal therapy, spiritual therapy in the form of listening to mural Al-Quran, and body-based manipulative methods such as sequences or ribs. Listening to the Koran, reading and memorising it can increase life expectancy in patients with palliative radiotherapy who have cancer [15].

Herbal therapies used generally come from parts of plants and grains which are processed by boiling namely, leaves of the “Dewa”, leaves of bin along, betel leaves, soursop leaves, Dayak onions, garlic, nutmeg and aloe vera, colour leaves. Also, oil and “rice parem” grated are one of the treatments performed by patients. Karo oil is generally used by participants as one of the local wisdom that is only found in the North Sumatra region. The rice parem is also used by cancer patients as one of the treatments to reduce the effects of heat on the patient's stomach after chemotherapy. This param is made from rice and special spices which are processed in such a way that it causes a cold effect.

In this study, participants chose the medical treatment as the final choice in overcoming cancer. Erku stated that most cancer patients do not experience the side effects of using complementary and alternative therapies, and will continue to use them during cancer treatment. This occurs because the forms of complementary and alternative therapies used by patients in the study are generally based on clear evidence [16]. Research conducted by Temuci and Ortabag found that compared to the quality of life of cancer patients using CAM, there were no differences in physical activity, psychological and daily life [17]. All cancer patients in this study said that the handling of CAM used in general did not directly affect the cure of cancer suffered. The condition of the disease is even worse. Therefore, participants chose to stop using inappropriate CAM handling such as going to a dukun, a smart person or using a sensei.

This situation made participants decide to return to medical treatment. When the participants came to medical treatment again, the cancer was at an advanced stage. This finding is in line with the results of a study by Kim et al., stated that several factors related to cancer patients stop complementary and alternative therapies because of the side effects of therapy and the ineffectiveness of complementary and alternative therapies in overcoming cancer [18].

Some cancer patients generally choose complementary therapies, and these alternatives are due to disbelief in medical treatment. Patients claim to have carried out the treatment in more than three hospitals but to diagnose cancer has not been detected or do not believe in the diagnosis of cancer that has been determined. Also, other patients said that treatment at the hospital was not satisfactory due to improper handling of health workers in overcoming signs of symptoms that arise; even two participants chose to seek treatment abroad. Cancer patients seeking treatment tend to choose more than 6 health facilities because of feelings of disbelief, dissatisfaction with diagnoses of disease and others [19].

To overcome the side effects of medical therapy, patients usually use complementary therapies that support main therapies such as the use of milk, vitamins, and supplements from certain herbal products, as well as a source of vitamins from fruits and vegetables. In a study conducted by Thomas et al., stated that this complementary treatment was likely to succeed in one patient but was not effective in other patients*. But complementary medicine is supportive therapy for existing conventional medicine. Also, the challenge for integrative treatment in oncology patients is efficacy and safety, prioritising based on the results of research, education, communication and appropriate arrangements [20].*

Most cancer patients use complementary therapies to increase endurance [17]. The use of herbal products that already have permission from the Food Drug Supervisory Agency (BPOM) so that their use is safe, but for the right dose of use for cancer itself is not clear. The use of these herbal products is limited to increasing stamina, reducing nausea and other side effects of medical therapy and not intended to cure cancer. The fruits that are often consumed are beet fruit, dragon fruit, guava fruit, papaya which is believed to have high anti-oxidants. But the use of herbs, both fruit and vegetables, vitamins and other foods must be considered because not all herbs match certain people.

According to research conducted by Johnson,

Park, Gross, and Yu, explained that integrative medicine is not the same as an alternative treatment because it is integrated and complementary can be combined with conventional/medical treatment while alternative treatment is a substitute for medical treatment. The results of this study found that cancer patients who chose alternative medicine as the main treatment without being helped with medical treatment had a greater risk of death [21]. Evidence-based integrative therapy is recommended in breast cancer patients but requires further research for good results [22].

Therefore, the participants in this study generally prefer to combine complementary treatments that are in line with the medical treatment they carry out, namely radiotherapy, chemotherapy, and hormone therapy. Five participants admitted that they still had not consulted the use of complementary treatment to health workers or doctors. Participants argued that complementary treatments that they use, such as milk and juice products do not need to be consulted. In seeking treatment and treatment of cancer, it is quite long and is influenced by external sources. External sources obtained by participants came from family and friends, cancer survivors, social media, health workers and culture in the community. Research conducted by Muhamad, Afshari, & Kazilan that cancer survivors need the support of their family members for information about survival strategies related to emotions, lifestyle, and food to help patients in making decisions [23]. Therefore, the family plays an important role in influencing the patient to choose the right treatment.

The involvement of cancer survivor associations can help cancer survivors obtain appropriate treatment. Unfortunately, some participants said that there was an official association of cancer patients in the city of Medan, North Sumatra, who could provide guidance and counselling as well as a gathering place for cancer survivors.

The development of information technology now influences the selection of appropriate cancer treatment. This development certainly must be followed by the intelligence of social media users/communities to select and filter the correct information by looking at trusted sources and confirming to existing health practitioners. Also, there is a need for national policies to provide information, both social and public media, which are needed to meet different patient information needs. Health care providers must recognise that cancer patients will continue to need information at all stages, including those related to complementary therapies and appropriate alternatives [24].

According to Tariman and Szubski, there is a need for a good evolution of the relationship between patient doctors and nurses in making patient decisions to choose the right treatment. Nurses must have full awareness of their professional role to involve themselves in patient decisions, resolve obstacles faced by patients and use evidence-based interventions to reduce uncertainty in decision-making in patients choosing treatment [25].

All participants said there was involvement of health workers, especially nurses and doctors while seeking treatment. Generally, health workers do not recommend the use of complementary treatments and alternatives as patient choices except milk and fruit juice. But besides that, nurses still found that suggested the use of alternative therapies that were not appropriate to replace medical therapy recommended by doctors. This is certainly not appropriate and fatal.

The obstacles and obstacles that cancer patients undergo in seeking and undergoing treatment vary greatly from financial constraints, weak body conditions, transportation, and others. This certainly can affect the psychology of cancer patients in seeking treatment such as the emergence of feeling tired, tired, hopeless and even resigned. These feelings emerge as negative feelings that can affect a patient's motivation to recover. According to the Irish Cancer Society (2012), cancer can affect emotional patients such as shock and disbelief, loss of self-control, sadness, resentment, anger, isolation and even cause anxiety and depression.

This spiritual support can be through prayer or guidance from Pastors, Rabbis, Imam, priests or religious leaders. This is supported by research carried out by Ahmadi, Darabzadeh, Nasiri, and Askari found that spirituality and religiosity are positively related to the well-being of cancer patients in seeking treatment [26].

In terms of religiosity, 12 participants were Muslim, and 3 participants were Protestant. One of the participants lives in a halfway house sheltered by a religious zakat body in the city of Medan, claiming that he has received psychological and spiritual support since living in that place. At this halfway house, patients are listened to the Quranic mural every day, get religious lectures every week, and get Ruqiah services every month. Also, participants are advised to always recite the dhikr and muhasabah themselves if the psychological condition is not stable. Protestant participants say they always say a prayer if the psychological condition is unstable. One in 3 patients said that they rarely attend church services due to the condition of the disease, but the participants claimed to do still the prayer led by one family member. Praying is a way that can be done individually, a spiritual feeling between individuals and God, providing peace for cancer patients [27].

Besides that, all participants admitted that when feelings of sadness and fatigue they would always try to remember God by praying, reciting, praying or praying according to religion and belief, and seeking support from people who knew more about religion.

In this study, there was a lack of the involvement of the health centre as basic health services, which played an important role in early detection of cancer. Participants generally said that they had never received counselling and pre-cancer examinations at the local health centre. Participants were not exposed to the dissemination of health centre officials regarding pre-cancer examinations such as IVA and SADANIS at the Puskesmas. Fourteen participants came to the health centre only when making referrals to the hospital. Of the 11 participants with breast and cervical cancer, 10 said they did not get the initial examination at the basic health centre after signs and symptoms.

Whereas in the Regulation of the Minister of Health of the Republic of Indonesia Number 29 of 2017 concerning changes to Minister of Health Regulation No. 34 of 2015 concerning the prevention of breast cancer and cervical cancer there is a role of health centre in the effort of early detection of breast and cervical cancer by involving trained midwives and trained doctors before refer patients to advanced health services or hospitals. Early detection services for cervical cancer and breast cancer can be done in primary care or health centres [28]. Whereas, in the follow-up, the patients referred to the health centre have not yet developed a clear path. The lack of clarity in the flow of handling follow-up of cancer patients makes health workers as implementers at the health centre level unable to implement this action, so the role of the health centre in preventing cancer is felt to be less than optimal [29].

The existence of gaps in the field with regulations issued by the Ministry of Health makes the explanation that the basic health centre is less optimal in tackling this cancer. This also relates to the quality of human resources, namely, health workers. Health workers in each service should have a special ability to detect this cancer from signs or symptoms that appear as early as possible. The reality is not in line with Minister of Health Regulation Number 29 of 2017 that the unavailability of health workers is trained midwives who have an early role who can refer to trained doctors at the Puskesmas level, and if they cannot be handled, they can be referred to further services [30]. Also, other health workers who diagnose the existence of cancer are less skilled so that patients can go to various health services both at home and abroad to seek treatment.

In conclusion, advanced cancer patients have experience using complementary therapies and inappropriate alternatives before using a medical treatment on the grounds of distrust of medical treatment and advice from the environment around patients. This study could be implicated in the field of nursing education as a reference for implementing education for patients in choosing the appropriate treatment. Cancer patients should be more concerned about the use of complementary and medical treatment appropriate to seek information to health care. For policy-makers should be able to perform any action against healthy institutions by applicable regulations. This study has limitations that the interview method cannot represent the type of cancer that has limitations in communication. This research can be further developed into more specific types of cancer or one type of complementary or alternative treatment.

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Log-Linear Modelling of Effect of Age and Gender on the Spread of Hepatitis B Virus Infection in Lagos State, Nigeria

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Keywords: Hepatitis B; Log-linear; Modeling; Transmission; AIC

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Abstract

BACKGROUND: The effect of age and gender on the transmission of any infectious disease can be of great important because the age at which the host contact the disease may be a determinant on the rate at which the disease will spread.

AIM: The purpose of this research is to model the significant effect of age and gender on the spread of hepatitis B virus using data collected from Lagos State, Nigeria.

MATERIAL AND METHODS: The data that was used for this research is a ten years data covering the period of 2006 to 2015, which was collected from Nigeria Institute of Medical Research (NIMR). A log-linear modelling approach was employed using R programming language software. Akaike Information Criterion (AIC) method of model selection was used in selecting the best model.

RESULTS: It was discovered from the analysis that both factors (age and gender) have a significant effect on the spread of hepatitis B infection. This means that the age at which an individual is tested positive to hepatitis B virus will affect the spread of the disease. In choosing the best model among the four models that were developed, model AY: GY (age & year: gender and year) was found to be the best model.

CONCLUSION: Age and gender were found to act as a risk influencer that could have a great effect on the transmission of hepatitis B virus infections in Lagos state, Nigeria.

Introduction

The word hepatitis comes from the Ancient Greek word *hepar* (root word *heat*) meaning 'liver', and the Latin *itis* meaning inflammation. Hepatitis, therefore, means injury to the liver with inflammation of the liver cells [1], [2].

According to the World Health Organization, “Hepatitis is an inflammation of the liver. The condition can be self-limiting or can progress to liver cancer. Hepatitis viruses are the most common cause of hepatitis in the world, but other infections, toxic substances (e.g. alcohol, certain drugs), and autoimmune diseases may also cause hepatitis”.

It has been discovered from the literature that there are various types of Hepatitis [3] classified these various types into five major categories, namely: A, B, C, D and E, respectively. These viruses are not related to each other. They differ in their structure, the way they spread among individuals, the severity of symptoms they can cause, the way they are treated, and the outcome of the infection [3]. Among these categories, the most dangerous is Hepatitis B because it leads to chronic disease condition in hundreds of millions of people [4], [5], [6].

Hepatitis B has been described as one of the major infectious diseases in the world today because over 750,000 deaths are attributed to it annually [7], [8], [9]. Hepatitis B infection is a global healthcare problem with particularly high prevalence in developing countries in sub-Saharan Africa and South-East/Central Asia. Statistics shows that, about 350-400 million individuals worldwide suffer from chronic Hepatitis B virus infection which is a dominant cause of cirrhosis and hepatocellular carcinoma (HCC): [10], [11], [12], [13], [14], [15], [16], [17].

It has been discovered, that age and gender may be risk factors in the transmission of hepatitis B virus [18], [19], [20]. This study aimed to investigate the influence of age and gender on the prevalence of hepatitis B infections among the people of Lagos State, Nigeria. By clearly indicating the characteristics of hepatitis B and its associated risk factors, we intend to develop log-linear models and choose the best model among the developed models.

Material and Methods

The log-linear model which is used in the analysis of contingency tables is a generalised linear model for counted data, and the variety of associations and interaction terms in log-linear models can easily be described by the goodness of fit tests. The methodology of the log-linear model for the analysis of contingency tables is described in many articles and book such as [21], [22], [23], [24], [25].

Consider an *I x J* contingency table. The log-linear model is represented by:

$$\log(M_{ij}) = \lambda_0 + \lambda_i^1 + \lambda_j^2 + \lambda_{ij}^{12} \quad (1)$$

For all i and j, under the constraints of the λ term to sum to zero over any subscript such as:

$$\sum_{i=1}^{I} \lambda_i^1 = 0, \qquad \sum_{j=1}^{J} \lambda_j^2 = 0 \quad ,$$
$$\sum_{i=1}^{I} \lambda_{ij}^{12} = \sum_{j=1}^{J} \lambda_{ij}^{12} = 0 \quad (2)$$

The log-linear model given above is called the saturated model or full model for the statistical dependency between Y_1 and Y2.

By analogy with analysis of variance models, we define the overall mean by:

$$\lambda_0 = \frac{1}{IJ} \sum_{i=1}^{I} \sum_{j=1}^{J} \log M_{ij} \quad (3)$$

The main effects of Y_1 and Y_2 by

$$\lambda_i^1 = \frac{1}{J} \sum_{j=1}^{J} \log M_{ij} - \lambda_0 \quad (4)$$

$$\lambda_i^1 = \frac{1}{I} \sum_{j=1}^{J} \log M_{ij} - \lambda_0 \quad (5)$$

And the two-factor effect between Y_1 and Y_2 by

$$\lambda_{ij}^{12} = \log M_{ij} = (\lambda_i^1 + \lambda_j^2) - \lambda_0 \quad (6)$$

Then the main and two-factor effects are determined by the odds and odds ratio, and can be written by:

$$\lambda_i^1 = \frac{1}{IJ} \sum_{i^1=1}^{I} \sum_{j=1}^{J} \log \frac{M_{ij}}{M_{i^1 j}}, \quad (7)$$

$$\lambda_i^2 = \frac{1}{IJ} \sum_{i=1}^{I} \sum_{j^1=1}^{J} \log \frac{M_{ij}}{M_{ij^1}} \quad (8)$$

And

$$\lambda_{ij}^{12} = \frac{1}{IJ} \sum_{i^1=1}^{I} \sum_{j^1=1}^{J} \log \frac{M_{ij} M_{ij}}{M_{i^1 j} M_{ij^1}} \quad (9)$$

For the independence model that Y_1 is statistically independent of Y_2, the cell probability Mij can be factorised into the product of marginal probabilities M_{i+} and M_{+j}, that is,

Mij = Mi+ M+j,

Where and

$$M_{i+} = \sum_{j=1}^{J} M_{ij}$$

Then the two-factor effect is:

$M_{+j} = \sum_{i=1}^{I} M_{ij}$.

$$\lambda_{ij}^{12} = \frac{1}{IJ} \sum_{i^1=1}^{I} \sum_{j^1=1}^{J} \log \frac{M_{i+} M_{+j} M_{i^1+} M_{+j^1}}{M_{i^1+} M_{+j} M_{i+} M_{+j^1}} = 0$$

, (10)

So that the log-linear model for the independence model is expressed by:

$$\log M_{ij} = \lambda_0 + \lambda_i^1 + \lambda_j^2, \text{ for all I and j (11)}$$

For an I x J x K contingency table, the saturated log-linear model for the contingency table is:

$$\log M_{ijk} = \lambda_0 + \lambda_i^1 + \lambda_j^2 + \lambda_k^3 + \lambda_{ij}^{12} + \lambda_{ik}^{13} + \lambda_{jk}^{23} + \lambda_{ijk}^{123}$$

(12)

for all i, j and k.

The λ terms satisfy the constraints;

$$\sum_{i=1}^{I} \lambda_i^1 = \sum_{j=1}^{J} \lambda_j^2 = \sum_{k=1}^{K} \lambda_k^3 = 0, \quad (13)$$

$$\sum_{i=1}^{I} \lambda_{ij}^{12} = \sum_{j=1}^{J} \lambda_{ij}^{12} = \ldots = \sum_{k=1}^{K} \lambda_{jk}^{23} = 0, \quad (14)$$

$$\sum_{i=1}^{I} \lambda_{ijk}^{123} = \sum_{j=1}^{J} \lambda_{ijk}^{123} = \sum_{k=1}^{K} \lambda_{ijk}^{123} = 0, \quad (15)$$

We define the λ terms as follows:

The overall mean is given by:

$$\lambda_0 = \frac{1}{IJK} \sum_{i=1}^{I} \sum_{j=1}^{J} \sum_{k=1}^{K} \log M_{ijk.} \quad (16)$$

The main effects of Y_1, Y_2, and Y_3 are:

$$\lambda_i^1 = \frac{1}{JK} \sum_{j=1}^{J} \sum_{k=1}^{K} \log M_{ijk.} - \lambda_0 \quad (17)$$

$$\lambda_j^2 = \frac{1}{IK} \sum_{i=1}^{I} \sum_{k=1}^{K} \log M_{ijk.} - \lambda_0 \quad (18)$$

$$\lambda_k^2 = \frac{1}{IJ} \sum_{i=1}^{I} \sum_{j=1}^{J} \log M_{ijk.} - \lambda_0 \quad (19)$$

Each interaction effect is given by:

$$\lambda_{ij}^{12} = \frac{1}{K}\sum_{k=1}^{K} \log M_{ijk.} - (\lambda_i^1 + \lambda_j^2) - \lambda_0 \quad (20)$$

$$\lambda_{ik}^{13} = \frac{1}{J}\sum_{j=1}^{J} \log M_{ijk.} - (\lambda_i^1 + \lambda_k^3) - \lambda_0 \quad (21)$$

$$\lambda_{jk}^{23} = \frac{1}{I}\sum_{i=1}^{I} \log M_{ijk.} - (\lambda_j^2 + \lambda_k^3) - \lambda_0 \quad (22)$$

and,

$$\lambda_{ijk}^{123} = \log M_{ijk.} - (\lambda_{ij}^{12} + \lambda_{ik}^{13} + \lambda_{jk}^{23}) - (\lambda_i^1 + \lambda_j^2 + \lambda_k^3) - \lambda_0 \quad (23)$$

Results

The summary of the data that was used for this research is presented in Table 1. The data covers the period of ten years (2006 – 2015) of those that are tested positive to hepatitis B virus in Lagos state, Nigeria.

Table 1: Classification of the data according to the attributes of age and gender of the patients and the year they were diagnosed with having the disease

Age Interval	Gender	Year										Total
		2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	
Less than 20	Male	2	4	2	4	5	3	3	1	2	1	27
	Female	5	7	8	6	7	5	7	3	4	2	54
21-30	Male	45	60	132	101	72	68	52	65	29	20	644
	Female	93	112	191	163	118	103	90	110	50	34	1064
31-40	Male	208	171	330	289	284	269	107	231	147	108	2826
	Female	435	451	719	633	697	694	345	575	341	265	4545
41-50	Male	181	158	280	236	153	168	139	207	99	71	1692
	Female	377	331	562	525	368	386	310	398	110	94	3461
51-60	Male	30	38	80	71	51	47	39	44	11	7	418
	Female	129	173	275	224	195	207	139	153	57	31	1583
61-70	Male	8	13	19	15	11	10	8	12	5	4	105
	Female	31	39	68	50	39	45	28	35	20	15	370
71 and Above	Male	3	4	6	4	5	3	4	3	2	1	35
	Female	5	7	9	8	7	6	6	7	5	3	36
Total		1552	1568	2753	2329	2012	2014	1277	1844	882	656	16887

Log-Linear Fitted Models

The summary of the log-linear fitted models for the data presented in table 1concerning the effect of age, gender and the year of being tested positive is presented below:

Table 2: Model 1: Age: Gender: Year (A: G: Y)

Statistics:	X^2	df	P (> X^2)
Likelihood Ratio	72.28144	63	0.1981745
Pearson	73.03648	63	0.1815950

Model 1 presented in table 2 above considered when no association exists among the variables under consideration. The model is written mathematically as:

$$\log(m_{ijk}) = \lambda + \lambda_i^A + \lambda_j^G + \lambda_k^Y \quad (24)$$

Table 3: Model 2: Age and Year: Gender and Year (AY: GY)

Statistics:	X^2	df	P (> X^2)
Likelihood Ratio	235.6358	60	0.0034421
Pearson	299.4320	60	0.0110223

Model 2 presented in table 3 above considered two-way association between each variable with the year of being tested positive. The model is written mathematically as:

$$\log(m_{ijkl}) = \lambda + \lambda_i^A + \lambda_j^G + \lambda_k^Y + \lambda_{ik}^{AY} + \lambda_{jk}^{GY} \quad (25)$$

Table 4: Model 3: Age and Gender: Year (AG: Y)

Statistics:	X^2	df	P (> X^2)
Likelihood Ratio	227.5998	69	0.081745
Pearson	224.8027	69	0.034568

Model 3 presented in table 4 above considered two-way association between the two variables together the year of being tested positive. The model is written mathematically as:

$$\log(m_{ijkl}) = \lambda + \lambda_i^A + \lambda_j^G + \lambda_k^Y + \lambda_{ik}^{AG} \quad (26)$$

Table 5: Model 4: Age: Gender: Year (A: G: Y)

Statistics:	X^2	df	P (> X^2)
Likelihood Ratio	49.95058	54	0.0312744
Pearson	49.97675	54	0.0302744

Model 4 presented in table 4 above considered three-way association among the variables. The model is written mathematically as:

$$\log(m_{ijkl}) = \lambda + \lambda_i^A + \lambda_j^G + \lambda_k^Y + \lambda_{ij}^{AG} + \lambda_{ik}^{AY} + \lambda_{jk}^{GY} \quad (27)$$

Table 6: Aic Values for The Models

MODEL	G^2	AIC	P-VALUE
Model 1	72.28144	122.05	0.1981745
Model 2	235.63583	117.37	0.0034421
Model 3	227.59983	122.67	0.081745
Model 4	49.95058	127.04	0.0312744

Discussion

Comparing the p-values of all the models with 0.05 level of significance; model 1, which is the model that represents no association among the variables under consideration, is the only model that is not significant. Model 2, 3 and 4 that established an interaction among the variables are all significant. Therefore, modelling the effect of age and gender on the spread of HBV virus infection in Lagos state for the period of ten years of 2006 to 2015 shows that, both variables (age and gender) have a significant effect on the spread of the disease (Table 2, 3, 4 and 5).

On the other hand, choosing the best model among various log-linear models developed model: age and year: gender and years was discovered to be the best model since the AIC value (117.37) for the model are the lowest and the highest using likelihood ratio test (235.63583) (Table 6). This means that age

and gender are not independent of the spread of the disease that is, the effect of age on the spread of the disease is not independent of the effect of gender on the spread of the disease.

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Public Health

The Factors of Affect Indoor Air Quality Inpatient at Private Hospital, Pekanbaru, Indonesia

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Abstract

BACKGROUND: Air quality in inpatient rooms influenced by several factors. Room not qualified health can cause disease and can spread through equipment, the material used, food and drink, health workers, patients and visitors.

AIM: The purpose of this study is to know the factors that affect air quality in the inpatient room at a private hospital, Pekanbaru.

METHODS: The research is quantitative analytic by design cut latitude study. Samples from 120 nurses were selected overall sampling. The data do with the measurement directly, interviews and observation using a questionnaire. Data analysed by using the chi-square with significance p-value less than 0.05.

RESULTS: The quality of the air in inpatient rooms has met the standard. Variables are affecting air quality in inpatient rooms in the hospital significantly with p-value more than 0.05 is temperature, dust, the germ, density occupancy, sanitation room. While the moisture, standard operating procedures and behaviour is not significant.

CONCLUSION: The factors that significantly affect indoor air quality inpatient hospitalisation are temperature, dust, germ numbers, occupancy density, room sanitation.

Introduction

Human activity can change chemical composition in the air structures and concentration and on the chemical substances to increase, especially if that activity is done in a room with a bad air circulation system. Air quality in space not only affected by chemical pollution but also by physical of environmental factor such as temperature and moisture [1].

Air quality in space is the air inside a building inhabited or occupied to a period of at least 1 hours by people with various health status different; Air quality in the room is the air inside a building inhabited or occupied to a period of at least 1 hours by people with various health status [2]. Well, air quality in space defined as air free from irritation, free of pollution discomfort or health inhabitant of disruption. Room temperature and moisture also affect the comfort and health of the inhabitants [3].

One of the rooms that could potentially have a problem of indoor air pollution is the hospital. The hospital as a health service for the community must have inpatient ward qualified, health good air quality, construction and facility. In a room that did not qualify, health disease can spread via, equipment, food and drink, health workers, and visitors [4].

The Indonesian government has set the requirements of environmental health hospital in Minister for Health Decree No.1204 / MENKES / SK / X / 2004 which is air that is in should be free inside the germ pathogens by the number was no more than 500 CFU/m^3 air and dust levels of particulate matter less than 10 micron average with 8 hour or 24 hours does not exceed 150 µg/m^3 and does not contain

asbestos dust. [5]. The results study of Abdullah in public hospitals haji Makassar south Sulawesi, shows that more than 91% the number of germs and 71-87% the quality of the physical environment health was not up to the required by Indonesian decree number 1204 / MENKES / SK / X / 2004 [6].

In Pekanbaru city hospitals, this research has never been found, so researcher assumes that the topic of this research is very important to study and the researcher hopes that with this study, we can know the existing conditions of air quality in hospital that is in the hospital and so that it does not affect the health and environment of the hospital. The difference between this study and earlier studies is that there are several variables examined in this study rarely examined by previous studies, and usually, other researchers only focus on the physical environment of the hospital.

Based on the results of observations made at three private C class hospitals in the city of Pekanbaru, there were several findings, including the temperature of class 2 and 3 hot rooms. This is because the number of patients in the room does not meet the standards, the extent of the inpatient room cannot accommodate a large number of patients so that the condition of the room becomes less conducive. And natural ventilation is not available for the exchange of air circulation so that the condition has the potential to cause health problems.

Based on the background, the researcher was interested in conducting this research. The purpose of this study was to find out the factors that influence air quality in the inpatient room at a private hospital in Pekanbaru City.

Methods

This study is a quantitative analytic with a cross-sectional design. The population is all in-patient room nurse at a private hospital in Pekanbaru. Sample in this research as many as 120 nurses who were chosen with a total of sampling. The data was undertaken in two ways: first, measuring directly inpatient rooms that are variable temperature used a thermometer, moisture used a hygrometer, dust used a Low Volume Air Sampler (LVS), The germ used a microbiology air sampler, and density dwelling uses laser distance meters. Second, with the interview and observation consisting of a variable behaviour, knowledge, standard operating procedures. Variable behaviour, consisting of 5 questions variable knowledge consisting of 9 questions, variable standard operating procedures are consisting of 4 questions. Used the validity and has tested realibilitas. Coding all data was undertaken then analysis to see characteristic nurse, the percentage the frequency and the factors that affect air quality in the inpatient ward at Private Hospital in Pekanbaru. Data analysis use the chi-square with significance p-value less than 0,05 by SPSS 21 version.

Results

Characteristics of respondents

Total respondents were 120, as many as 65.9% (n = 79) was 20-30 years old, 28.3% (n = 24) was 31-40 years old and 5.8% (n = 7) 41-50 years old. Most of respondents 95 % (n = 114) were women and 5% (n = 6) of them were men. As many as 60% (n = 72) were diploma and the others 40% (n = 48) were bachelor. Most of respondents working less than 5 years 92.5% (n = 111) and only 7.5% working more than 5 years (n = 9). It's showed in the Table 1.

Table 1: Characteristics of respondents

No	Variable	Frequency (n= 120)	Persentage (%)
1	Age		
	20-30 years old	79	65.9
	31-40 years old	24	28.3
	41-50 years old	7	5.8
2	Sex		
	Man	6	5
	Woman	114	95
3	Education		
	Diploma	72	60
	Bachelor	48	40
4	Working Duration		
	≥ 5 years	9	7.5
	< 5 years	111	92.5

The percentage of air quality in the inpatient ward of the hospital and to for variables affecting

Air quality in the inpatient ward hospitals that had to meet the standards as many as 65% (n = 78) and does not fit the standard 35% (n = 42). As in figure 1 below:

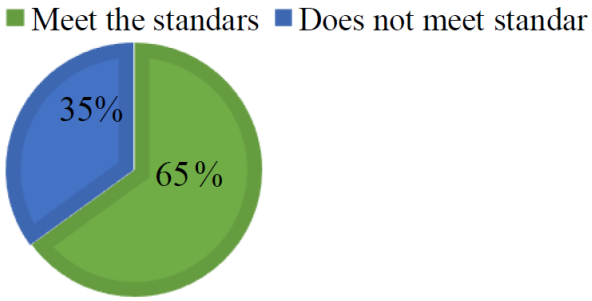

Figure 1: Percentage of Indoor Inpatient Room Air Quality of Hospital

The temperature that had been measured categorised into two if measurement result 22-24°C category, if the measurement results it less than 22°C and more than 24°C category does not meet the standard. The results of the measurement of

temperature obtained that 34.2% (n = 41) were standard and the others 65.8% (n = 79) temperatures do not meet the standard.

Moisture that had been measured categorised into two is that if the measurement results 45-60 % categorised as based on standard if the measurement result less than 45% and more than 60% was a category does not meet the standard. The measurement result moisture indicated that 39.2 % (n = 47) who meet the standards and 60.8 % (n = 73) moisture that does not meet the standard.

The dust that had been measured categorised into two is that if the measurement results in less than 150 μg/m^3 including meet the standard if the measurement results more than 150 μg/m^3 category does not meet the standard. The measurement result indicated that dust 62.5% (n = 75) who meet the standards and 37.5 % (n = 45) dust which does not meet the standard.

The germs that had been measured categorised into two is that if the measurement result 200-500 CFU/m^3 including meet standards, if the measurement result less than 200 CFU/m^3 and more than 500 CFU/m^3 category does not meet the standard. The measurement result obtained the germs that 56.7% (n = 68) who meet the standards and 43.3% (n = 52) the germs do not meet the standard.

The occupancy density that had been measured categorised into two is that if the measurement results 8 m^2 including meet the standards, if the measurement result less than 8 m^2 in the category does not meet the standard. The measurement result obtained the occupancy density that 55.8% (n = 67) who meet the standards and 44.2% (n = 53) the occupancy density does not meet the standard.

Respondent's standard operating procedures about air quality was calculated with the correct scores of 4 items question if the answer more than 75% were corrected then concluded in accordance category. On the other hand, if the answer, less than 75% were category not in accordance. The results showed that the percentage of the standard operating procedures that 45% (n = 54) appropriate and 55% (n = 66) standard operating procedures that are not appropriate.

Respondent's sanitation the room about air quality was calculated with the correct scores of 9 items questions if the answer more than 75% were corrected then concluded in meet standards. On the other hand, if the answer, less than 75% were category does not meet the standard. The results showed that the percentage of sanitation the room that 55% (n = 66) who meet the standards and 45% (n = 54) sanitation the room does not meet the standard.

Respondent's behaviour of air quality was calculated with the correct scores of 5 items question if the answer more than 75% were corrected then concluded in the category of good if the answer less than 75% categorised not good. The results showed that the percentage of the behaviour that 60.8% (n = 73) of conduct good and 39.2% (n = 47) of behaviour that is less than good (Table 2).

Table 2: The Percentage Variables Affecting Indoor Air Quality

No	Dependent Variable	Frequency	Percentage (%)
1	Temperature		
	Does not meet standards	79	65.8
	Meet standards	41	34.2
2	Humidity		
	Does not meet standards	73	60.8
	Meet standards	47	39.2
3	Dust		
	Does not meet standards	45	37.5
	Meet standards	75	62.5
4	The germ		
	Does not meet standards	52	43.3
	Meet standards	68	56.7
5	Occupancy density		
	Does not meet standards	53	44.2
	Meet standards	67	55.8
6	Standard operating procedures		
	Not in accordance	66	55.0
	Accordance	54	45.0
7	Sanitation the room		
	Does not meet standards	54	45.0
	Meet standards	66	55.0
8	Behavior		
	Not good	47	39.2
	Good	73	60.8

Factors affect air quality in inpatient rooms at the hospital

Table 3 show variables are affecting air quality in inpatient rooms at the hospital. The research results indicated that variables affecting the air quality in inpatient rooms at the hospital, that is: the significant temperature, dust, the germs, occupancy density, sanitation the room like a table below:

Table 3: Factors that affect air quality in inpatient rooms at the hospital

Factors	Air quality in inpatient rooms at the hospital				
	Does not meet standards	Meet standards	Total	P value	POR (95% CI)
Temperature					
-Does not meet standards	58 (73.4%)	21 (26.6%)	79 (100%)	0.007	2.900 (1.316-6.390)
-Meet standards	20 (48.8%)	21 (51.2%)	41 (100%)		
Humidity					
-Does not meet standards	52 (71.2%)	21 (28.8%)	73 (100%)	0.074	2.000 (0.929-4.304)
-Meet standards	26 (55.3%)	21 (44.7%)	47 (100%)		
Dust					
-Does not meet standards	39 (86.7%)	6 (13.3%)	45 (100%)	0.001	6.000 (2.271-15.853)
-Meet standards	39 (52.0%)	36 (48.0%)	75 (100%)		
The germ					
-Does not meet standards	44 (84.6%)	8 (15.4%)	52 (100%)	0.001	5.500 (2.257-13.403)
-Meet standards	34 (50.0%)	34 (50.0%)	68 (100%)		
Occupancy density					
-Does not meet standards	44 (83.0%)	9 (17.0%)	53 (100%)	0.001	4.745 (2.003-11.239)
-Meet standards	34 (50.7%)	33 (49.3%)	67 (100%)		
Standard operating procedures					
-Not in accordance	40 (60.6%)	26 (39.4%)	66 (100%)	0.265	0.648 (0.301-1.392)
-Accordance	38 (70.4%)	16 (29.6%)	54 (100%)		
Sanitation the room	43 (79.6%)	11 (20.4%)	54 (100%)	0.002	3.462 (1.525-7.860)
-Does not meet standards	35 (53.0%)	31 (47.0%)	66 (100%)		
-Meet standards					
Behaviour	33 (70.2%)	14 (29.8%)	47 (100%)	0.337	1.467 (0.670-3.210)
-Not good	45 (61.6%)	28 (38.4%)	73 (100%)		
-Good					

Discussion

Nurses in hospitals are predominantly female with ages 20-30 years and working period less than 5 years with Diploma education, this is in accordance with the early history of professional nursing that began in Florence nightingale that is based on the love of a mother or females but to live and length of employment usually a nurse with age and length of employment old more experienced in do the work. This is supported by the results of the study Megawati that age and length of working very influence the performance nurses of the hospital [7].

For diploma education this is supported by the theory Notoadmodjo, that people having higher education will know higher the comparison with peoples having low education and through education a person can improve ripeness intellectual so that it can decide between acting [8].

Air quality in inpatient rooms at the hospital is adequate to suggest that air quality in the room was uncontaminated from physical harm, chemical or biological to pollute air quality. However, it does not rule out the possibility that inpatient rooms are free of diseases that cause symptoms of health problems in patients, health workers, and visitors.

The result of this research can define it as many as 79% temperature does not meet the standard is a lot of room that has been exceeding that of 24°C condition of inpatient rooms feels hot, the high temperature can be a disturbing role in regulating the way a metabolic reaction for the organism. High cold temperature frustrates the patient condition and causes inconveniences. This is in line with an average temperature of research Lisa that obtained from the measurement of in-patient hospital room Syekh Kabupaten Gowa more than 30°C that has been exceeding the value of the standard [9].

The research results show there an effect that welfare between temperature and the quality of the air as it when the temperature exceeds the standard he will affect the quality of the air in the inpatient ward, this is in accordance with the results of the study Fang state that there are significant links of temperature and moisture on air quality, the air is considered far less caused by a rise can be received by temperatures and humidity, so that it will have a negative impact [10].

The humidity just 39.2% complete the standards, to the humidity is relatively low less than 20% can cause drought mucous membrane, membrane while high humidity would increase the growth of microorganisms. This fits in with research conducted Nizar the average results of moisture measurements when researching RSUD Prof. Dr Margono Soekarjo Purwekerto as many as 68,25% did not meet the requirements of the Minister of Health Decree No.1204 / Menkes / S / X / 2004 [11].

The dust of 62.5% meets the standard; this condition requires maintenance of the room so that the dust inside the inpatient room still meets the standards. This is supported by Laila research that the concentration of dust in the air the library FB and FC still qualified while in the FA library have exceeded the limits [12].

Because the particles dust is small and does not look by the eye and to find out more than dust standard or not to be done directly measure the same as conducted by the results of this research as to when the dust has more than a standard will impact on negatively. This health research results together with the research Lisa that levels of dust on the mend did not exceed 150 mg/$m^{3,}$ so levels of dust the room on the study is based on standard [9].

But from the results of Mahmoud's research, it was stated that air quality in space, especially health care facilities (HCFs) was strongly influenced by outside sources, especially traffic. For the highest level of total suspended particles (TSP) and less than 10 microns (PM) in hospitals with locations where there is a lot of human activity. The level of the particulate maker (PM and TSP) is higher than the Air Quality Guidelines (AQG) so that the occupants of the room must know about the sources and effects of contaminants and ventilation systems that are in the room to reduce particulates in the room [13].

The germ, as many as 56.7% qualified standards where the spread of the germ the media involved the environment such as air and vector as an intermediary or vehicle. As the results of the study Adysaputra in space surgery RSUDP, Dr Wahidin Sudirohusodo Makasar found staphylococcus aureus germs the main cause of nosocomial infections derived from the upper part of the respiratory channel [14].

In this study, density dwelling was qualified standard as many as 55.8%, according to northern European studies that there was a correlation between increased temperature about 23°C, density inhabitant and ventilation to indoor uncomfortable. When temperature more than 28°C need to use a neutralizer such as air conditioner or fan. Results shows that there is significant influence between density dwelling and air quality in space, having the increasing number of a number of people in a room will affect air quality in space. This is supported by the study of Yousef that the quality of the environment in the room could affect the welfare and comfort residents and issues such as sick building syndrome thermal comfort to the quality of indoor air, as comfortable as visual and acoustic so that it is necessary designs of buildings by considering the parameters of welfare [15]. Same as research of Huisman that adverse effects of the physical environment very proved to be relevant to the process of healing and welfare for patient and the patient family and health workers, with the development of

healthcare facilities (HCF) approach in the design and construction executive future such as a seven-bed single to their own generation, identical room, and lighting [16].

As many as 45% standard operating procedures were not appropriate and the rules to be applied at the hospital is not optimal and it has to do with the manners nurse during inpatient care in maintaining the air quality, if not applied standard operating procedures at the hospital nurses will affect behavior. In this study the standard operating procedures is a significant relation exists with the air quality in space, this is different to the theory that behavior nurse in maintaining and preventing nosocomial infections is an important factor to prevent nosocomial infections in hospitals. According to the theory of Notoatmojo that behavior domain influenced by knowledge, attitudes and the act of, so that on this research nurse behavior is strongly influenced by the knowledge that is largely obtained through the eyes and ears. Knowledge of the realm of cognitive constituted the domain that is very important in shaping the act of someone or overt behaviour [8]. According to its research of Beiyu that behavior could affects air quality in rooms and with the good manners being measurable and knowledge can develop a system building with smart city [17].

Results it can be concluded that temperature and moisture are not adequate and variable this is significantly influenced the quality of the air in the inpatient room at the hospital, it is closely related to the can caused by the high number of temperature and moisture in the room. Dust, the germ, density dwelling, standard operating procedures, sanitation and behaviour still room standard, but it is possible that this condition free from an impairment of health due to an impact caused. Health and suggested to the management hospital to anticipate this must be cleaned every day, inpatient rooms monitoring the condition of the maintenance water conditioner regularly and periodically to be able to maintain the quality of air in a room.

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Review Article

Regional Policy for Disaster Risk Management in Developing Countries Within the Sendai Framework: A Systematic Review

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Abstract

The recently concluded World Conference on Disaster Risk Reduction (WCDRR) in Sendai, Japan and the Sendai Framework for Disaster Risk Reduction 2015–2030 (SFDRR) have set renewed priorities for disaster risk reduction (DRR) for the next 15 years. This framework is the main guiding instrument for Disaster Risk Management (DRM) within the scope of sustainable development and the eradication of poverty. Disaster management policies and practices should be based on an understanding of risks, not just on an ideological level. Gap and key challenges identified include Still weak coordination, cooperation and linkages among the sectors related to DRR, Lacks of skills in loss assessment and post disaster needs, lack of strategic research agenda, absence of consensus regarding terminology, and limited coordination between stakeholders. The aim of this study was to gain an understanding of why disaster risk reduction efforts undertaken by regional policy often fail to improve future disaster responses. These findings can be used to help guide to improve regional policy in disaster risk reduction processes. This research is a systematic review study by collecting articles that are relevant to International Journal of Disaster Risk Science. From the analysis, we found that all four priorities for action in the Sendai Framework are relevant to Disaster Risk Management (DRM) field as follows: 1). Understanding disaster risk; 2). Strengthening disaster risk governance to manage disaster risk; 3). Investing in disaster risk reduction for resilience and 4). Enhancing disaster preparedness for effective response and to "Build Back Better" in recovery, rehabilitation and reconstruction.

Introduction

The Sendai Framework used for disaster risk reduction which goal started from 2015 until 2030 notes that over 700 thousand people have lost their lives, 1.4 million have been injured, and approximately 23 million have been made homeless. Overall, more than 1.5 billion people have been affected by disasters. The total economic loss was more than USD 1.3 billion. Also, between 2008 and 2012, 144 million people were displaced by disasters [1]. In the last decade, Asia has experienced 1730 natural disasters, which is 39 % of all-natural disasters in the world and almost 50 % of the total disaster losses and also the impact in an economic estimated loss of approximately USD 752 billion [2].

The impacts of disaster can disrupt the progress and developmental efforts of nations, often pushing them many years back [3]. That a major cause of these natural disasters is the changing climate, which affects human lives while exceeding the economic toll [4]. In support of this argument, stated that "the techniques to efficiently discover, collect, organize, search, and disseminate real-time disaster information have become national priorities for efficient crisis management and disaster recovery tasks" [5]. Although it may not be possible to entirely prevent all disasters, it is well acknowledged that an effective use of innovative technology can, to a great extent, reduce the magnitude of loss in life and property. Indeed, emerging technological innovations

including social media, location-based systems, radio frequency identification, and big data analytics (BDA) are considered as powerful tools that may help stakeholders during the disaster management cycle [6].

The research results revealed significant progress in integrating climate change adaptation into the policy and regulatory frameworks of the three relatively new fields of (a) disaster risk reduction, (b) environmental management and (c) urban planning. It is concluded that to achieve greater and more coherent integration of climate change adaptation (CCA) and, improve the way climate-related risks are dealt with, urban authorities need to systematically review current policies and regulations to assess the synergies and gaps [7].

Disaster management has been defined as the body of policy and administrative decisions, the operational activities, the actors and technologies that pertain to the various stages of a disaster at all levels. The literature about disaster management is becoming wide. Even a cursory review of the literature would identify that scholars of disaster management claim different theoretical foundations and argue different theoretical frameworks. It is necessary to conduct a systematic review to find out the literature on disaster management and to know the latest issues. Review with systematic review will give the decision to strong literature because based on searching the source of information from trusted articles.

Taking into account the experience gained through the implementation of the Hyogo Framework for Action, and in pursuance of the expected outcome and goal, there is a need for focused action within and across sectors by States at local, national, regional and global levels which is better known as this thing following four priority areas: Priority 1 (understanding disaster risk); Priority 2 (strengthening disaster risk governance to manage disaster risk); Priority 3 (investing in disaster risk reduction for resilience) and Priority 4 (enhancing disaster preparedness for effective response and to “Build Back Better” in recovery, rehabilitation and reconstruction) [1].

This study aimed to gain an understanding of why disaster risk reduction efforts were undertaken by regional policy often fail to improve future disaster responses. These findings can be used to help guide to improve regional policy in disaster risk reduction processes.

This paper is structured as follows: the next section describes the methodological approach used to conduct the review. This is followed by an analysis of the data collected, and then by a discussion of the results obtained and an outline of suggestions for future research on regional policy disaster risk reduction. The final section reports the conclusions.

Methods

This research is a systematic review study by collecting articles that are relevant to the International Journal of Disaster Risk Science. This research was conducted by reviewing articles from 2012 to December 2017. Impact factor (IF) is one measure that shows the average citations to articles published by a scientific journal within a certain period (for example: IF within 2 years, or IF within 5 years). IF is often a benchmark for researchers to select target journals by considering the possibility of citation of the article. The higher the IF, the greater the chance for citations to a published article in the target journal. This results in a comprehensive set of articles on selected topics. However, it is possible to skip some papers inadvertently. For the literature review, we have used the four-stage protocol (Figure 1): a) search done in the title and abstract field of database Scopus, Proquest, Pubmed with keyword: policy AND disaster risk management AND disaster risk reduction. Data analysis using guidelines from the preferred reporting item from the Systematic Reviews and Meta-Analysis (PRISMA); b) the selected article is then filtered, irrelevant title of study excluded; c) further review of the full-text articles assessed for eligibility and d) full-text articles are taken and reviewed individually by all authors for additional filtering. Remaining records are abstracted for analysis.

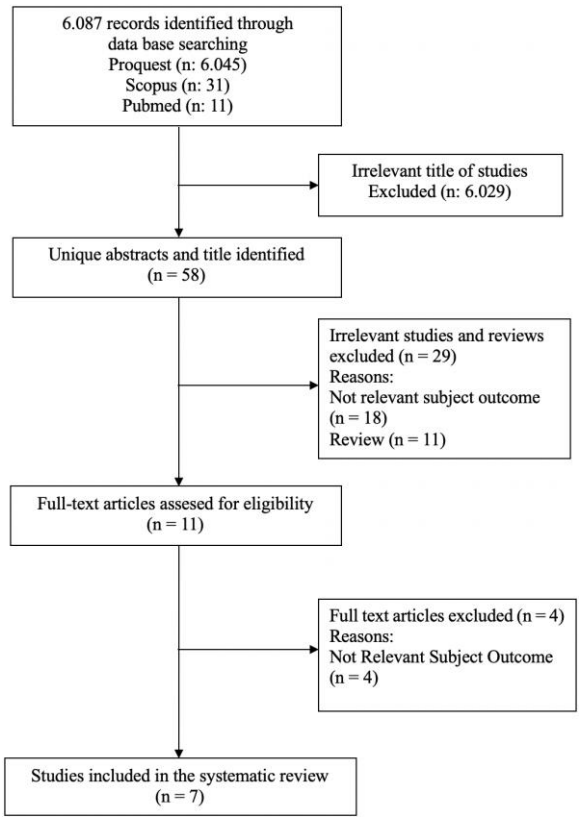

Figure 1: Prisma Flow Diagram

Results

In this section, we use methods to classify selected articles by author, year, title, research methodology, and the results of research (Table 1):

Table 1: Article Result of Systematic Review

No	Authors, year	Method	Outcome
1	Moshodi T, 2016 [8]	A qualitative research design, Study of the phenomenon	In particular, the study showed that in key disaster management activities and Processes, such involvement in institutional bodies for risk management, disaster risk assessment, training and awareness is very limited, or no stakeholder management is currently taking place. This lack of participation and inputs from sinkhole affected communities in the Merafong Local Municipality (MLM) understanding of the extent of the problem and which communities are deemed priority areas for risk reduction interventions
2	Ahmedab Z, 2013 [9]	Critical review.	This study has found that the Act emphasises mainly on institution building and action plan development for mitigating disasters in the country. Strategies have been developed to integrate Disaster Risk Reduction (DRR) in development policies and practices. However, due to bad governance, lack of political. Commitment, rampant corruption, economic constraints and overambitious plans, these policies and plans have not been implemented effectively. It has also transpired that Disaster Risk Management (DRM) policies have been implemented in an ad hoc and uncoordinated manner.
3	Baytiyeh H, 2017 [10]	Literature review.	This research demonstrates that Socio-cultural characteristics can profoundly influence the success or failure of the implementation of disaster risk reduction (DRR) strategies in divided sectarian societies. Despite recent progress in response management and disaster awareness, the lack of policies intended to institutionalise DRR and the neglect of integrating socio-cultural characteristics into DRR strategies. However, due to the lack of policies institutionalising DRR, such actions have remained inadequate, short-lived, fragmented, localised efforts ineffective at reducing the impacts of future large-scale disasters, such as earthquakes.
4	Niekerk D V, 2015 [11]	Qualitative and Quantitative Design	The research found that African countries have been making steady progress in implementing disaster risk governance against theoretical indicators. It is evident from the research that significant national political commitment to disaster risk reduction exists in most countries. Certain gaps and challenges are, however, still hampering better progress in the reduction of disaster risks. For disaster risk reduction to become a reality, national political support is needed (which mostly drives a legislative reform process). National commitment and involvement by African governments in international (global, regional and sub-regional) disaster risk reduction processes should be sought, with an emphasis on cross-border disaster risk reduction through appropriate protocols.
5	Alcayna T, 2016 [12]	A descriptive study on direct observations of and conversations	Results: Numerous activities in community-based resilience and Disaster Rsik Reduction (DRR) have been identified across the whole disaster continuum. Important gaps in research and practice remain. Discussion results: The Philippines is a leading regional actor in disaster risk management. However, a full picture of who is doing what, how, where, and when on resilience and disaster preparedness does not exist.
6	Pal I, 2017 [13]	The research descriptive study evaluation	The study principally examines the effectiveness of the Indian policy about the disaster management in achieving its intended outcome, i.e. Achieving effective mitigation and response to a disaster thereby minimising the casualties and losses to the community caused by the cyclone Phailin, India in 2013. Policies and legislation related to risk management are paramount towards defining the efficiency of the on-ground implementation of the Disaster Management Plan.
7	Sihvola KP and Chimpuku SV, 2016 [14]	Semi-structured interviews and document review.	Governance challenges and the historical burden have resulted in overlapping policy processes, as both policies incorporate the other field, and creates a threat of parallel national-level structures, thereby increasing potential inefficiencies in governance and policy implementation. The importance of developing a horizontal integration implementation of the DRR and Climate Change Adaptation (CCA) policies strategy before policy formulation processes to avoid the potential of inefficiencies became evident.

Discussion

From the analysis, we found that there are only a few studies based on theory. Disaster risk management policies and practices should be based on an understanding of disaster risk across all dimensions of vulnerability, capacity, people and exposed assets, hazard and environmental characteristics. Such knowledge can be utilised for risk assessment before the disaster, for prevention and mitigation and the development and implementation of adequate preparedness and effective response to disasters. The following describes the challenges and opportunities of disaster risk reduction policy research with theoretical insights by Sendai Framework.

Understanding disaster risk

Correct understanding of the risk-based on science, technology and local wisdom. Including the availability of detailed multi-threat assessment of risk for all areas, all spatial planning has used risk assessment.

In their approach to disaster risk reduction, States, regional and international organisations and other relevant stakeholders should take into consideration the key activities listed under each of these four priorities and should implement them, as appropriate, taking into consideration respective capacities and capabilities, in line with national laws and regulations. In the context of increasing global interdependence, concerted international cooperation, an enabling international environment and means of implementation are needed to stimulate and contribute to developing the knowledge, capacities and motivation for disaster risk reduction at all levels, in particular for developing countries [1].

To date, disaster management has been limited to preparedness and response, with little understanding of the need for risk reduction and post-disaster recovery. However, the introduction of the 2005 Hyogo Framework for Action (HFA) 2005-2015 results in a global paradigm Shifting from limited emergency measures to a more comprehensive approach to disaster management [15]. Sendai Disaster Risk Reduction Framework (SFDRR) 2015-2030, which replaces the HFA 2005-2015, reinforces the need for a broad approach to DRM. SFDRR emphasises the need to strengthen disaster risk reduction (DRR) and the establishment of a national health system strengthening as a means of achieving DRR [1].

Drawing from experience in disaster response and consistent with HFA principles, the World Health Organization (WHO) realises the importance of an all-peril and overall healthy approach to the health sector of Disaster management. WHO articulates its core commitment to health disaster risk management (DRM) at World Health Assembly Resolution 64.10 [16] and 65.20 [17].

Meanwhile, to strengthen DRR efforts within the Regional / Cross-border, Indonesia plays an active role through the *Association of Southeast Asian*

Nations (ASEAN) and is committed to managing DRR together. One example is through Indonesia's commitment as one of three early warning providers within the Indian Ocean Tsunami Warning System (IOTWS). Also, the AHA Center, based in Jakarta and fully facilitated by the Government of Indonesia. Furthermore, Indonesia also has laws and policies on disaster management that have incorporated many aspects emphasised by the ASEAN Agreement on Disaster Emergency Response (AADMER) and actively participates in the *ASEAN Committee on Disaster Management* (ACDM), *Indian Ocean Rim Association* (IORA) forums.

The previous study known 58% of countries assessed has established a disaster risk management coordination unit (DRM) in their Ministry of Health (MOH). Most have dedicated DRM Health Department staff (88%) and national level DRM committees (71%). Only Fourteen (58%) countries have health DRM sub-commissions that use multi-sector disaster risk reduction platforms. Less than 40% have conducted surveys such as disaster risk analysis, hospital safety index, and health mapping of resource availability. The main challenges in implementing the strategy are political will, and inadequate commitment generates poor funding for DRM health, weak health systems, and lack of scientific evidence on DRM mainstreaming and disaster risk reduction in long-term health system development programs [18].

The effectiveness of the Indian policy about the disaster management in achieving its intended outcome it's achieving effective mitigation and response to a disaster thereby minimising the casualties and losses to the community caused by the cyclone Phailin, India in 2013 [19]. Policies and legislation related to risk management are paramount towards defining the efficiency of the on-ground implementation of the Disaster Management Plan.

Strengthening disaster risk governance to manage disaster risk

National, regional and global disaster risk governance is essential for effective and efficient management of disaster risk. Clear vision, plans, competencies, guidance and coordination across sectors and the participation of relevant stakeholders are required. Strengthening disaster risk governance for prevention, mitigation, preparedness, response, recovery and rehabilitation is necessary to encourage collaborative mechanisms and partnerships across agencies and for the use of instruments relevant to disaster risk reduction and sustainable development [1].

To achieve this, it is important: To mainstream and integrate disaster risk reduction within and across all sectors and review and promote the coherence and further development, as appropriate, of national and local frameworks of laws, regulations and public policies, which, by defining roles and responsibilities, guide the public and private sectors in: (i) addressing disaster risk in publically owned, managed or regulated services and infrastructures; (ii) promoting and providing incentives, as relevant, for actions by persons, households, communities and businesses; (iii) enhancing relevant mechanisms and initiatives for disaster risk transparency, which may include financial incentives, public awareness-raising and training initiatives, reporting requirements and legal and administrative measures and (iv) putting in place coordination and organizational structures. To adopt and implement national and local disaster risk reduction strategies and plans, across different timescales, with targets, indicators and time frames, aimed at preventing the creation of risk, the reduction of existing risk and the strengthening of economic, social, health and environmental resilience; To carry out an assessment of the technical, financial and administrative disaster risk management capacity to deal with the identified risks at the local and national levels [1].

Governance challenges and the historical burden have resulted in overlapping policy processes, as both policies incorporate the other field, and creates a threat of parallel national-level structures, thereby increasing potential inefficiencies in governance and policy implementation. The importance of developing a horizontal integration implementation of the Disaster Risk Reduction (DRR) and Climate Change Adaptation (CCA) policies strategy before policy formulation processes to avoid the potential of inefficiencies became evident [14].

I am improving the governance system in disaster management through the application of principles of participation, justice and equity, professionalism, independence, efficiency in resource use and targeted/effective. Including LGs can manage risks: DRR policies, professional human resources, adequate budgets, multi-threat risk assessments and integrated planning, Secure culture has been built on individual residents living in disaster-prone areas, and Risk assessment through districts is comprehensive and updated regularly.

In Asia, local governments provide services to their communities and act as implementing agents for most DRR work. All levels of local authority in Asia have various capacities. In general, local authors play an important role in regional development planning, promoting comprehensive school safety, encouraging disaster-resistant towns and villages through community-based DRB at the local level, and promoting the development of community-based support networks [20].

Since 2007, the Indonesian government has developed a strong framework for strengthening disaster risk management in the country by issuing several laws, regulations, plans and policies. In the field of data collection, analysis, management and use

of disaggregated data on disaster areas, Indonesia has launched an online system called DIBI in 2008. The presence of DIBI provides an opportunity to identify trends, risks and vulnerabilities in the future come.

Through this process, local/municipal governance in DRR activities is strengthened, and stakeholder roles and responsibilities are identified, classified, and ultimately done. The most important set of actors are government and local institutions. Local governments are fully responsible for the safety of their citizens and communities. Help is far from enough to play beyond just a complementary role in overcoming risk management challenges [21].

The local level is very important, not only because it is more discerning to the citizens but because it is the basic environmental management warehouse and regulatory function that is essential for effective DRR [22]. Many commentators pointed out that the most important change in this framework is its emphasis on disaster risk management and no longer Disaster Management.

Other research results indicate that African countries have made steady progress applying disaster risk governance to theoretical indicators. This continent contains some international best practices that can be learned by other countries. Certain gaps and challenges, however, still hamper better progress in disaster risk reduction. There is a need for multi-layered ownership and an understanding of disaster risk and its cross-sectoral nature, with strong community involvement [23].

Investing in disaster risk reduction for resilience

Public and private investment in disaster prevention and disaster reduction through structural and non-structural measures that are essential to enhance economic, social, health and cultural resilience of individuals, communities, countries and their assets, as well as the environment. This is to encourage innovation, growth and job creation. These measures are through effective financing and contribute to the rescue, prevent and reduce losses and ensure effective recovery and rehabilitation. [1].

The majority of the economic losses that have occurred in Asian disasters have recently been borne out by the private sector when compared to the public sector. As Asia has emerged, as a global business centre with an extensive supply chain network, the impact of disasters is no longer limited within national boundaries. More than 70% of the capital investments are made by the private sector globally, and in Asian countries, it is important to secure these investments, which will adversely affect regional, national and local economies in the event of significant losses due to natural disasters. Future increases in the privatisation of basic services and critical infrastructure are often predictable, which places the responsibility on private sector groups to be actively involved in DRR in Asia [24].

Total investment for DRR activities has increased sharply from Rp 2.6 trillion in 2006 to nearly Rp 10 trillion in 2012, according to a UNDP study; it is believed that DRR investment in Indonesia is larger considering that some activities are attached to sectoral programs. During 2006 to 2011, two-thirds of the investment for DRR was allocated for mitigation and disaster prevention activities, followed by preparedness and research activities, education, and training.

The resulting study has found that the Act emphasises mainly on institution building and action plan development for mitigating disasters in the country. The Act does not directly mention disaster risk reduction, and there are no directions about the budgetary mechanisms and extent of funds from disaster risk management (DRM) in the country. The DRM in Pakistan is reactive, and there is a need for revision of PDMA 2010 to make it proactive [9].

In line with its commitment to SFDRR, Indonesia recognises the need to develop its commitment to DRR more thoroughly and sustainably, based on success, lessons learned from past targets, and challenges in the past. Projection is used in planning based on an analysis of the patterns that are formed in the present and the past as well as considering the uncertainty/scenario phenomenon that can occur. According to the [25], a good understanding of the decision-making context is crucial in determining the types, resolutions, and characteristics of information required for vulnerability assessment, adaptation, and the impact of natural disasters. [26] Emphasize that the choices that make up the planning process consist of three levels: a) the objectives and criteria available; b) identification of options that conform to the desired constraints and options and c) implementation guidance on the options taken.

One of the challenges in applying the concept of disaster risk reduction and climate change is how to determine the right formulation in translating a concept into an operational State Policy. The future environmental conditions will be much different from the present and "uncertainty" or the uncertainty of the scale of change that will occur to confirm that no one type of planning approach can answer the whole problem. The encourage to use flexibility of planning approaches that can be modified quickly if the situation suddenly changes. Integration of program/activity planning does not necessarily solve the problem [27]. The major environmental planning challenge lies in the scale of natural resource management and the scale of implementation of international activities and cooperation in promoting the adaptive capacity to achieve sustainable development objectives [28]. The capacity for each

stakeholder engages in relying on the quality of the local policy culture [29].

The results show that the low penetration of government instruments for disaster risk reduction in Mexico has led to high community dependence on post-disaster measures. [30]. The Sendai Strategy for Disaster Risk Reduction 2015-2030 leads us to improve understanding of disaster risks in the various dimensions of vulnerability characteristics, strengthening disaster risk management, and readiness to "rebuild for the better." The main objectives are strengthening disaster risk management to manage disaster risk and investing in disaster risk reduction for toughness [1].

The above policy references, both nationally and globally, provide a strong impetus to focus on disaster risk reduction. However, the implementation of disaster risk reduction programs in disadvantaged areas still faces several internal challenges: 1) lack of regulation and weakness of disaster risk reduction policies such as the lack of compilation of Local Action Plans (RAD), Spatial and Regional Plans (RTRW) based on disaster risk, and other supporting policies; 1) the weakness of the disaster risk reduction planning system; 2) weak institutional capacity and disaster risk reduction activists, 3) lack of access and information related to disaster risk reduction; 4) lack of capacity building activities such as technical guidance, socialization, workshops, training, simulations, and so on; 5) minimization of local government budget allocation in disaster risk reduction investment activities.

Enhancing disaster preparedness for effective response and to "build back better" in recovery, rehabilitation, and reconstruction

Stable disaster risk growth, including increased people and exposed assets, combined with past disaster learning, demonstrates the need to strengthen disaster preparedness and response further, taking action to anticipate events, integrate disaster risk reduction in preparedness and ensure response capacity and effective recovery at all levels. Empowering women and people with disabilities for public leadership and promoting gender equality and access to a generally accepted response, rehabilitation and reconstruction rehabilitation approaches are key. Disaster has shown that the recovery, rehabilitation and reconstruction phase need to be prepared ahead of the disaster, this is an important opportunity to rebuild better, including by integrating disaster risk reduction into development measures, making the nation and community resilient to disaster [1].

The results show that the implementation of HFA by the local government is one of the important areas for the international community to support and cooperate. Such recognition and efforts are also promoted through international initiatives such as the ISDR World Campaign for the PRC "Making Cities Resilient" (UNISDR 2010), which promotes local governments from around the world to take action in implementing DRR activities [31].

Governments and communities that can respond effectively to disaster and bounce back after the disaster and build a better life. Including Government and community members in areas with high self-reliance capacity, Government and community members in the regions can conduct post-disaster recovery and reconstruct better, and There are effective mechanisms of cooperation in disaster response ranging from community, regional, national to the regional level.

The research demonstrates that, despite recent progress in response management and disaster awareness, the lack of policies intended to institutionalise DRR and the neglect of integrating socio-cultural characteristics into DRR strategies have undermined the effectiveness of Lebanon's disaster response capacities. The author highlights the important role of religio-political organisations in influencing socio-cultural factors and contributing to DRR implementation [10].

Other research results: Numerous activities in community-based resilience and Disaster Risk Reduction (DRR) have been identified across the whole disaster continuum. Important gaps in research and practice remain. Discussion results: The Philippines is a leading regional actor in disaster risk management. However, a full picture of who is doing what, how, where, and when on resilience and disaster preparedness does not exist [12].

Conclusions

Based on a review of the literature on regional policies in resolving issues related to disaster risk reduction, it is important to improve local government capacity as well as to improve community resilience through measures taken by local governments through the four priority actions in the relevant Sendai Framework with disaster risk reduction policy, as follows: 1). Understanding disaster risk; 2). Strengthening disaster risk governance to manage disaster risk; 3) and investing in disaster risk reduction for resilience and 4). Enhancing disaster preparedness for effective response and to "Build Back Better" in recovery, rehabilitation and reconstruction.

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