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Correlations between Insulin Receptor Substrate-1 with Phosphoinositide 3-Kinase and P38 Mitogen-Activated Protein Kinase Levels after Treatment of Diabetic Rats with Puguntano (*Curanga Fel-Terrae* [Merr.]) Leaf Extract

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Keywords: Puguntano; IRS-1; PI3K; p38 MAPK; T2DM

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Abstract

BACKGROUND: Defects in post-receptor insulin signalling are the major cause of insulin resistance in type 2 diabetes mellitus (T2DM).

AIM: This study aimed to investigate the correlations between insulin receptor substrate (IRS)-1 with phosphoinositide 3-kinase (PI3K) and p38 mitogen-activated protein kinase (MAPK) levels after puguntano (*Curanga fel-terrae* [Merr.]) leaf extract treatment in a rat model of T2DM.

METHODS: A combination of high-fat diet-feeding (HFD) and multiple low dose intraperitoneal injections of streptozotocin was used to induced T2DM in 48 Wistar rats, which were then randomly divided into control and treatment groups (n = 24 per group). Puguntano leaf extract was administered to the treatment group once daily (200 mg/kg.bw) for 10 days. IRS-1, PI3K and p38 MAPK levels were measured in skeletal muscle using sandwich ELISAs in control group after becoming T2DM and in the treatment group after 10 days of puguntano treatment. Data were analysed using the Wilcoxon test and Spearman's correlation.

RESULTS: IRS-1, PI3K and p38 MAPK levels were significantly higher in the treatment group than in the control group. There were also significant positive correlations between IRS-1 with PI3K and p38 MAPK levels ($r = 0.375$, $p = 0.035$; $r = 0.552$, $p = 0.003$; respectively) after the treatment.

CONCLUSION: This study demonstrated significant positive correlations between IRS-1 with PI3K and p38 MAPK levels after puguntano leaf extract treatment of T2DM rats.

Introduction

Insulin resistance is a fundamental pathophysiologic defect in type 2 diabetes (T2DM), and this leads to reductions in glucose uptake and utilisation in skeletal muscle, the tissue that is responsible for the majority of the postprandial glucose disposal [1]. Defects in insulin signal transduction are generally regarded as the underlying cause of this insulin resistance [2].

Insulin exerts its intracellular effects via signal transduction pathways that are activated following binding to insulin receptors on the plasma membrane [2], [3]. The insulin receptor consists of two extracellular α subunits and two β subunits that possess tyrosine kinase activity, which is activated upon insulin binding. Post-receptor signalling involves the phosphorylation of insulin receptor substrate (IRS)-1 and IRS-2, and of the β subunit itself (autophosphorylation) by the receptor tyrosine kinase [4]. Phosphorylation of IRS-1 leads to the activation of the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) and/or the Ras/Raf/mitogen-activated protein kinase (MAPK) signalling pathway. The PI3K/Akt

signalling pathway is the primary pathway stimulating glucose uptake, which occurs via glucose transporter-4 (GLUT-4) [1], [2], [3]. Activation of Akt initiates the translocation of GLUT-4 from its intracellular storage site to the plasma membrane, where it acts as a facilitative glucose transporter [5].

Multiple post-receptor intracellular defects have been identified in insulin-resistant skeletal muscle, including in the IRS/PI3K pathway, which therefore represents a promising therapeutic target for T2DM [6], [7]. Some data also show that p38 MAPK is necessary for insulin-stimulated glucose uptake through GLUT-4, but the role of p38 MAPK in the regulation of glucose transport in skeletal muscle is controversial [8]. However, another previous report has suggested that therapeutic targeting of p38 MAPK activity remain potential approaches for the treatment of T2DM [9].

The currently available therapeutic options for T2DM have some limitations, and many natural products and herbal medicines have been recommended for the treatment of this disease [10]. Puguntano (*Curanga fel-terrae* [Merr.]) The leaf has long been used as traditional medicine by the inhabitants of Tiga Lingga Village, Dairi, North Sumatera Province of Indonesia, for the treatment of diabetes [11]. Its secondary metabolites are thought to mediate its beneficial effects because tannins increase muscle glucose uptake by enhancing PI3K, activating p38 MAPK, and increasing GLUT-4 translocation [12]; flavonoids increase GLUT-4 translocation by activating the PI3K/Akt pathways [13]; triterpenoids increase the activation of IRS-1 [14]; and saponins increase GLUT-4 expression via the PI3K/Akt pathway [15]. A previous study has shown that puguntano improves glucose metabolism and ameliorates insulin resistance, alongside an increase in expression of adiponectin receptor (AdiporR) in diabetic rats [16]. Furthermore, another previous study has demonstrated that quercetin which contains a flavonoid compound, activates both the PI3K/Akt and MAPK pathways in skeletal muscle [17].

This study aimed to determine the correlations between insulin receptor substrate (IRS)-1 with phosphoinositide 3-kinase (PI3K) and p38 mitogen-activated protein kinase (MAPK) levels after puguntano *(Curanga fel-terrae* [Merr.]*)* leaf extract treatment in a rat model of T2DM.

Material and Methods

Forty-eight specific-pathogen-free 8-week-old male Wistar rats weighing 180-200 g were used in the present study. The rats were housed under a natural light cycle at 22-25°C. Diabetes was induced by feeding a high-fat diet (HFD) for 5 weeks, followed by two intraperitoneal injections of streptozotocin (30 mg/kg; Sigma-Aldrich, Munich, Germany). After this, fasting plasma glucose was measured in blood obtained from a lateral tail vein using a glucometer, and rats with a fasting plasma glucose (FPG) of 200 md/dL were deemed to have diabetes [18]. The study was approved by the Ethics Committee of Universitas Sumatera Utara, Medan, Indonesia (Reference 42/TGL/KPEK FK USU-RSUP HAM/2018).

After verifying the presence of diabetes in the rats, they were randomly divided into a control group and a treatment group (n = 24 per group), which was treated with 200 mg/kg/day ethanolic extract of puguntano leaves using an orogastric cannula for 10 days. Control rats were sacrificed on the day their diabetes was confirmed, while the puguntano-treated rats were sacrificed after 10 days treatment period was complete.

After anaesthesia with ketamine, the rats were decapitated, and blood was obtained from the left ventricle for the measurement of FPG by spectrophotometry and fasting insulin using a sandwich ELISA. Gastrocnemius muscles were dissected for the subsequent measurement of IRS-1, PI3K, and p38 MAPK levels. Insulin resistance was assessed using the homeostasis model assessment–insulin resistance (HOMA-IR) equation, which is calculated using fasting insulin and glucose concentrations [19]. The study was conducted in the Molecular Genetics Laboratory, Medical Faculty of Universitas Padjajaran. The ethanolic extract of puguntano leaves was obtained by maceration methods in the Department of Biological Pharmacy, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia [20].

Skeletal muscle samples were homogenized in ice-cold homogenization buffer (100 mM Tris, pH 7.4; 150 mM NaCl; 1 mM EGTA; 1 mM EDTA; 1% Triton X-100; 0.5% Sodium deoxycholate) supplemented with phosphatase and protease inhibitor cocktails, and 1 mM polymethyl sulfonyl fluoride, immediately before use. The homogenates were then frozen at -80°C and subsequently used to determine IRS-1, PI3K, and p38 MAPK levels using kits supplied by Qayeebio (China).

Statistical Analysis

Statistical analysis was performed using SPSS 22.0 software. All data are expressed as the mean ± standard deviation, and the Wilcoxon test was used to compare the groups. The relationships between IRS-1 with PI3K and p38 MAPK levels were analysed using Spearman's correlation. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Body weight and FPG levels in the treatment group was significantly lower than in the control group as shown in Table 1.

Table 1: Body weight and FPG levels in the control and treatment groups

Variable	Groups		p
	Control (n = 24)	Treatment (n = 24)	
Body weight (g)	386 ± 20	245 ± 35	0.001
FPG (mg/dl)	355 ± 105	136 ± 33	0.001

Data are expressed as mean ± standard deviation. FPG: fasting plasma glucose. Wilcoxon test. P < 0.05 is statistically significant.

IRS-1, PI3K and p38 MAPK levels were significantly higher in the treatment group than in the control group as shown in Table 2.

Table 2: IRS-1, PI3K and p38 MAPK levels in control and treatment groups

Variable	Groups		p
	Control (n = 24)	Treatment (n = 24)	
IRS-1 (ng/mL)	0.28 ± 0.11	0.52 ± 0.21	0.001
PI3 Kinase (ng/mL)	14.22 ± 2.03	18.23 ± 5.20	0.0015
p38 MAPK (ng/mL)	20.81 ± 3.02	23.70 ± 4.04	0.0025

Data are expressed as mean ± standard deviation; IRS-1: insulin receptor substrate-1; PI3K: phosphoinositide 3-kinase; p38 MAPK: p38 mitogen-activated protein kinase; Wilcoxon test; P < 0.05 is statistically significant.

There was a significant positive correlation between IRS-1 with PI3K and p38 MAPK levels in skeletal muscle of rats after treatment with puguntano leaf extract as shown in Table 3.

Table 3. Correlations between IRS-1 with PI3K and p38 MAPK levels in skeletal muscle after treatment with puguntano leaf extract

Variable	r	p
PI3 Kinase (ng/mL)	0.375	0.035
p38 MAPK (ng/mL)	0.552	0.003

Data were analysed using Spearman's correlation; PI3K: phosphoinositide 3-kinase; p38 MAPK: p38 mitogen-activated protein kinase; P < 0.05 is statistically significant.

Discussion

The incidence of T2DM is increasing worldwide, with more rapid increases occurring in developing countries. Therefore, it is of great importance to study the pathogenesis of T2DM and search for effective and economical treatments [2]. Skeletal muscle is a key site of peripheral insulin resistance in T2DM [6], [21], and this tissue, therefore, represents an important target for potential anti-diabetic substances [22].

This present study has demonstrated significantly lower body weight and FPG levels in the skeletal muscle of T2DM rats that were administered with puguntano leaf extract. These findings are consistent with lower levels of obesity and hyperglycemia and with the results of a previous study demonstrating that treatment with puguntano extract tends to reduce body weight and significantly reduces FPG in patients with newly diagnosed T2DM [23].

Two signal transduction pathways mediate insulin-stimulated glucose transport in skeletal muscle. The binding of insulin to its receptor causes tyrosine phosphorylation of IRS-1 and IRS-2 [22], [24], which activates the PI3K/Akt signaling pathway that include IRS, PI3K, Akt, AS160 and GLUT-4, and MAPK signaling pathway which is necessary for insulin-stimulated glucose uptake through GLUT-4 [8], [22].

In this study, we have shown significantly higher IRS-1, PI3K and p38 MAPK levels in puguntano extract-treated rats than in controls, which may have been caused by one or more secondary metabolites of the tannins, flavonoids, triterpenoids, and saponins that are present in the leaf extract. This finding was consistent with those of some previous studies that evaluated the efficacy of another similar plant products in muscle or muscle cell lines. Rajendran et al. demonstrated that quercetin, which contains a flavonoid compound, increases IRS-1, IRS-2, PI3K, Akt, p38 MAPK, adenosine monophosphate-activated protein kinase (AMPK) and GLUT-4 expression in L6 myotubes [17]. The administration of a mulberry *(Folium Mori)* leaf extract containing flavonoids and polyphenols to T2DM rats caused significant increases in IRS-1, PI3K p85a and GLUT-4 expression through activation of the IRS-1/PI3K signalling pathway in skeletal muscles [25]. Furthermore, administration of *Momordica charantia* extract, which contains triterpenoids, increased glucose uptake in C2C12 myotubes by increasing the activation of IRS-1 and downstream signaling pathways, resulting in GLUT-4 translocation [14]. Previous research has also shown that a ginsenosides extract from *Panax ginseng,* containing a triterpenoid saponin compound, increases the expression of the insulin receptor, IRS-1, PI3Kp85, phosphorylated Akt and GLUT-4 in the skeletal muscle of diabetic rats [26]. Cinnamon (*Cinnamomum cassia*) extract, which contains tannins, also caused increases in IRS-1, PKB, PI3K and protein kinase C (PKC) gene expression in the skeletal muscle of diabetic Wistar rats [27]. Finally, a study of the effects of guava (*Folium Psidii Guajavae Psidiumguajava L*.) leaf extract in diabetic rats demonstated increases in expression of IRS-1, Akt, and PI3K p85, which was suggested to be mediated through the tannins, flavonoids, pentacyclic triterpenoids, and/or other chemical compounds it contains [6]. Together, these findings demonstrate that secondary metabolites in a variety of plant extracts can influence the expression of key insulin signaling intermediates, potentially ameliorating defects in insulin sensitivity, and increase glucose uptake into skeletal muscle cells.

The potential role of p38 MAPK in the regulation of glucose transport in skeletal muscle has been controversial, even though a previous study has

shown that p38α and p38β MAPK activity is required for insulin-stimulated glucose uptake [8]. Jiang et al. demonstrated that inhibition of p38α and p38β MAPK reduces insulin-stimulated glucose uptake in L6 myotubes, but GLUT-4 translocation is not affected, leading them to hypothesise that a p38 MAPK-dependent signalling pathway may regulate GLUT-4 activation [26]. Consistent with this finding, other studies showed that inhibitors of p38α and p38β MAPK do not affect GLUT-4 translocation, suggesting that p38 MAPK may increase the intrinsic activity of GLUT-4 in response to insulin stimulation [28,29]. Also, a study by Lawan et al. demonstrated that inhibition of p38 MAPK/c-jun n-terminal kinase (JNK) module signalling in skeletal muscle promotes insulin resistance and metabolic dysfunction [30].

The present study is the first to demonstrate that after puguntano leaf extract treatment there are significant positive correlations between IRS-1 with PI3K and p38 MAPK levels in the skeletal muscle of T2DM rats. We have shown that puguntano leaf extract increases the muscle expression of PI3K/Akt and MAPK pathway intermediates and ameliorates hyperglycemia. These effects of puguntano are similar to those reported by Rajendran et al., who demonstrated that the effect of quercetin is not predominantly through the PI3K signalling pathway, but instead through AMPK and its downstream target p38 MAPK in L6 myotubes.

This was the first study to show an antidiabetic effect of quercetin mediated through activation of multiple therapeutic targets for T2DM (in both the PI3K/Akt and MAPK pathways) and manifesting in an increase in glucose uptake, achieved through greater GLUT-4 expression and translocation [17].

In conclusion, puguntano leaf extract treatment caused an increase in the expression of several post-receptor insulin signalling intermediates in the skeletal muscle of T2DM rats, and there was a significant positive correlation between IRS-1 with PI3K and p38 MAPK levels. These changes are likely to be accompanied by an amelioration of insulin resistance in this tissue, but further studies are required to fully elucidate the molecular mechanisms associated with the anti-diabetic effects of puguntano leaf extract.

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The Influence of Mesenchymal Stem Cell Wharton Jelly toward *Prostaglandin E2* Gene Expression on Synoviocyte Cell Osteoarthritis

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Abstract

BACKGROUND: Pharmacological therapy in the management of OA causes many new health problems due to side effects caused by long-term use of drugs, such as long-term use of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) will cause gastric ulcers and impaired kidney function. In OA pathogenesis, *PGE2* gene is involved in the inflammation process.

AIM: This study aims to identify the influence of Wharton Jelly Mesenchymal Stem Cell (MSC-WJ) on *PGE2* expression gene in synoviocyte by in vitro.

MATERIAL AND METHODS: The method used in this study is the co-culture method of primary cells and stem cells in the appropriate media. This research is pure experimental research. The sample used came from synovial tissue of osteoarthritis patients who underwent Total Knee Replacement (TKR) surgery. This study was divided into 6 groups treated with 4 replications. The expression analysis of the Prostaglandin E2 gene was done using qPCR (Real-Time Polymerase Chain Reaction). The expression analysis of the *Prostaglandin E2* gene was carried out before and after the co-culture with Wharton's Jelly and continued with the analysis of statistical data processing using the SPSS.15 program. *PGE2* gene expression data were processed using the Kruskal-Wallis test and continued with the Mann-Whitney test with a 95% confidence level.

RESULTS: The results showed that Mesenchymal Stem Cells Wharton Jelly could reduce the expression of *Prostaglandin E2* gene after co-culture for 24 hours and 48 hours in synoviocyte cells osteoarthritis significantly compared with the control group. The administration of Mesenchymal Stem Cells for 24 hours reduced the expression level of *PGE2* gene by 0.61 times compared to the control group ($p < 0.05$) and the administration of Mesenchymal Stem Cells for 48 hours decreased the expression level of *PGE2* gene by 0, 47 times compared to the control group ($p < 0.05$).

CONCLUSION: This study concluded that MSC-WJ in OA synoviocyte significantly reduced the expression of the *PGE2* gene ($p < 0.05$).

Introduction

According to the American College of Rheumatology, osteoarthritis is a heterogeneous condition in the joints characterized by the process of degradation, repair, and inflammation that occurs in the connective tissue, the vulnerable layer of joints, synovium, and subchondral bone. According to the World Health Organization (WHO) in 2004, the prevalence of osteoarthritis sufferers in the world reached 151.4 million and around 27.4 million people in the Southeast Asia region [1].

At the molecular level, an imbalance between catabolic and anabolic activity in which the major injury response occurs in joint cartilage results in osteoarthritis. When the pro-inflammatory response occurs in the cartilage, some types of prostanoid enzymes such as cyclooxygenase (COX) will be produced and released in excessive amounts. Cyclooxygenase activation will increase the production of MMP, inhibit the expression of Prostaglandin E2 (*PGE2*) and collagen genes and will stimulate the apoptosis process.

Studies conducted by Hardi *et al.,* (2002) and Shimpo *et al.,* (2009) have analyzed the role of *PGE2* in chondrocytes. Pro-inflammatory cytokines IL-1β stimulate and produce *PGE2* in large quantities, and this will induce the degradation process of osteoarthritis [2], [3]. Molecularly, IL-1β will increase the expression of the cyclooxygenase 2 (*COX-2*) gene and prostaglandin E synthase-1 (*mPGE-1*) microsomal at mRNA and protein levels. So an increase in *PGE2* production is related to mPGES-1 and COX-2 derivatives from osteoarthritis chondrocytes stimulated by IL-1β. A better understanding of the pathogenesis of OA has recently been obtained, among others, thanks to increased knowledge about joint-prone biochemistry and molecular biology, which is expected to be able to manage OA patients with a variety of more appropriate and safer therapies. Therapy that can cure osteoarthritis with satisfactory results has not been found to date. There are limitations in terms of joint cartilage availability, difficulties in isolation, expansion of chondrocytes and differentiation of chondrocytes isolated in culture making cell-based OA therapy change to the use of Mesenchymal Stem Cells (MSC) which can be a potential source of cells for cartilage repair [4]. Mesenchymal Stem Cells (MSC) have been considered as alternative promising cell sources for cartilage repair. Stem cells or stem cells are stem cells that can form new cells. Wharton's Jelly is one type of Mesenchymal Stem Cells that can differentiate into chondrocytes which are the main joint-prone cells that are very necessary for the treatment of osteoarthritis, where cartilage tissue is damaged or worn.

Based on the description above, the use of stem cells derived from Wharton Jelly Mesenchymal Stem Cells can be used as an alternative for the treatment of osteoarthritis; the authors are interested in conducting in vitro studies of mesenchymal stem cells against cells that are isolated directly from the synovial tissue and fluid of OA patients. In synovial tissue, it contains pro and anti-inflammatory factors, so co-culture of OA cells with mesenchymal stem cells will reduce proinflammatory factors and increase anti-inflammatory factors which in turn will indicate improvements made by cells. Stem cells to osteoarthritis cells.

Material and Method

This study was a pure experimental study which was divided into 6 treatment groups with 4 number of replications. Group I was an Osteoarthritis synoviocyte cell (OA) control group which was incubated for 24 hours, group II was an OA synoviocyte cell control group which was incubated for 48 hours, group III was a Mesenchymal Stem Cell Wharton Jelly (MSC-WJ) group incubated for 24 hour, group IV was incubated for 48 hours in Mesenchymal Stem Cell Wharton Jelly (MSC-WJ) group, group V was synoviocyte-MSC-WJ cell co-culture group which was incubated for 24 hours and group VI was cell co-culture group synoviocytes-MSC-WJ incubated for 48 hours. The number of cells used in each treatment group was 10^5 cells, each for synoviocyte and MSC-WJ cells. Mesenchymal Stem Cell Wharton Jelly comes from IMERI (Indonesian Medical Education and Research Institute) Faculty of Medicine, University of Indonesia. Synoviocyte cells are derived from synovial tissue of grade IV Osteoarthritis undergoing Total Knee Replacement surgery at Dr. M. Djamil Padang, Indonesia. The synoviocyte cells taken for treatment are the results of the 3rd phase cell culture.

Isolation of OA primary cells

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

Coculture of stem cells with OA primary cells

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

Table 1: Primer Design

No. Primer Nucleotide Sequence	NM NCBI Accession Number Gene	Amplicon Size
1. PGE-2 F 5'-TCAAGATGTACGTGGTGGCC-3'	NM_004878.4	203 bp
2. PGE-2 R 5'-CAGAAAGGAGTAGACGAAGCC-3'	NM_004878.4	203 bp
3. HPRT1 5'-CCTGGCGTCGTGATTAGTGAT-3'	NM_000194.2	158 bp
4. HPRT1 5'-CCCATCTCCTTCATCACATCTC-3'.	NM_000194.2	158 bp

RNA extraction and cDNA synthesis

RNA was extracted from the isolates of synovial tissue grade IV from OA patients with TRIzol® (Invitrogen, USA) according to manufacture's protocol. The quantity of RNA was calculated by NanoDrop. Synthesis of cDNA was performed by using iScript cDNA Syntesis Kit (BioRad, USA) on thermal cycler C1000 (BioRad, USA) Reverse Transcriptase PCR (RT-PCR) devices. The reaction of cDNA synthesis was 5 µg total RNA, 1 x RT buffer, 20

pmol oligodT, 4 mM dNTP, 10 mM DTT, 40 U TMII RTase and H2O-DEPC SuperScript enzymes with a total volume of 20 µl. The cDNA synthesis was performed at 52°C for 50 min according to the manual kit protocol (Biorad, USA).

PCR Gradient Amplification

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

Measurement of gene concentration

The measurement of gene concentration in this study was the relative quantification method [5].

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

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

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

The comparison of gene expression levels = $2^{\Delta\Delta CT}$. The measurement of gene concentration was by using LightCycler® software program referred to Livak formula (concentration in picogram size).

Statistical analysis

Data will be presented in the form of tables and graphs, as well as the results of the expression of the *PGE2* gene. In the *PGE2* gene expression level, data normality test was performed using the Shapiro Wilk Test and data homogeneity with the Levene Test. Criteria for testing decisions on the Shapiro Wilk Test that is if the p-value > 0.05 then said the data is normally distributed, while the decision criteria in the Levene Test is if the value of p > 0.05, then the data is said to be homogeneous. For the *PGE2* gen expression, the data is not normally distributed and homogeneous, so the non-parametric Kruskal Wallis test is carried out and continued with the Mann Whitney Test [6]. Data is processed using SPSS 15 statistical analysis.

Research Ethics

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

Results

Sample Characteristics

The result of isolation of synoviocyte cells obtained from synovial tissue is a fibroblast-shaped cell grown in a culture medium on a plate. Synovial cell morphology is presented in Figure 1. In each passage, a uniform cell morphology with cell shape is like fibroblast cells, has a nucleus located in the middle and attaches to the base of the plate containing the medium.

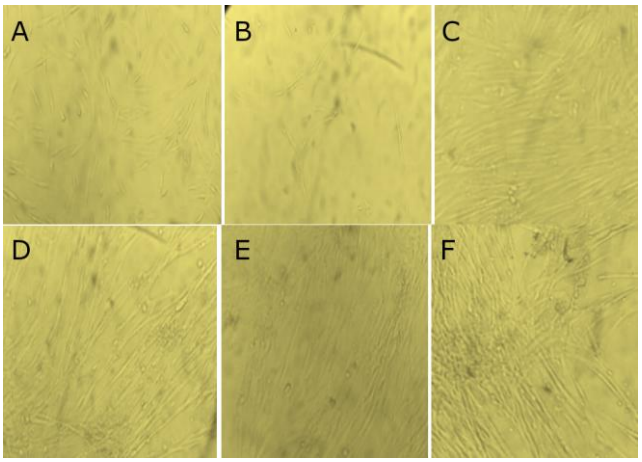

Figure 1. Morphology cells; A) 24-hour synoviocyte cell; B) 48-hour synoviocyte cell; C) MSC-WJ 24 hours; D) MSC-WJ 48 hours; E) synoviocyte co-culture MSC-WJ 24 hours; F) synoviocyte co-culture MSC-WJ 48 hours

Real-time PCR Optimization of PGE2 gene

From the results of the optimization on Real-time PCR obtained graphical form as shown in Figure 2.

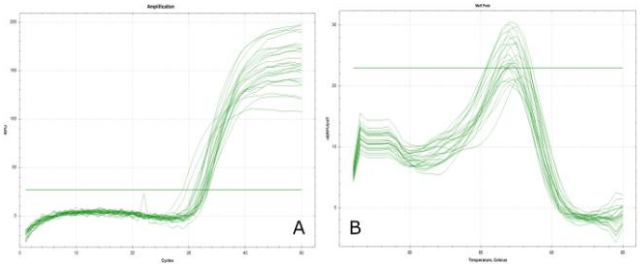

Figure 2. Graph of QPCR primary optimization results of PGE2 gene; A) Graph of amplification curve results on qPCR; B) Homogeneous melting peak graphs from the results of qPCR

From Figure 2 it can be seen that on the amplification curve on qPCR 50 cycles, the *PGE2* target gene is amplified with both Figure 2 (A) and from the melting peak graph Figure 2 (B) shows a 1 peak curve that clearly shows that from the optimization results *PGE2* gene primer obtained sharp and homogeneous peaks. This proves that the primers used are specific primers. To confirm the results of this optimization, the electrophoresis

method was done on 0.5% agarose gel loaded with 1 x TBE. After optimization using real-time PCR, then electrophoresis is done to see the optimized primary base pass as shown in Figure 3.

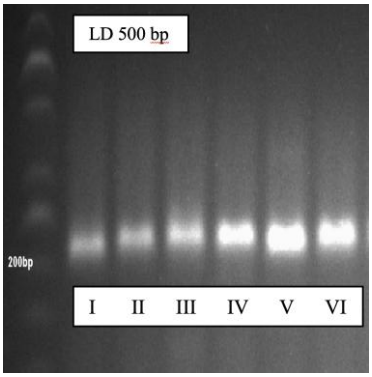

Figure 3. The results of electrophoresis of the PGE2 gene

From these results it was found that the *PGE2* primer was specific; this was marked by a single ribbon print during electrophoresis. To ensure qPCR is carried out and from data sequencing, it is found that the *PGE2* primer is specific at 203 bp.

Expression of PGE2 Gene

From the results of the research obtained, before the analysis is carried out, a preliminary test is carried out for the basic assumptions of normality and homogeneity of the data (Appendix 3). The results of the normality test with the Shapiro Wilk Test obtained a significant value of > 0.05 in all treatment groups, meaning that the data was normally distributed. To test variant homogeneity based on the Levene Test is 0.03 < 0.05, this means that the *PGE2* gene research data has not a homogeneous variant. Because of the results of the preliminary test, the data is normally distributed and not homogeneous, so the data is processed by the Kruskal Wallis test and continued with the Mann Whitney Test ($p < 0.05$)

From Figure 4 in the box-plot diagram, it can be seen that homogeneously distributed data is seen from the median value in the diagram that is located in the middle, but from the results of the Levene, Test results are not homogeneous ($p \leq 0.05$).

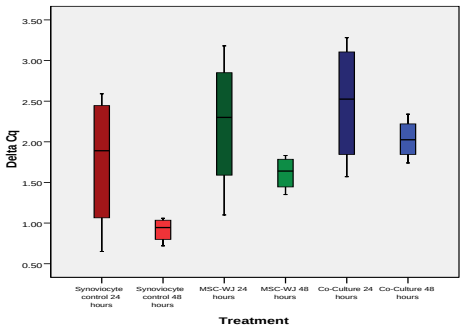

Figure 4. PGE2 expression in various treatment groups (box-plot diagrams with median values)

Figure 5. Histogram level of PGE2 gene expression in various treatment groups

If seen from Table 3 and Figure 5 it appears that the *PGE2* gene expression from the lowest to the highest obtained results in a row is in group VI, followed by groups V, IV and III, while for group I and II the expression level is the highest which is used as a standard *PGE2* expression.

Table 2: Effect of Mesenchymal Stem Cell on *PGE2* gene expression levels in osteoarthritis synoviocyte cells

Groups	Gene expression	
	average	Assymp Sig
24 hours-synoviocyte control	1,00 ± 0,00	0,00
48 hours-synoviocyte control	1,00 ± 0,00	
24 hours-MSC-WJ	0,73 ± 0,01	
48 hours-MSC-WJ	0,62 ± 0,01	
24 hours *Co-Culture*	0,61 ± 0,02	
48 hours *Co-Culture*	0,47 ± 0,01	

From Table 3 it can be seen that there are significant differences in *PGE2* gene expression between group I with groups II, III, IV, V and VI ($p < 0.05$), meaning that there are significant differences in *PGE2* gene expression between synoviocyte control groups 24 hours with 48-hour synoviocyte control group 24 hours and 48 hours MSC-WJ control group 24 hours and 48 hours co-culture group. The expression of *PGE2* gene between group II and group III, IV, V and VI found a significant difference ($p < 0.05$), where from the calculation of the Livak-Schmittgen Formula (2011) it was found that gene expression in group II was higher than groups III, IV, V and VI as shown in Table 3. *PGE2* gene expression between group III and the group I, II, IV, V, and VI found significant differences ($p < 0.005$), where the value of gene expression in group III was greater than in groups IV, V and VI.

Table 3: Analysis of the influence of Wharton Jelly Mesenchymal Stem Cell on *PGE2* gene expression

Groups	*PGE2* gene expression (ng/µl)					
	I	II	III	IV	V	VI
I	-	1.00	0.01	0.01	0.01	0.01
II	1.00	-	0.01	0.01	0.01	0.01
III	0.01	0.01	-	0.02	0.02	0.02
IV	0.01	0.01	0.02	-	1.00	0.04
V	0.01	0.01	0.02	1.00	-	0.02
VI	0.01	0.01	0.02	0.04	0.02	-

*) significantly difference ($p < 0,05$); I = 24-hour synoviocyte control group; II = 48-hour synoviocyte control group; III = 24 hours Mesenchymal Stem Cell Wharton Jelly (MSC-WJ); IV = 48 hours of Mesenchymal Stem Cell Wharton Jelly (MSC-WJ); V = Synoviocyte Co-Culture Group-24 hours MSC-WJ; VI = 48-hour synoviocyte co-culture group-MSC-WJ.

The expression of the *PGE2* gene between group IV and group V had no significant difference ($p > 0.05$), although the expression level in group IV was higher compared to group V (Table 3). For group IV, when compared with groups I, II, III and VI from the Mann Whitney test results there was a significant difference ($p < 0.05$) with a higher expression level in group IV compared to group V and VI. The expression level between groups V and VI found a significant difference ($p < 0.05$) with group V expression values higher than group VI.

Discussion

Sample Characteristics

Synoviocyte cells are fibroblast-shaped cells grown in a culture medium on a plate. In each passage in various treatment groups, uniform cell morphology with cell shape such as fibrous cells is obtained, having a nucleus located in the middle and attached to the base of the plate containing the medium. According to Shikichi *et al.,* (2000), the synovial membrane consists of a superficial layer containing two to three layers of cells below it called synovial intima. In the part of the synovial membrane cavity, there are thick intima cells and subintima which function as a link between tissues and are rich in blood vessels [7]. The main function of the synoviocytes that make up the synovium membrane is to provide various molecules of lubricants such as glycosaminoglycans, oxygen, and plasma proteins, nutrients for joint spaces and susceptibility to joints and chondrocytes [8]. Synovial cell morphology before and after treatment had similar characteristics, both in the synoviocyte control group, in the control group, and after synoviosit co-culture and Mesenchymal Stem Cell Wharton Jelly (MSC-WJ) treatment.

Synoviocyte cells morphologically, including fibroblast cells (fibroblast-like synoviocyte) are bipolar or multipolar cells, having a long and growing cell shape attached to the substrate. Fibroblasts Like Synoviocyte can be cultured from synovial tissue of TKR (Total Knee Replacement), synovectomy or synovial biopsy. After being digested with the collagenase enzyme, the cells attached to the flash are composed of synovial fibroblasts and synovial macrophages. Fibroblast Like Synoviocyte proliferation can be repeated up to the third passage, which in this condition contains 95% homogeneous cells. The use of fibroblast-like synoviocyte after the 9th post is not recommended anymore because there has been a decline in cell quality [9].

Mesenchymal Stem Cells (MSCs) are adult stem cells that have morphology such as fibroblasts (fibroblast-like) and the ability to differentiate MSCs into several types of connective tissue cells that make these cells as candidates for cell sources in the treatment of tissue regeneration [10]. From the results of the study, morphological images of culture synoviocytes were very similar to Mesenchymal Stem Cell Wharton Jelly, because these two cells are fibroblast-like cells. Another great advantage of this cell culture is cell homogeneity which appears in the morphological picture of the two types of cells cultured. According to Listyorini (2001), a measure of success that can be used in making this cell culture is the absence of contamination in culture and cell success in multiplying. The advantage of cell culture is that it is easily homogeneous, easy to characterize, easily controlled at certain temperatures, osmotic pressure and pH [11].

PGE2 gene expression

Based on the research that has been done, it can be seen that the lowest regulated *PGE2* gene in group VI which is the treatment group of synoviocyte cell and MSC-WJ co-culture for 48 hours is by the initial hypothesis that Wharton Jelly Mesenchymal Stem Cell can reduce expression from the *PGE2* gene. The expression of the *PGE2* gene in the 48-hour synoviocyte cell and MSC-WJ co-culture treatment group decreased by 0.47 times *PGE2* was relatively lower compared to the control group, whereas in group V which was the treatment group of synoviocyte cell co-culture and MSC-WJ for 24 hours decreased by 0.61 times *PGE2* was relatively lower compared to the control group.

There are several related studies that can explain the results of this study. At the time of the inflammatory process, the *PGE2* gene which is a gene that plays a role in the inflammatory response to the pathogenesis of osteoarthritis. Activation of the *PGE2* gene involves the role of NFKβ as a cytokine regulating the occurrence of an inflammatory response, activation of NFKβ will trigger the expression of genes that induce articular joint damage resulting in osteoarthritis. This is in line with the results of this study that the high ΔCq value of the *PGE2* gene in the osteoarthritis synoviocyte control group will decrease the expression of the *PGE2* gene [12]. The results of the 24-hour MSC-WJ group also showed high *PGE2* expression levels; this is in line with recent studies that show that prostaglandin E2 enzymes play a role in the process of self-renewal of MSC-WJ [13]. During the differentiation process from MSC, there will be an increase in *PGE2* gene activity and expression. In a study conducted by Lu *et al.,* (2017) it was reported that there was an increase in *PGE2* gene expression during the differentiation process of human Mesenchymal Stem Cells. From the results of incubation for 24 hours in vitro showed significant migration from MSC compared to the control group. This shows that *PGE2* is one of the cytokines needed by MSC in the differentiation process [14].

The Human Mesenchymal Stem Cell is a promising and potential agent in cell therapy. MSC is a progenitor cell that can differentiate into osteoblasts and contribute to modulate the immune response in bone regeneration therapy [15], [16], [17], [18]. The results of *PGE2* gene expression in the synoviocyte co-culture group and 48 hours of MSC-WJ were significantly lower compared to 24-hour synoviocyte and MSC-WJ co-culture groups. This can be caused by the effect of the immunomodulatory effect of MSC-WJ which has begun to work on osteoarthritis synoviocyte cells which begin to control *PGE2* gene expression. This is consistent with the results of a study which states that bone marrow-derived Mesenchymal Stem Cells can modulate the effects of proinflammatory cytokines on human corneal epithelial cells [19]. According to research Ryan *et al*., (2014) Mesenchymal Stem Cell immunomodulation effects can reduce the expression level of prostaglandin E2 gene after co-culture in vitro at a dose of 2 x 10^6 cell/well [20]. The results of this study reinforce previous research conducted by Bull *et al.,* (2012) which stated that the Mesenchymal Stem Cells effect on cartilage cell explants taken from grade IV knee osteoarthritis patients could reduce the expression level of prostaglandin E2 gene by 50% relative to the control group. after incubation for 48 hours [21]. This study can explain the influence of Mesenchymal Stem Cells Wharton Jelly on osteoarthritis synoviocyte cells through *PGE2* gene expression parameters which play an important role in the inflammatory process in the pathogenesis of osteoarthritis. In general, MSC-WJ can reduce *PGE2* gene expression which is a proinflammatory cytokine in osteoarthritis. The results of this study can also be useful as a reference for the use of stem cells, especially MSC-WJ as a promising osteoarthritis therapy in the future.

The results showed that there was no effect of Mesenchymal Stem Cells on synoviocyte cell morphology in all treatment groups and Mesenchymal Stem Cells Wharton Jelly can reduce the expression of prostaglandin E2 gene after co-culture for 24 hours and 48 hours in synoviocyte cells osteoarthritis significantly compared with the control group. The administration of Mesenchymal Stem Cells for 24 hours reduced the expression level of *PGE2* gene by 0.61 times compared to the control group ($p < 0.05$) and the administration of Mesenchymal Stem Cells for 48 hours decreased the expression level of *PGE2* gene by 0.47 times compared to control group ($p < 0.05$).

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Basic Science

Immunohistochemical Expression of Androgen Receptors (AR) in Various Breast Cancer Subtypes

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Keywords: Androgen receptors; Breast cancer; Hormonal status; Immunohistochemistry; Luminal; Triple-negative

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Abstract

BACKGROUND: Breast carcinoma ranks the first among malignant tumours in females and is the chief cause of cancer-related mortality. Androgen in implicated in the induction of proliferation and growth of mammary cells through binding to their corresponding receptors. Androgens influence the risk of acquiring breast cancer through either direct binding to androgen receptors (AR) or indirectly through their transformation to estradiol or competing for steroid binding proteins.

AIM: To study the expression of AR in various breast cancer subtypes and to elucidate its clinical significance by correlating it with clinicopathological parameters.

METHODS: One hundred and fifty breast cancer cases were studied using AR immunohistochemistry, and its expression was correlated with different clinicopathologic parameters and with ER, PR, Her-2/neu and Ki 67 expression.

RESULTS: AR was expressed in 91 breast carcinoma cases out of 150 examined. There was a statistically significant correlation between AR expression and tumour size, mitotic count, tumour necrosis, infiltrative borders, the hormonal status of the tumour and subsequently luminal subtypes ($p < 0.05$). A subset of studied TNBC (34.6%) also expressed AR. On the other hand, there was no significant correlation between AR expression and other clinicopathological parameters.

CONCLUSION: Positive AR immunostaining was associated with favourable prognostic factors and luminal subtypes (A&B). Also, a subset of TNBC cases showed positive AR expression. These results introduce the current potent, next-generation AR- antagonist as possible target therapy in breast cancer. Further researches on AR expression in breast cancer are recommended on a larger scale with follow up and survival to validate the current results.

Introduction

Breast carcinoma occupies the first rank among malignant tumours in females [1]. It is the second most common cause of cancer-related death in women, exceeding 1.7 million reported cases per year all over the world. Relative difference in incidence is noted between various regions as high occurrence was recorded in North America and the North of Europe, followed by the South region of Europe and Latin America, and lowest records were reported in Asia and Africa, but with a high tendency for rising incidence lately due to increased affluence of some of these regions [2].

In Egypt, breast cancer ranks second among different malignancies [1]. In 2013, breast cancer cases were estimated as 18192 [3] and raised to 23081 in 2018 [1].

Breast cancer is a nonuniform disease with diverse clinical backgrounds, histomorphology, outcome and response to treatment regimens. Moreover, neoplasms sharing similar histopathologic features can differ in their responses to therapy and finally, have different prognosis. This can be attributed to molecular diversity among histologically similar tumours [4].

Steroid hormones induce the growth of mammary cells by attaching to their corresponding

receptors, resulting in the clonal proliferation of both non-neoplastic and neoplastic cells. These signals can act directly by affecting hormone receptor-positive cells, or through the induction of growth factors elaboration that acts indirectly on receptor-negative cells. Three major receptors belong to this steroid superfamily; estrogen receptor is (ER), progesterone receptor (PR) and androgen receptor (AR) [5].

The fundamental role of estrogen and progesterone receptors in breast cancer prognosis and therapy management is well known and established. In contrast, few data is known about the exact role of the androgen receptor (AR) in breast tumorigenesis. The androgen receptor (AR) is more widely expressed in breast cancers than other steroid receptors [6], [7], [8].

Androgens influence the risk of acquiring breast cancer through either direct binding to AR or indirect through their transformation to estradiol or competing for steroid binding proteins [9].

In the post-menopausal period, women develop falling in the estrogen levels, and subsequently, adrenal androgens become the dominant stock in replenishing estrogen to cells. This new metabolic pathway represents the main source of estrogens and circulating androgen levels are implicated in the rising rates reported for breast cancer [10], [11].

Currently, investigators suggest that AR (+) tumours have favourable characteristics and that tumours expressing both AR and ER are associated with better outcome [12], [13].

To date, controversy exists among epidemiological, clinical, and preliminary clinical data on the basic role of androgens and of ARs in (ER)-negative breast carcinoma. However, results reported from most preliminary clinical researches suggest that activated ARs, initiate and induce the proliferation and growth, especially in HER2 positive cell lines, due to the crosstalk between AR and HER2 pathways. The proposed mechanism of action is that androgens are bidirectional: mainly proliferative, as androgens are the main harbingers of estrogens, but also anti-proliferative, because stimulated AR limits the ER activity [14].

In cases of ER-negative disseminated breast cancer, AR expression is noted in a subset defined as ‘molecular apocrine’ tumours and is associated with lower 5-year survival [15], [16].

The subcategory of TNBC positively expressing AR has been termed as the luminal androgen receptor (LAR) subtype. The prognostic and predictive value of AR in TNBC remains a challenging topic of research [17].

AR can be a promising candidate for target therapy in breast cancer [15]. The AR antagonist (enzalutamide) implicated in prostate cancer treatment, has shown promising results in some patients with advanced TNBC whose tumours were AR-positive [18].

Methods

One hundred and fifty cases of breast carcinoma with a wide range of age were randomly retrieved from the pathology files of the Pathology Department, Ahmed Maher Teaching Hospital during the period from January 2013 to December 2016. Patients with pure in situ duct carcinoma were excluded. The study was approved by the local Ethics Committee of the General Organization of Teaching Hospitals and Institutes.

The collected specimens were tru-cut needle biopsy (n = 10), wide local excision with axillary evacuation (n = 85), and modified radical mastectomy (n = 55).

Five µm thick sections were prepared from Formalin-fixed paraffin-embedded blocks and stained with routine Hematoxylin and eosin for confirmation of histopathological diagnosis and further tumour subtyping, grading and, staging. Other data were assessed such as; foci of tumoral necrosis, lymphovascular tumour emboli, perineural invasion, lymphocytic response and status of lymph nodes were reported.

The immunohistochemical (IHC) staining procedure was done using an immunostainer (Shandon Sequenza) through the labelled streptavidin-biotin method with the following reagents: Citrate buffer, 10X, heat-induced epitope retrieval, (Thermo medical Catalog number: AP-9003-500), Hydrogen peroxide block (Lab Vision, USA, Catalog number: TA-060-HP), Ultravision large volume detection system (Lab Vision, USA, Catalog number: TP-060-HL) including Ultra V block, Biotinylated goat anti-polyvalent plus (link) & Streptavidin peroxidase plus (label) and DAB plus substrate system (Lab Vision, USA, Catalog number: TA-060-HDX) including DAB plus chromogen & DAB plus substrate. The primary antibodies were: AR: a mouse polyclonal antibody (Thermo Medical Catalog number: MS-433-R7), ER: a rabbit monoclonal antibody (Thermo Medical Catalog number: RM-9101-R7), PR: a rabbit monoclonal antibody (Thermo Medical Catalog number: RM-9102-R7), HER-2/neu: a mouse monoclonal antibody (Thermo Medical Catalog number: MS-730-R7) and Ki67: a rabbit polyclonal antibody (Thermo Medical Catalog number: RB-9043-R7).

The adjacent breast tissue served as a positive internal control for For ER, PR, Her-2/neu and Ki 67 while prostatic tissue was used as a positive

control for AR. The slides were at least examined by two pathologists, totally blind to the reported clinical data. The following cut-off values were used for scoring of IHC-stained slides:

- The examined sections were reported as ER-positive if more than or equal 1% of ER nuclear staining was noted in tumour cells [19].

- The examined sections were reported as HER-2/neu positive in score 3+; cases with more than or equal 10% of tumour cells showed intense complete membranous staining [19].

- The Ki-67 proliferative activity was determined through semi-quantitative scoring. A cut-off point 20% for the Ki-67 nuclear staining positivity was used to classify cases into either low proliferative (< 20%) or high proliferative (≥ 20**%**) [20].

- A combination of 4 IHC markers (ER, PR, HER-2/neu, and Ki-67) was used for further subtyping of breast carcinoma according to St. Gallen international expert Consensus, 2013 [21].

- The examined sections were reported as AR-positive when more than 1% of tumour cells showed positive nuclear immunostaining [22], [23].

Statistical Package for Social Science (SPSS 17.0 for Windows; SPSS Inc, Chicago, IL, 2010) was used for data analysis. Chi-Square test was used to examine the correlation between two qualitative variables and between one quantitative and one qualitative variable. P-value was set as significant (S) when ≤ 0.05.

Results

The patient's age showed a wide range of age from 31 to 73 years with a mean age of 55.86 ± 13.11 years. The studied patients were all females. Tumour size ranged from 1 cm to 11.5 cm with Mean ± SD 3.2 ± 1.8. Most of the cases were invasive duct carcinoma of no special type (NST) with a percentage of 85.3%.

After exclusion of the Tru-cut cases (10), the majority of tumors were histologically grade II (77.1%), average mitotic rate (1- 10/10 HPF) (53.5%), were negative for tumor necrosis, (89.3%) , had infiltrative tumor borders (71.4%), showed moderate lymphocytic response (37.1%), had positive lymphovascular emboli (88.6%), weres stage T2 (55%) and were N0 (48.6%).

Regarding the intrinsic subtypes; after exclusion of cases with equivocal Her-2/neu (21), 129 cases were classified; 40 cases were Luminal A (31%), 54 cases were were Luminal B (41.9%), 9 cases were Her-2 Enriched (7%), and 26 cases were triple negative (7%).

The majority of cases were AR positive (60.7%) where AR expression was positive in 39.2% of luminal A cases, 43% of luminal B cases, 6.3% of Her-2 Enriched and 11.4% of triple-negative cases. AR expression was more in the luminal subtypes (Figure 1).

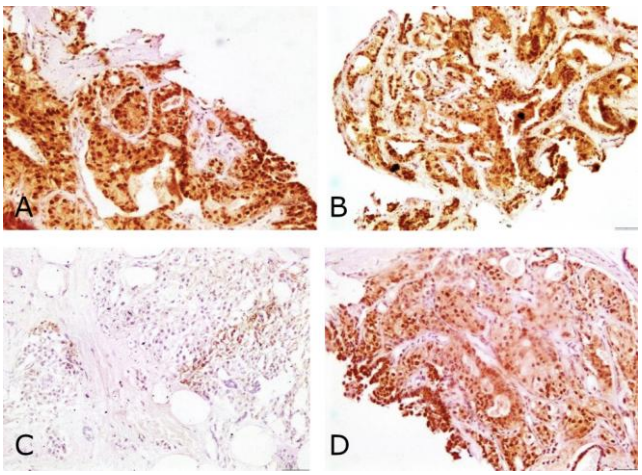

Figure 1: A) AR-positive nuclear expression (X200 original magnification) in Luminal B breast carcinoma; B) ER-positive nuclear expression (X100 original magnification); C) negative HER-2 neu membranous staining (X100 original magnification); D) high Ki-67 ≥ 20% (X200 original magnification)

On the correlation of AR expression with the clinicopathological parameters; there was a statistically significant correlation between AR status and both tumour size, and it's the mitotic count. AR expression was associated with tumours of smaller size and low mitotic count (P value: 0.017 and < 0.001 respectively).

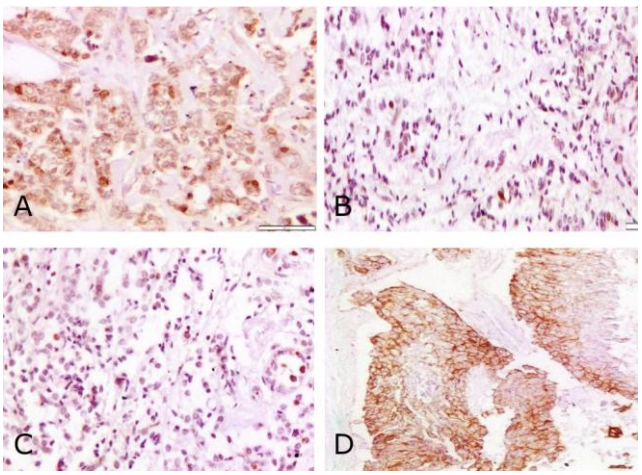

Figure 2: A) AR-positive nuclear expression (X200 original magnification) in HER-2 enriched breast carcinoma; B) ER-negative nuclear expression (X200 original magnification); C) PR negative nuclear expression (X200 original magnification); D) positive HER-2 neu complete membranous staining (X100 original magnification)

Also, there was a statistically significant correlation between AR status and tumour necrosis, tumour borders, its hormonal status and subsequently tumour subtype. AR expression was associated with

the absence of tumour necrosis, with infiltrative borders, with positive ER, positive PR and with luminal subtypes (P-value: < 0.001, 0.005, 0.004, 0.016 and 0.006 respectively) (Figures 1, 2, and 3).

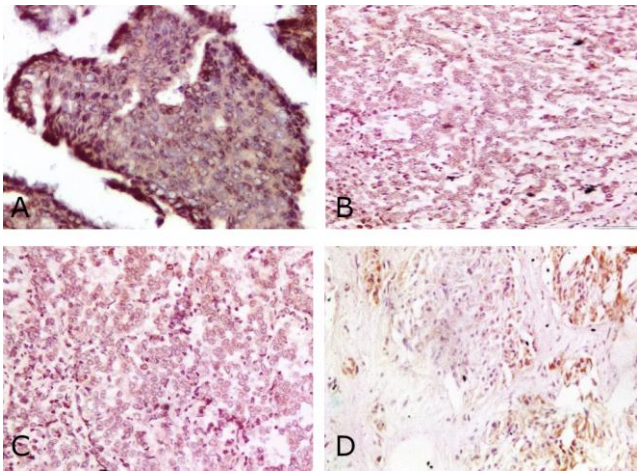

Figure 3: A) AR Negative nuclear expression (X200 original magnification) in Triple Negative breast carcinoma; B)ER-negative nuclear expression (X200 original magnification); C) PR negative nuclear expression (X200 original magnification); D) negative HER-2 neu membranous staining (X200originalmagnification)

No correlation could be found between AR status and patients age, tumour site, multifocality, histologic type, grade, lymphocytic response, lymphovascular invasion, perineural invasion, T stage, N stage, Her-2/neu status and ki 67 status (Table 1).

Table 1: Clinicopathological findings and their correlation with androgen receptors expression

	AR - n (%)	AR + n (%)	Total n (%)	P value
Right side	27 (45.8)	48 (52.7)	75 (50)	0.33
Left side	32 (54.2)	43 (47.3)	75 (50)	
Unifocal	49 (89.1)	75 (88.2)	124 (88.6)	0.924
Multifocal	6 (10.9)	10 (11.8)	16 (11.4)	
IDC (NST)	55 (93.2)	73 (80.2)	128 (85.3)	0.125
ILC	0 (0)	10 (11)	10 (6.7)	
Tubular/Cribriform/ mixed	3 (5.1)	4 (4.4)	7 (4.7)	
Others	1 (1.7)	4 (4.4)	5 (3.3)	
Grade I&II	50 (90.9)	84 (98.8)	134 (95.7)	0.087
III	5 (9.1)	1 (1.2)	6 (4.3)	
No mitosis	12 (21.4)	34(40.5)	46 (32.9)	< 0.001*
1-10/10 HPF	28 (50)	47 (56.9)	75 (53.5)	
> 10/10 HPF	15 (28.6)	4 (3.6)	19 (13.6)	
Necrosis abscent	42 (76.4)	83 (97.6)	125 (89.3)	< 0.001*
Present	13 (23.6)	2 (2.4)	15 (10.7)	
Infiltrative border	32 (58.2)	68 (80)	100 (71.4)	0.005*
Pushing	23 (41.8)	17 (20)	40 (28.6)	
No Lymphocytic response	9 (16.4)	23 (27.1)	32 (22.9)	0.14
With lymphocytic response	46(83.6)	62(73.9)	108(77.1)	
No vascular emboli	8 (16.5)	8 (27.1)	16 (11.4)	0.14
With vascular emboli	47 (83.5)	77 (72.9)	124 (88.6)	
No perineural invasion	55 (100)	84 (98.8)	139 (99.3)	0.83
With perineural invasion	0 (0)	1 (1.2)	1 (0.7)	
T Stage I & II	43 (78.2)	74 (87.1)	117 (83.6)	0.166
III&IV	12 (21.8)	11 (12.9)	23 (16.4)	
N Stage N0	25 (45.5)	43 (50.6)	68 (48.6)	0.55
N1, N2	30 (55.5)	42 (49.4)	72 (51.4)	
ER-	22 (37.7)	15 (16.7)	37 (24.7)	0.004*
ER+	37 (62.3)	76 (83.3)	113 (75.3)	
PR-	26 (45.3)	23 (25)	49 (32.7)	0.016*
PR+	32 (54.7)	69 (75)	101 (67.3)	
Her2-	38 (67.9)	76 (84)	114 (76)	0.084
Her2+	9 (15.1)	6 (6.2)	15 (10)	
Equivocal Her2	12 (17)	9 (9.9)	21 (14)	
Low Ki 67	17 (29.3)	37 (40.2)	54 (36)	0.139
High Ki 67	42 (70.7)	54 (59.8)	96 (64)	
Luminal A	9 (18)	31 (39.2)	40 (31)	0.006*
Luminal B	20 (40)	34 (43)	54 (41.9)	
Her-2 enriched Triple	4 (8)	5 (6.3)	9 (7)	
Negative	17 (34)	9 (11.4)	26 (20.2)	

Discussion

The chief role of androgen receptor signalling in neoplastic breast cells remains questionable. It has been reported in previous studies to be involved in the proliferation and growth of normal mammary cells [24].

This work studied the relation between AR expression and clinicopathological parameters in 150 cases of invasive breast carcinoma.

A significant correlation was found between AR expression and tumour size. This finding is in agreement with Ogawa et al., [25], Niemeier et al., [26], Collins et al., [27], and Aleskandarany et al., [28] studies which found that AR expression was higher in the smaller tumours. On the other hand, Gonzalez et al.29 and Samaka et al., [30] found no significant correlation between AR expression and the size of the tumour. Gonzalez et al., [29] used both tissue microarrays and immunohistochemistry and Samaka et al., [30] used a smaller number of cases.

AR expression is significantly associated with infiltrative borders of the tumour, and this is in concordance with Putti et al., [31]. But this is in contrast to Gonzalez et al., [29] study which found no significant relation between AR expression and the type of tumour borders. This discrepancy might be the result of their use of tissue microarray in testing for AR.

The mitotic count is significantly inversely correlated to AR expression where the cases with lower mitotic figures showed a higher percentage of AR expression. This is in harmony with Safarpour et al., [32] and Aleskandarany et al., [28].

AR expression was significantly associated with the absence of tumour necrosis, and this is was similar to Niemeier et al., [26] study results.

A significant correlation between AR expression and hormonal status was found. This is by Park et al., [6], Qi et al., [33], Safarpour et al., [32], Vera-Badillo et al., [34], and Chottanapund et al., [35]. On the contrary; Gonzalez et al.[29] found no correlation between AR status and the hormonal status of breast cancer. This might be the result of their use of tissue microarray in testing for AR.

As Ogawa et al., [25], Park et al., [6] and Qi et al., [33], no significant relation between AR expression and Her-2/neu expression could be found, though Agrawal et al., [36], Chottanapund et al., [35], and Samaka et al., [30] found that AR expression is more in tumours expressing Her-2/neu. This might be due to different primary antibodies used.

No significant relation between AR expression and Ki 67 expression was found as Vera- Badillo et al., [34], but in contrast to Qi et al., [33], and Samaka et al., [30], this might be due to their use of 14 % as

cut-off, not 20% as we used.

Regarding the correlation between AR expression and the subtypes, AR was expressed in significantly higher proportions of luminal breast carcinoma cases. This is by Collins et al., [27], Qi et al., [33], Aleskandarany et al., [28] and Samaka et al., [30].

In our study, the triple negative cases were 20.2%. This is within the documented range for triple negative cases (15-20%) of Kohler et al., a study [37]. AR was expressed in 34.6% of the TN cases, and that is within the wide range of 6.6 to 75% documented by Rampurwala et al., [38]. These subsets of patients are possible candidates for the promising anti-androgen target therapy.

No correlation could be found between AR status and patients age, tumour site, multifocality, histologic type, grade, lymphocytic response, lymphovascular invasion, perineural invasion, T stage, N stage, Her-2/neu status and ki 67 status.

Gonzalez et al., [29] also found no significant correlation between AR expression and histologic grade, lymphocytic response, lymphovascular invasion or N stage.

Ogawa et al., [25] *and* Collins et al., [27] found androgen receptor-positive tumours were lower grade and more often node-negative.

Soiland et al., [39] found no significant correlation between AR expression and patients age.

Park et al., [6] *revealed a* significant correlation between AR expression and both histologic type and grade.

Qi et al., [33] did not find a significant correlation between AR expression and both patients age and N stage.

Ruibal et al., [40] tested AR expression in 816 breast cancers using immunohistochemistry and found no relation between AR expression and the tumour multifocality.

Agrawal et al., [36] found no significant correlation between AR expression and both histologic type and N stage but found a significant correlation between AR expression and histologic grade.

Aleskandarany et al., [28] found that nuclear AR immunostaining was significantly associated with features favouring good prognosis including older age groups, smaller tumour size, lower histologic grade and lobular carcinoma.

Samaka et al., [30] found a significant relation between AR expression and the patient's age and no significant relation with histologic type, lymphovascular invasion or N stage.

In the highlight of the previously mentioned results, we conclude that positive AR immunostaining was associated with smaller tumour size, infiltrative margins, lower mitotic count, negative tumour necrosis, positive ER and PR expression and mainly luminal subtypes (A&B) and in a subset of TNBC cases.

The most used therapy for advanced BC (Tamoxifen-resistant-BCs and TNBCs) is based on the use of AR antagonists, such as bicalutamide and enzalutamide, a first- and second-generation AR antagonist, respectively [41], [42]. Both the antagonists have been used in clinical trials with positive results [43]. Other therapies for TNBC are based on the use of CYP17A1 inhibitors, such as abiraterone acetate and seviteronel. These inhibitors reduce the androgen production and 11the androgen levels. They are now being tested in phase 2 clinical trials [44], [45], alone or in combination with AR antagonists [46].

In conclusion, positive AR expression was associated with favourable prognostic factors and luminal subtypes (A&B). Also, a subset of TNBC cases showed positive AR expression. These results introduce the current potent, next-generation AR-antagonist as possible target therapy in breast cancer. Further researches on AR expression in breast cancer are recommended on a larger scale with follow up and survival to validate the current results.

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Clinical Science

The Difference of Serum Gastrin-17 Level Based on Gastritis Severity and Helicobacter Pylori Infection

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Abstract

BACKGROUND: Gastritis was defined as the histological presence of gastric mucosal inflammation. One of the most common aetiology was *H. pylori*. Gastrin-17 was a hormone that was secreted by G cells. *H. pylori* infection induced increased in gastrin-17 in gastritis. Therefore, this study was to investigate the relationship of gastrin-17 with gastritis severity and *H. pylori* infection.

AIM: To determine the difference in serum Gastrin-17 level based on gastritis severity and *H. pylori* infection.

METHODS: A cross-sectional study enrolling 45 patients with gastritis was conducted in Haji Adam Malik General Hospital between April and July 2018. Endoscopy and biopsy examinations were performed to confirm the diagnosis of gastritis. Gastritis severity was assessed using the Updated Sydney System. The presence of *H. pylori* infection was detected by a Campylobacter-like organism (CLO) examination. Gastrin-17 level and demographic data were also gathered. The analysis was done using Mann Whitney and Kruskal-Wallis test. P-value of < 0.05 was considered statistically significant.

RESULTS: Serum Gastrin-17 level was significantly different based on gastritis severity (P = 0.001 according to neutrophils infiltration and P = 0.023 according to degree of atrophy), *H. pylori* infection (P = 0.038), and combined gastritis severity and *H. pylori* infection (P < 0.001). Serum Gastrin-17 level was higher in subjects with severe neutrophils infiltration, without atrophy, and with *H. pylori* infection.

CONCLUSION: There was a significant difference in serum Gastrin-17 level based on gastritis severity and *H. pylori* infection.

Introduction

Gastritis is one of the most common digestive tract problems. Worldwide, the incidence of gastritis was 1.8-2.1 million, while in South East Asia, 583,635 per year. The incidence of gastritis in Indonesia itself is very high, which is 247,396 cases from 238.452.952 population [1]. Gastritis is defined as the histological presence of gastric mucosal inflammation. In acute gastritis, the microscopic finding is neutrophilic infiltration, while in chronic gastritis, mononuclear cells, mostly lymphocytes and plasma cells and macrophages dominate the microscopic findings [2], [13]. Chronic gastritis is classified into normal, mild, moderate, severe based on mononuclear inflammatory cells infiltration, neutrophils infiltration, atrophy, intestinal metaplasia [3], [4].

H. pylori infection plays an important role in the development of peptic ulcer in gastritis patients. *H. pylori* infection in developing countries was about 25-30%, where 5-27% were found in early childhood and 50-60% were found in adults aged more than 60 years old [1]. *H. pylori* reside within the mucous layer. The stomach is a dangerous environment for most other microorganism because of its low pH. The ability of *H. pylori* to flourish in the stomach has been attributed to a protective mechanism such as its production of urease, protecting bacteria from gastric acidity by creating a basic microenvironment. *H. pylori* can cause both acute and chronic gastritis in inadequately treated *H. pylori* patients; Chronic gastritis can progress to chronic atrophic gastritis.

Gastrin prohormone is produced by G cells

located within gastric antrum and corpus in response to vagal and gastrin-releasing peptide (GRP) stimulation secondary to ingestion of peptides, amino acids, gastric distention and an elevation of stomach pH. The prohormone is later processed to shorter peptides. Two major forms of gastrin are secreted which are Gastrin-17 and Gastrin-34. The major role of gastrin within gastric tissue is the regulation of acid secretion. In *H. pylori* infection, Gastrin levels are found to be consistently elevated, and normal physiological negative feedback control of secretion is lost. Furthermore, after *H. pylori* treatment, gastrin levels are decreased and normal feedback control of gastrin secretion is restored [4].

Given the high prevalence of gastritis, this study is aimed to determine the difference in serum Gastrin-17 level based on gastritis severity and *H. pylori* infection.

Methods

Study Design

A cross-sectional study was conducted in Haji Adam Malik General Hospital Medan, Indonesia between April and July 2018 following approval from the Ethics Committee of the Faculty of Medicine Universitas Sumatera Utara and Haji Adam Malik General Hospital.

Subject Recruitment

Individuals who were not pregnant, aged 18 years or older, and willing to take part in the study were enrolled in this study. Exclusion criteria included patients who had received *H. pylori* eradication therapy within the last 6 months or on antibiotic therapy commonly used in eradication therapy, concomitant use of proton pump inhibitors, H2 receptor antagonists, NSAIDs, steroids, and alcohol for the last 48 hours and patients with systemic disease.

Physical examination, routine blood count, liver and kidney function, blood sugar, amylase, and lipase evaluation, ECG, and abdominal ultrasound were conducted to assess the exclusion criteria. Subjects then underwent endoscopy and biopsy examination to establish the diagnosis of gastritis. All endoscopy examinations used scopes. Biopsy specimens were obtained from 5 places, including the greater and lesser curvature of the distal antrum, lesser curvature at incisura angularis, anterior and posterior wall of the proximal corpus. Additional biopsies were also done in suspicious regions that were not mentioned previously. Histopathologic examination was done by Anatomic Pathologists at Universitas Sumatera Utara blindly. Gastritis severity was determined using the Updated Sydney System.

Serum gastrin levels were measured in serum using the ELISA human gastrin-17 (BIOHIT OYJ, Laipattie, Fl-00880 Helsinki, Finland). Campylobacter-like Organism test (CLO) was performed to detect *H. pylori*. The changing of colour from yellow to red magenta, pink, or dark orange means positive *H. pylori* infection.

Statistical Analysis

Data from this study were analysed statistically using a descriptive study to obtain baseline characteristics. Mann Whitney U test was used to determine the difference in serum Gastrin-17 levels based on *H. pylori* infection, while the difference in serum Gastrin-17 levels based on gastritis severity was analysed using the Kruskal-Wallis test. The calculation was conducted at a 95% confidence interval and P-value of < 0.05 was considered significant.

Results

A total of 45 gastritis patients were enrolled in this study. There were 25 (55.6%) males. The mean age was 51.0 (SD 12.27) years, with mean Body Mass Index (BMI) of 23.0 (SD 4.02) kg/m^2. The majority of the ethnic background was Bataknese (71.1%).

Table 1: Baseline characteristics

Characteristics	n = 45
Gender, n (%)	
Male	25 (55.6)
Female	20 (44.4)
Mean age, years (SD)	51.0 (12.27)
Mean body mass index,kg/m^2 (SD)	23.0 (4.02)
Ethnic background, n (%)	
Acehnese	3 (6.7)
Bataknese	32 (71.1)
Javanese	10 (22.2)
Occupation, n (%)	
Housewife	11 (24.4)
Private employee	12 (26.7)
Government employee	4 (8.9)
Entrepreneur	18 (40)
Mean gastrin-17 level, pmol/mL (SD)	14.0 (12.92)
CLO, n (%)	
Positive	23 (51.1)
Negative	22 (48.9)
Chronic inflammation, n (%)	
Mild	23 (51.1)
Moderate	7 (15.6)
Severe	5 (11.1)
Neutrophil infiltration, n (%)	
Normal	22 (48.9)
Mild	18 (40.0)
Moderate	15 (33.3)
Degree of atrophy, n (%)	
Normal	36 (80.0)
Mild	4 (8.9)
Moderate	5 (11.1)

Mean serum Gastrin-17 levels in this study were 14.0 pmol/mL. The result of CLO examination showed 23 (51.1%) gastritis patients had positive results. The result of the histopathological

examination for chronic inflammation showed 51.1% of patients had mild inflammation. Based on neutrophils infiltration, 18 (40.0%) had mild infiltration and 15 (33.3%) had moderate infiltration. Based on the degree of atrophy 4 (8.9%) patients had a mild degree and 5 (11.1%) had a moderate degree (Table 1).

The difference in serum Gastrin-17 levels based on gastritis severity was shown in Table 2. There were statistically significant differences in serum Gastrin-17 levels based on gastritis severity according to neutrophils infiltration and degree of atrophy (P = 0.001 and 0.023, respectively). Patients with severe neutrophils infiltration had the highest serum Gastrin-17 level in their group, while patients without atrophy had the highest serum Gastrin-17 level in their group.

Table 2: Differences in Gastrin-17 levels based on gastritis severity

	N	Gastrin-17, median (min-max)	p*
Chronic inflammation			
Mild	23	6.0 (0.8-33.0)	0.806
Moderate	7	2.6 (0.8-40.0)	
Severe	15	19.5 (1.1-40.0)	
Neutrophil inflammation			
Normal	22	4.2 (0.8-40.0)	0.001
Mild	18	14.0 (1.1-40.0)	
Severe	5	27.6 (15-40.0)	
Atrophy			
Normal	36	13.65 (0.8-40.0)	0.023
Mild	4	5.85 (5.8-6.0)	
Moderate	5	1.5 (1.1-2.6)	

*Kruskal Wallis test.

There was a statistically significant difference in serum Gastrin-17 levels based on *H. pylori* infection (P = 0.038). Median serum Gastrin-17 levels are shown in Table 3.

Table 3: Serum Gastrin-17 levels differ based on *H. pylori* infection

CLO	Gastrin-17, median (min-max)	P*
Positive	14.2 (1.1-40.0)	0.038
Negative	9.0 (0.8-33.0)	

*Mann Whitney U test.

We also analysed the difference in serum Gastrin-17 level based on *H. pylori* infection in each gastritis severity group.

Table 4: Differences in serum Gastrin-17 levels based on *H. Pylori* infection in each gastritis severity group

Gastrin-17 Level, median (range)		N	*H. pylori* (+)	*H. pylori* (-)	P*
Chronic inflamation	Mild	23	5.8 (1.5-6.0)	10.0 (0.8-33.0)	0.218
	Moderate	7	8.6 (2.6-40.0)	1,65 (0.8-24.0)	0.157
	Severe	15	19.5 (1.1-40.0)	-	-
Neutrophil infiltrasion	Normal	22	5.8 (1.5-40.0)	2,2 (0.8-33.0)	0.480
	Mild	18	13.8 (1.1-40.0)	21.8 (10.3-26.1)	0.750
	Severe	5	27.6 (15-40.0)	-	-
Atrophy	Normal	36	19.8 (8.6-40.0)	9.7 (0.8-33.0)	< 0.001
	Mild	5	5.9 (5.8-6.0)	5.8	0.346
	Severe	5	1.5 (1.1-2.6)	-	

*Mann-Whitney U test.

Based on the statistical analysis, we found a significant difference in serum Gastrin-17 levels in patients without atrophy between positive and negative *H. pylori* infection with a P value of < 0.001. Significantly higher serum Gastrin-17 level was observed in patients with positive *H. pylori* infection without atrophy (Table 4).

Discussion

Gastrin is secreted by G cells, which are presented in gastric antrum and duodenum. Gastrin is secreted in response to vagal and gastrin-releasing peptide (GRP) stimulation secondary to ingestion of peptides, amino acids, gastric distention and an elevation of stomach pH. The secret gastrin into the systemic circulation is delivered to parietal cells and enterochromaffin-like cells (ECL) in gastric fundus and cardia. Gastrin stimulated parietal cells to secrete gastric acid and ECL to secret histamine which also results in gastric acid production [14].

H. pylori infection mostly is found in antrum at an early stage and in both corpus and antrum in the later stage of infection. It causes gastric inflammation which released cytokines (TNFα, IL1β, IFN gamma, IL8) [4], [12]. Gastric inflammation/gastritis are described by neutrophils infiltration level, lymphocytes level, present of intestinal metaplasia and atrophy. High level of TNFα is related to a severe degree of neutrophils infiltration. Because of its potent chemotactic and stimulatory activity on neutrophils and lymphocytes, high IL8 count is related to severe degree of chronic inflammation, neutrophils infiltration, atrophy and intestinal metaplasia [16]. Cytokines caused elevation of gastrin production. Increment of gastrin levels in *H. pylori* gastritis is also contributed by reduced somatostatin secreting D-cells [19]. In this study, Gastrin-17 levels were significantly higher in patients with positive *H. pylori* infection compared to negative *H. pylori* infection (p = 0.038). This result was supported by the previous study which was conducted by Park et al., [4] They reported fasting serum gastrin concentrations were significantly higher in patients with *H. pylori* infection compared to patients without infection (80.3 ± 23.5 vs 47.6 ± 14.1 pg/ml, p < 0.001).

In Sheykholeslami et al., study, Gastrin-17 was increased in corpus-predominant gastritis (p < 0.01) [5]. However in this study, Gastrin-17 level was significantly higher in severe neutrophil infiltration. Meanwhile, in chronic inflammation, Gastrin -17 was higher in the severe category but was not significantly different.

In our study, Gastrin-17 level tends to be lower in moderate atrophy (1.5 pmol/mL), and the highest value was observed in subjects without atrophy (13.63 pmol/mL) (p = 0.023). This result was consistent with a study that was done by Vaananen et al., [7], Ebule et al., [11]. In most cases, *H. pylori* colonisation occurs, causing peptic ulcer disease in

the antrum, gastric atrophy and achlorhydria in gastric corpus. *H. pylori* induce chronic inflammation eventually lead gastric to become atrophic. In atrophic gastritis, the mucosal gland is replaced by immature gland and epithelial cells which is intestinal type gland (intestinal metaplasia), fibrous tissue and/or pyloric type (resembling pyloric glands and epithelium that has no G cell inside) [17]. The decrement in G cell population causes gastrin production to decrease as the atrophic progress [8], [15]. Serum Gastrin-17 is significantly reduced in antral atrophy and coexistence of corpus atrophy [18].

In conclusion, we found a significant difference in serum Gastrin-17 level based on gastritis severity and *H. pylori* infection. Serum Gastrin-17 level is higher in subjects with severe neutrophil infiltration, without atrophy, and with *H. pylori* infection.

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Basic Science

The Effect of Mesenchymal Stem Cell Wharton's Jelly on ADAMTS-4 and iNOS Levels in Osteoarthritis Rat Model

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Abstract

BACKGROUND: Osteoarthritis (OA) is one of the most common diseases among the elderly. OA occurs due to an imbalance between degradation and synthesis in articular joint tissue, causing changes in joint components such as cells, matrices and molecular production. Therefore, knowledge of cartilage-degrading enzymes such as ADAMTS-4 and iNOS is needed.

AIM: This study aims to prove the effect of Mesenchymal Stem Cell Wharton Jelly on decreasing ADAMTS-4 levels as cartilage-degrading enzymes and increasing levels of iNOS which showed the immunosuppressive potential of MSC-WJ in cases of osteoarthritis in vivo.

MATERIAL AND METHODS: This research is an experimental study with the design of Post-test-Only Control Group Design. The sample consisted of 16 OA rats as a control group and 16 OA rats treated with MSC-WJ as a treatment group. OA induction is done by injection of monosodium iodoacetate (MIA) into the intra-articular right knee. Giving MSC-WJ is done in the third week after MIA induction. The serum ADAMTS-4 and iNOS levels were measured after 3 weeks treated with MSC-WJ using the ELISA method. The statistical test used is an independent t-test. The value of $p < 0.05$ was said to be statistically significant.

RESULT: The result showed that serum ADAMTS-4 levels were lower in the group treated with MSC-WJ than in the control group, but not statistically significant ($p > 0.05$). Serum iNOS levels were higher in the group treated with MSC-WJ than in the control group ($p < 0.05$).

CONCLUSION: This study concluded that MSC-WJ significantly reduced ADAMTS-4 levels and increased the serum iNOS levels of OA rats.

Introduction

Osteoarthritis (OA) is a disorder of the joint joints characterised by cell stress and extracellular matrix degradation triggered by micro and macro injuries. This disease manifests first as molecular disorder followed by anatomical abnormalities, and physiology (characterised by cartilage degradation, bone remodelling, osteophyte formation, joint inflammation and loss of normal joint function), which can lead to disease [1].

Molecular disorders in osteoarthritis activate the pro-inflammatory pathway, causing an increase in expression of inflammatory mediators such as IL-1β and TNF-α. A Disintegrin-like and Metalloproteinases with Thrombospondin Motifs-4 (ADAMTS-4) is aggrecanase which is responsible for aggrecanolysis in OA [2], [3], [4)]. Fan *et al.,* (2005) reported that IL-1β can improve ADAMTS-4 regulation in both normal human chondrocytes and OA [5]. Bondeson *et al.,* (2007) showed that IL-1β increased regulation of ADAMTS-4 in synovial OA in human fibroblasts [6].

Mesenchymal Stem Cells (MSC) has the

potential for multipotent differentiation for regenerative medicine [7] and has the strong immunosuppressive capacity, so it has therapeutic potential for various inflammatory-related diseases [8], [9], [10]. The immunosuppressive ability of MSC is shown by the increase in nitric oxide (NO) products which play a major role in inhibiting T-cell proliferation [10]. Inducible nitric oxide synthase (iNOS) is an enzyme that plays a role in NO synthesis. Inducible nitric-oxide synthase induced MSC after activation by IFN IF and TNFα, IL-1α or IL-1β. MSC of $iNOS^{-/-}$ rat has a low ability to suppress T-cell proliferation [8].

This study aims to prove the effect of Mesenchymal Stem Cell Wharton Jelly on decreasing ADAMTS-4 levels as cartilage-degrading enzymes and increasing levels of iNOS which showed the immunosuppressive potential of MSC-WJ in cases of osteoarthritis in vivo.

Material and Methods

Animal and Experimental Design

Male white rats (*Rattus novergicus*) with a weight ranging from 200-250 grams as experimental animals placed in clean, disinfected and pathogen-free cages and given standard food in the form of pellets and drinking in ad libitum. Trial animals adapted first for 1 week before treatment. Induction of osteoarthritis conducted with 300 μg intra-articular injection of monosodium iodoacetate (MIA) (Sigma Aldric, USA) in 50 μl of saline solution (0.9% NaCl) sterile [11] singly into the right knee joint rats anaesthetized by intraperitoneal injection of xylazine 10 mg/kg and ketamine 20 mg/kg uses insulin syringe with a needle (needle) 27G [12]. 32 osteoarthritis male white rats (three weeks after MIA induction) were divided into 2 treatment groups (n = 16): Control group and MSC-WJ group. MSC-WJ group is given 50 μl MSC-WJ with a dose of 1 x 10^6 cells into the right knee joint and a control group given 50 μl complete medium after anaesthetized. Rats were sacrificed after 3 weeks of treatment. Serum and knee joint were taken and then analyzed.

Analysis of Flow Cytometry

Mesenchymal Stem Cell Wharton Jelly was obtained from the Indonesian Medical Education and Research Institute (IMERI) Faculty of Medicine, University of Indonesia. Based on the analysis of flow cytometry, MSC-WJ used for this therapy had CD73-APC cell surface expression 99.8%, CD105-PerCP-Cys5.5 95% and CD90-FITC 99.9%. Photocell was taken use Nikon Ti-S microscope. Scale bar: 500 μm.

Measurement of serum ADAMTS-4 and iNOS by ELISA

Blood was taken from sinus periorbital and centrifuged at 3000 rpm for 15 minutes. The collected serum was stored at -80°C until measurement. Serum ADAMTS-4 and iNOS levels were measured by an ELISA kit (Bioassay Technology Laboratory, China). All samples are measured in duplicate.

Examination of ADAMTS-4 Levels (Work protocol based on rat ADAMTS-4 ELISA Kit)

Prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature. Determine the number of strips required for the assay. Insert the strips in the framers for use. The unused strips should be stored at 2-8°C. Add 50 μL standard well. Add 40 μL sample to sample wells and then add 10 μL anti-ADAMTS-4 antibody to sample wells, then add 50 μL streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a shaker. Incubate 60 minutes at 37°C. Removed the sealer and wash the plate 5 times with wash buffer. Soak wells with at least 0,35 ml wash buffer for 30 seconds to minute for each wash. For automated washing, aspirate all wells and wash 5 times with wash buffer, overfilling wells with wash buffer. Blot the plate onto paper towels or other absorbent material. Add 50 μL substrate solution A to each well and then add 50 μL substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark. Add 50 μL stop solution to each well; the blue colour will change into yellow immediately. Determine the optical density (OD value) of each well immediately using a microplate reader set a 450 nm within 30 min after adding the stop solution.

Examination of iNOS Levels (Work protocol based on rat iNOS ELISA Kit)

Prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature. Determine the number of strips required for the assay. Insert the strips in the framers for use. The unused strips should be stored at 2-8°C. Add 50 μL standard well. Add 40 μL sample to sample wells and then add 10 μL anti-iNOS antibody to sample wells, then add 50 μL streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a shaker. Incubate 60 minutes at 37°C. Removed the sealer and wash the plate 5 times with wash buffer. Soak wells with at least 0,35 ml wash buffer for 30 seconds to minute for each wash. For automated washing, aspirate all wells and wash 5 times with wash buffer, overfilling wells with wash buffer. Blot the plate onto paper towels or

other absorbent material. Add 50 µL substrate solution A to each well and then add 50 µL substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark. Add 50 µL stop solution to each well; the blue colour will change into yellow immediately. Determine the optical density (OD value) of each well immediately using a microplate reader set a 450 nm within 30 min after adding the stop solution.

Research Ethics

This study was already passed the ethics clearance and has been approved by the Ethics Committee of the Faculty of Medicine, Andalas University, Padang with registration number: 549/KEP/FK/2017.

Statistical analysis

Data are presented in mean and elementary forms. The statistical analysis used is SPSS 18.0. The statistical test used is an independent t-test. The value of p <0.05 was said to be statistically significant.

Results

OA rats were divided into 2 groups, namely the control group and the group treated with MSC-WJ (Figure 1). Examination of the levels of ADAMTS-4 and iNOS was carried out in the serum of rats by ELISA.

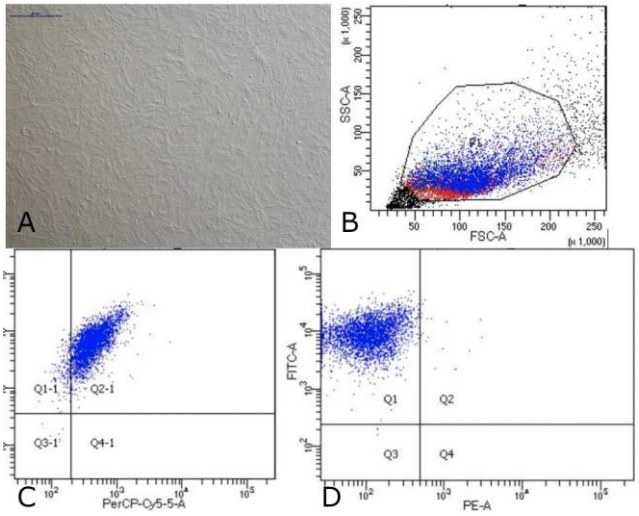

Figure 1: Data on Characteristics of Mesenchymal Stem Cells Wharton Jelly; A) Cells MSC-WJ reach confluence. Scale bar: 500 µM. Photographs of cells taken using a Nikon Ti-S microscope; B) Data flow cytometry. Forward scatter (FCS) plot&side scatter (SSC) plot. Population gated events (P1): 20,000; C) Cell surface markers expression: CD73-APC 99.8% and CD105- PerCP-Cy5.5 95%; D) Cell surface markers expression: CD90-FITC 99.9% and Lin (-) - PE 0.4%

ELISA examination

The blood obtained from the centrifuged animal is then obtained serum. Serum before analysis was stored in a refrigerator temperature of -80°C. The serum obtained was determined by ADAMTS-4 and iNOS levels, carried out in the Biomedical laboratory, Faculty of Medicine, Andalas University.

The results of the measurement of ADAMTS-4 and iNOS levels were carried out in normal rat, and the mean levels of ADAMTS-4 and iNOS were 27.92 ng/ml and 20.86 ng/ml. Based on the results of the normality test the data shows that the two research variables namely ADAMTS-4 and iNOS are normally distributed (p > 0.05). Thus, the parametric test (free t-test) can then be carried out.

Effect of MSC-WJ on ADAMTS-4 levels in serum of OA rats

The results of the measurement of ADAMTS-4 levels by ELISA method showed that the serum ADAMTS-4 levels of OA rats treated with MSC-WJ were lower than those not treated which can be seen in Figure 2.

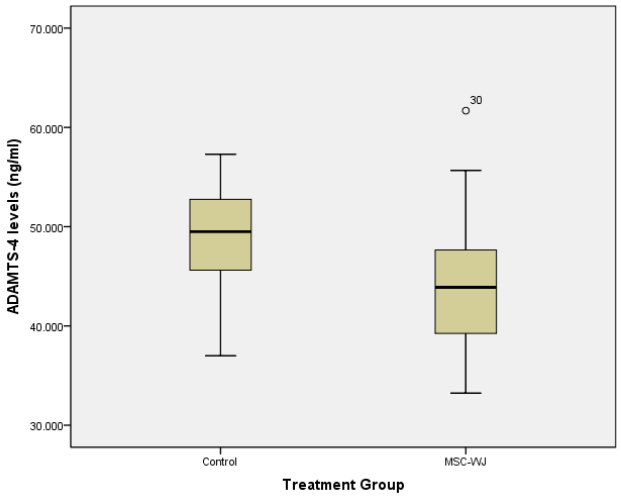

Figure 2: Boxplot graph of rat serum ADAMTS-4 levels

The difference in ADAMTS-4 levels between the serum of rats treated with MSC-WJ and not treated bivariate test can be seen in Table 1.

Table 1: Mean differences in ADAMTS-4 levels by group

Groups	ADAMTS-4 Levels (ng/ml) (Mean ± SD)	P value
Control	47.63 ± 5.32	0.114
MSC-WJ	43.89 ± 7.50	

Table 1 showed that there are differences in levels of ADAMTS-4 based on treatment. Decreased levels of ADAMTS-4 in the group treated with MSC-WJ from the control group. Statistically, the differences were not significant (p > 0.05).

Effect of MSC-WJ on iNOS levels in serum of OA rats

The results of measurement of iNOS levels by ELISA method showed that the serum iNOS levels of OA rats treated with MSC-WJ were lower than those not treated with bivariate tests which can be seen in Figure 3.

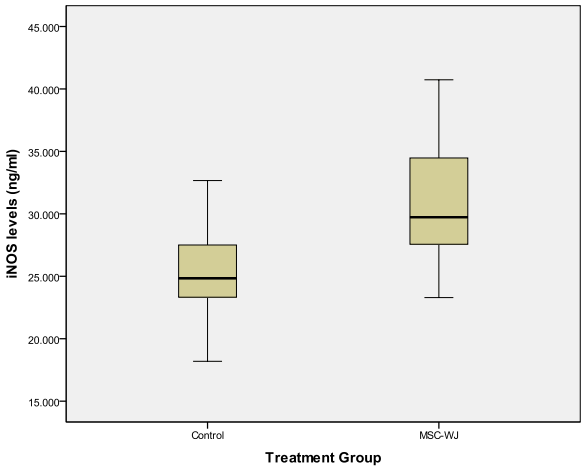

Figure 3: Boxplot graph of rat serum iNOS levels

The difference in iNOS levels between the serum of rats treated with MSC-WJ and not treated bivariate test can be seen in Table 1.

Table 2: Mean differences in iNOS levels by group

Groups	iNOS Levels (ng/ml) (Mean ± SD)	P value
Control	24.96 ± 3.56	0.000
MSC-WJ	30.79 ± 4.64	

Table 2 showed that there are differences in levels of iNOS based on treatment. Increased levels of iNOS in the group treated with MSC-WJ from the control group. Statistically, the differences were significant ($p < 0.05$).

Discussion

Aggrecanase-1 (ADAM-TS-4)

Aggrecanase-1 (ADAMTS-4) is mainly expressed in the active form in the osteoarthritis cartilage and plays an important role in the degradation of aggrecan in the cartilage of human osteoarthritis. ADAMTS-4 is overexpressed in human cartilage OA, and the expression of ADAMTS-4 in articular chondrocytes directly correlates with the degree of damage to the articular cartilage in OA. According to Naito *et al.,* (2007), ADAMTS-4 is an aggrecanase expressed in human OA cartilage and plays a key role in aggrecan degradation in humans OA [13].

Increased regulation of the expression of the ADAMTS-4 gene (aggrecanase-1) in OA was induced by IL-1. Interleukin-1β activates the NF-kB cascade in chondrocytes and kills almost all anabolic pathways, including collagen type II and aggrecan synthesis [14] and increases catabolic pathways.

The results of this study indicate that the levels of ADAMTS-4 serum OA rats treated with MSC-WJ were lower than those not treated, but the difference was not significant. While the results of the research by Shu *et al.,* (2016) showed that synovial ADAMTS-4 expression decreased significantly in OA joints after 6 weeks injected MSC compared with those not injected MSC [15].

The results of van Buul *et al.,* (2012) show that MSC can reduce IL-1β gene expression in synovial and cartilage tissue (16). MSC can increase anti-inflammatory cytokines which can inhibit the NF-kB cascade, thereby reducing catabolic pathways [17]. This causes MSC-WJ to have the ability to reduce serum ADAMTS-4 levels, which is one of the catabolic factors responsible for the occurrence of OA [17]. Although in this study there was a tendency to decrease ADAMTS-4 levels after MSC-WJ treatment, it did not reach statistical significance. This is probably due to the not optimal incubation time for MSC-WJ.

Inducible nitric oxide synthase (iNOS)

Inducible nitric oxide synthase (iNOS) is an enzyme responsible for the production of nitric oxide (NO), the main proinflammatory and destructive mediator in osteoarthritis (OA). INOS expression increases regulation by inflammatory cytokines including IL-1β, IL-17, TNF-α, IFN-γ [18].

Mesenchymal stem cells can act as an anti-inflammatory by reducing the production of proinflammatory cytokines which will directly inhibit the function and proliferation of T cells. Also, MSC has a potent immunosuppressive capacity. In inflammatory conditions, MSC of rats expresses high levels of iNOS, which inhibits immune cell proliferation and function [19]. Immunosuppressive effects occur through enzymatic actions such as inducible nitric oxide synthase (iNOS) and Indoleamine 2, 3-dioxygenase (IDO), and through the production of human leukocyte antigen class I (HLA-G) and prostaglandin E2 (PGE2) [20], [21].

The results showed that the iNOS levels of serum OA mice treated with MSC-WJ were higher than those not treated. Research by Li *et al.,* (2013) found that iNOS expression peaked at 1 week after being given HUC-MSC transplants in acute tubular necrosis (ATN) rat and then decreased to near normal values after 4 weeks of transplantation [22]. Yun *et al.,* (2016) in their study found that MSC treatment of animals trying OA after 2 months can stimulate a decrease in the regulation of expression of inflammatory cytokines such as iNOS [17]. While the

results of the study of Cosenza *et al.,* (2017) in vitro, Mesenchymal stem cells reduce iNOS gene expression [23]. Xu *et al.,* (2018) also obtained results of a decrease in iNOS expression using umbilical cord mesenchymal stem cells (UC-MSC) [24].

The increase in iNOS levels in this study was due to the immunosuppressive nature of MSC. The immunosuppressive function of MSC is caused by IFNγ along with one of three other proinflammatory cytokines, TNFα, IL-1α, or IL-1β. This cytokine combination provokes the expression of several chemokines and inducible nitric oxide synthase (iNOS) by MSC [9].

The presence of proinflammatory cytokines, MSC facilitates the high expression of iNOS which stimulates NO secretion, thus causing inhibition of T cell proliferation [25]. According to Ren *et al.,* (2008), both in vivo and in vitro studies showed that iNOS-deficient MSC showed reduced inhibiting ability [9]. High NO concentrations can suppress immunity modulation and cause immune cell apoptosis through inhibition in the cell cycle phase G0/G1 [26], inhibition of phosphorylation of the transducer signal and activator of transcription 5 (STAT5) and signal transducers in T cells [27]. The results of this study showed an increase in iNOS levels after being treated by MSC-WJ. This situation shows that MSC-WJ is immunosuppressive.

This study concluded that MSC-WJ significantly reduced ADAMTS-4 levels and increased the serum iNOS levels of OA rats.

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Clinical Science

The Impact of Goal-Directed Fluid Therapy in Prolonged Major Abdominal Surgery on Extravascular Lung Water and Oxygenation: A Randomized Controlled Trial

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Keywords: Fluid therapy; Extravascular lung water

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Abstract

BACKGROUND: A growing interest had been paid to goal-directed fluid therapy (GDT) in abdominal surgery; however, its impact on the respiratory profile was not well investigated.

AIM: We evaluated the impact of GDT on postoperative extravascular lung water and oxygenation after prolonged major abdominal surgery.

METHODS: A randomised, controlled study was conducted in Kasr Alainy hospital from April 2016 till December 2017 including 120 adult patients scheduled for prolonged major abdominal surgery. Patients were randomised into either GDT group (n = 60) who received baseline restricted fluid therapy (2 mL/Kg/hour) which is guided by stroke volume variation, or control group (n = 60) who received standard care. Both study groups were compared according to hemodynamic data, fluid requirements, lung ultrasound score, and PaO2/fraction of inspired oxygen ratio (P/F ratio),

RESULTS: Intraoperatively, GDT group received less volume of fluids and showed higher intraoperative mean arterial pressure compared to the control group. Postoperatively, lung ultrasound score was lower, and P/F ratio was higher in the GDT group compared to the control group. The number of patients who showed a significant postoperative increase in LUS was higher in the control group 44 (73%) patients versus 14 (23%) patients, P < 0.001).

CONCLUSIONS: Using stroke volume variation for guiding fluid therapy in prolonged, major abdominal operations were associated with better hemodynamic profile, less intraoperative fluid administration, lower extravascular lung water and better oxygenation compared to standard care.

Introduction

Major abdominal surgery is characterised by fluid shifts that need meticulous assessment for the volemic status [1], [2]. Inadequate fluid replacement in hypovolemic patients would result in impaired peripheral organ perfusion that might be un-noticed; this would seriously result in postoperative organ dysfunction. Over-infusion of unnecessary fluids in euvolemic patients would result in tissue oedema. Fluid overload would also increase extravascular lung water and impair gas exchange. These unfavourable complications are more likely to happen during lengthy operations. The aim of goal-directed therapy (GDT) is to guide intraoperative fluid replacement using functional hemodynamic targets instead of traditional clinical signs [3], [4]. The impact of GDT on hemodynamic parameters and surgical outcomes was previously investigated; however, its impact on the respiratory profile was only evaluated during thoracotomy [5]. No studies to the best of our knowledge had evaluated the impact of fluid restricted-GDT on extravascular lung water and oxygenation.

Stroke volume variation (SVV) is one of the dynamic parameters used for the evaluation of fluid responsiveness [6]. SVV depends on heart-lung interaction in mechanically ventilated patients. Positive pressure ventilation provokes cyclic changes in stroke volume due to decreased preload

(decreased venous return) in addition to increased afterload (increased trans-pulmonary pressure) [6]. SVV is a frequently used target during GDT in the operating room in high-risk patients [7], [8] as well as moderate and low-risk patients [4]. In the operating room, SVV was measured using Vigileo/FloTrac continuous pulse contour monitor [7], [8], [9], [10], trans-esophageal Doppler [11] and recently, using electrical cardiometry [12], [13], [14], [15].

This work aims to evaluate the impact of GDT on extravascular lung water and oxygenation after prolonged major abdominal surgery.

Methods

A randomised controlled trial was conducted in Cairo University hospital after obtaining research ethics committee approval (N-16-2016). The study was registered at clinical.trials.gov registry system (clinical trial identifier: NCT02845310) on 21 July 2016. The study was conducted between September 2016 and June 2017. An online randomiser was used to by a statistician to create patient codes. Closed, sealed, opaque, sequentially-numbered envelopes were used for concealment. The envelopes were opened by a research assistant. The study included 120 adult patients, aged between 18 years and 65 years, scheduled for major abdominal surgery with an anticipated duration of 180 minutes or more. Patients with cardiac arrhythmias, impaired cardiac contractility, patients with body mass index above 40 kg/m^2, and patients with neck or chest lesions that impair the application of cardiometry electrodes were excluded from the study.

Management of anaesthesia

Upon arrival to the operating room, patients received midazolam (0.05 mg/Kg) and ranitidine (50 mg), and full monitors were applied (ECG, pulse oximetry, and non-invasive blood pressure monitor were applied before induction of anaesthesia; whilst, invasive blood pressure monitor and capnography were applied after induction of anaesthesia). Electrical cardiometry device (ICON; Cardiotonic, Osypka; Berlin, Germany) was applied to the patient through 4 electrodes at the following sites: (1) Below the left ear; (2) Above the midpoint of the left clavicle; (3) Left mid-axillary line on the horizontal level of the xiphoid process; (4) two inches inferior to the third electrode.

Induction of general anaesthesia was achieved using propofol (2 mg/Kg), and fentanyl (2 µg/Kg). The endotracheal tube was inserted by the aid of atracurium (0.5 mg/Kg) after 2-3 minutes of positive pressure ventilation. Anaesthesia was maintained by isoflurane (1-1.5%) and atracurium (10 mg/30min). Morphine (0.1 mg/Kg intravenous bolus) and Ketorolac (30 mg intravenous infusion) were administrated after induction of anaesthesia. Arterial and right internal jugular central venous catheters were inserted. Mechanical ventilation was adjusted at the following settings: Tidal volume 8 mL/Kg, PEEP 5 cm H_2O, and respiratory rate titrated to maintain end-tidal CO_2 at 30-35 mmHg. By the end of the operation, isoflurane was discontinued, and the residual neuromuscular blocking agent was reversed by neostigmine (0.05 mg/Kg), and atropine (0.02 mg/Kg) and the patient was extubated and transferred to the post-anaesthesia care unit.

Fluid therapy

After induction of anaesthesia, all patients received an initial bolus of 5 mL/Kg lactated Ringer's solution. Then, patients were randomised into either the GDT group and control group.

GDT group: In this group, fluid therapy was restricted to 2 mL/Kg/hour. SVV was evaluated every 10 minutes, and a fluid bolus of 3 mL/Kg lactated Ringer's solution was infused to reach a target SVV less than 10%. If the total volume of fluid boluses reached 20 ml/Kg, no additional boluses were infused unless there was evident blood loss or hypotension. If mean arterial pressure (MAP) was not achieved despite reaching 20 mL/Kg infused fluids, norepinephrine infusion was planned to start in the central venous line with an initial dose of 0.01 mcg/kg/min.

Control group: In this group, lactated Ringer's solution was infused at a rate of 6 mL/Kg/hour. Additional boluses of lactated Ringer's (200 mL) were infused if MAP was below 65 mmHg and CVP was below 8 mmH_2O. Norepinephrine infusion was planned to start at an initial dose of 0.01 mcg/kg/min if MAP was below 65 mmHg and CVP above 8 mmH_2O.

In both groups, Packed RBCs were transfused if 1) Estimated blood loss was more than 20% of whole blood volume with MAP lower than 65 mmHg. 2) blood haemoglobin level was lower than 7 g/dL. 3) Continuous blood loss with MAP < 65 mmHg. Bleeding patients who did not meet the criteria for Packed RBCs transfusion were resuscitated by lactated Ringer's solution at a ratio of lactated Ringer's solution: blood loss = 3:1).

Lung ultrasound examination

Lung ultrasound was performed by a skilled operator who was blinded to the study group. A Mindray device (DC-N6, with a phased array transducer, model P4-2, 3-6 megahertz) was used for 12-region lung ultrasound examination according to the following protocol [16]. We used the following definitions: 1) B-line: “laser-like vertical hyperechoic artefact which extends between the pleural line and

the bottom of the screen, and moves with respiration". 2) B-7 lines: these lines are characterised by being 7 mm apart, and they denoted the presence of interstitial oedema. 3) B-3 lines: these lines are characterised by being 3 mm apart, and they denoted the presence of alveolar oedema. 4) Lung consolidation: "sub-pleural, hypoechoic, wedge-shaped, tissue-like structure".

All the 12 spaces were screened vertically, and each hemithorax was sub-divided into 6 areas (2 anterior areas, 2 lateral areas, and 2 posterior areas).

Lung ultrasound score (LUS) was then calculated [16]:

- The B-line score was estimated for each area according to the following protocol: zero: no lines, 1: B-7 lines, 2: B-3 lines, 3: consolidation.

- LUS was further calculated (ranging from 0 to 36) as the sum of B-line score of the 12 zones.

Primary outcome

Our primary outcome was LUS which was evaluated two times: a baseline preoperative measurement and a postoperative measurement which was obtained in the post-anesthesia care unit. The change in LUS delta-LUS was defined as the difference between the two measures: Postoperative LUS-baseline LUS. The number of patients with increased postoperative LUS by 3 or more was also compared between both groups.

Secondary outcomes

Intraoperative fluids: total intraoperative fluid requirements, number of fluid boluses, number of patients requiring vasopressors, and urine output

Hemodynamic data: MAP, heart rate, and central venous pressure (evaluated every 5 minutes starting from the baseline preoperative reading till patient discharge from the post-anesthesia care unit)

Demographic data: age and gender.

Surgical data: surgical duration, blood loss, and type of operation.

Postoperative data: postoperative *p*H, HCO_3, PCO_2, P/F ratio (defined as PaO_2 / Fraction of inspired oxygen), blood haemoglobin, length of ICU stay, and incidence of surgical complications.

Statistical analysis and sample size calculation

Our primary outcome was LUS in the post-anesthesia care unit. In a pilot study on 10 patients, the mean postoperative LUS in patients undergoing prolonged major abdominal surgery under standard care was 5 ± 0.9. Using MedCalc Software version 14.10.2 (MedCalc Sofware bvba, Ostend, Belgium), we calculated a sample size that would detect a mean difference of 10% (i.e. 0.5) in LUS between both study groups. The minimum number needed to have a study power of 80% and an alpha error of 0.05 was 104 patients (52 patients per group). This number was increased to 120 patients (60 patients per group) to compensate for possible drop-outs.

Statistical package for social science (SPSS) software, version 15 for Microsoft Windows (SPSS inc., Chicago, IL, USA) was used for data analysis. Categorical data were presented as frequency (%) and analysed using the chi-square test. Continuous data were checked for normality using the Shapiro-Wilk test and was presented as mean (standard deviation) or median (interquartile range) as appropriate. Continuous data were analysed using either unpaired t-test or Mann Whitney as appropriate. Repeated measures were analysed using analysis of variance (ANOVA) for repeated measures with post-hoc pairwise comparisons using the Bonferroni test. A P value less than 0.05 was considered statistically significant.

Results

One hundred and twenty patients were available for final analysis (Figure 1). The mean age of our patients was 50 ± 13 years, and the mean surgical duration was 4 ± 0.7 hours. Seventy-four (62%) of our patients were males.

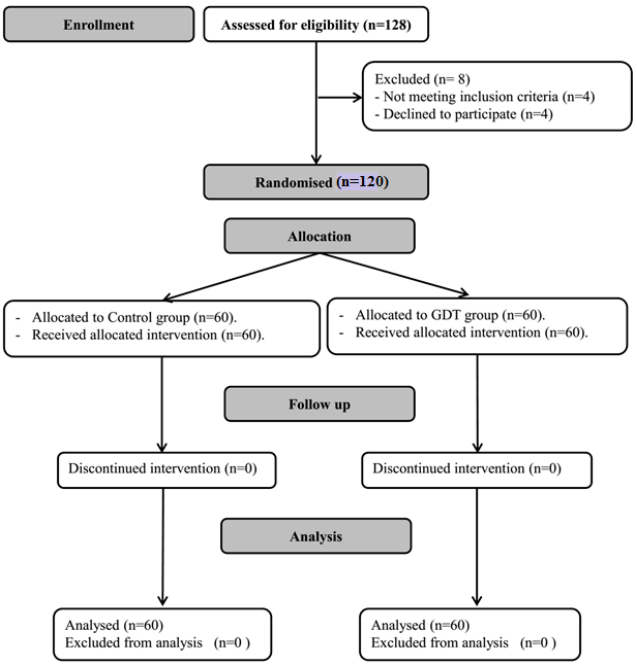

Figure 1: CONSORT chart showing patient recruitment; GDT: goal-directed therapy

The surgical procedures in our patients were

Whipple's operation (12%), gastrectomy (13%), colorectal resection (45%), common bile duct exploration (25%), and abdominal exploration (4%).

Demographic data (age, gender, and co-morbidities) and baseline measurements were comparable between both study groups (Table 1).

Table 1: Demographic data and baseline characteristics. Data are presented as mean (standard deviation) and frequency (%)

	GDT group (n = 60)	Control group (n = 60)	P value
Age (years)	49 (13)	50 (12)	0.25
Male gender	36 (60%)	38 (63%)	0.85
Diabetes	6 (10%)	7 (12%)	0.95
Hypertension	11 (18%)	10 (17%)	0.92
Smoking	9 (15%)	11 (18%)	0.84
Baseline hemodynamic data			
Heart rate (bpm)	86 (16)	84 (12)	0.4
Mean arterial pressure (mmHg)	93 (13)	90 (14)	0.2
Central venous pressure	7.3 (2.5)	7.1 (2.2)	0.61

GDT: Goal-directed therapy. * denotes statistical significance.

The GDT group had a slightly shorter surgical duration and lower blood loss compared to the control group (Table 2).

Table 2: Intraoperative data. Data are presented as mean (standard deviation) and median (quartiles)

	GDT group (n = 60)	Control group (n = 60)	P value
Surgical duration (hours)	4.3 (0.5) *	4.7 (0.7)	< 0.001
Blood loss (mL)	535 (159) *	645 (227)	0.008
Blood loss per hour (mL)	125 (34)	135 (37)	0.08
Urine output (mL)	400 (400, 500) *	500 (400, 700)	< 0.001
Urine output per hour (mL)	100 (125, 150) *	140 (100, 165)	< 0.001
Total crystalloids (mL)	1512 (478) *	3048 (784)	< 0.001
Total crystalloids per hour (mL)	354 (101) *	637 (85)	< 0.001
Number of fluid boluses	1.38 (2.2)	1.4 (2.2)	0.97

GDT: Goal-directed therapy. * denotes statistical significance.

The GDT group received markedly lower intraoperative fluids and showed higher MAP compared to the control group (Figure 2).

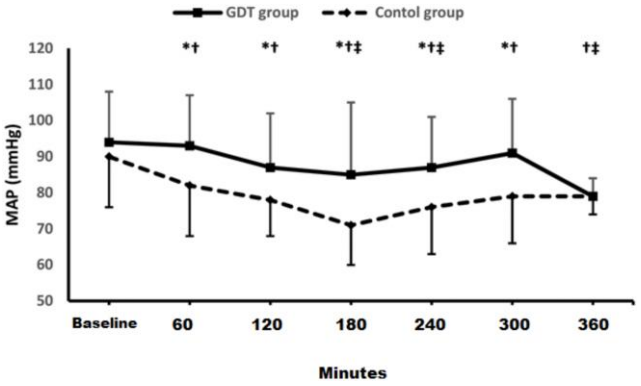

*Figure 2: Mean arterial pressure; GDT: goal-directed therapy; MAP: mean arterial pressure; * denotes statistical significance between both groups; † denotes statistical significance within GDT group compared to the baseline reading; ‡ denotes statistical significance within control group compared to the baseline reading*

Heart rate and CVP readings were comparable between study groups. None of our patients received vasopressors. Postoperatively, the GDT group had lower LUS and higher P/F ratio compared to the control group (Table 3).

Table 3: Postoperative data. Data are presented as mean (standard deviation) and median (quartiles)

	GDT group (n = 60)	Control group (n = 60)	P value
Baseline LUS	0 (0,2)	0 (0,2)	0.95
Post-operative LUS	2.4 (2.2) *	4.2 (2.7)	< 0.001
Delta LUS	1.6 (1.6) *	3.3 (2)	< 0.001
Post-operative P/F ratio	368 (37) *	353 (39)	0.04
Post-operative *p*H	7.36 (7.34, 7.38)	7.36 (7.33, 7.38)	0.78
Post-operative Pco_2 (mmHg)	34.4 (3.4)	34.7 (3.6)	0.73
Post-operative HCO_3 (mg/dL)	23 (3)	22 (3)	0.12
Postoperative hemoglobin (g/dL)	12.7(1.4)	12.9(1.6)	0.44

The number of patients who showed significant postoperative increase in LUS was higher in the control group [44/60 (73%)] patients versus 14/60 (23%) patients, P < 0.001). Postoperative arterial blood-gas analysis (HCO_3 and *p*H) was comparable between both groups (Table 3). Length of postoperative ICU-stay and frequency of postoperative complications were also comparable between both groups.

Discussion

We reported that restricted SVV-guided fluid protocol during prolonged major abdominal operations resulted in less fluid administration, higher MAP, less lung congestion, and better oxygenation compared to traditional, standard fluid therapy. We reported favourable respiratory outcomes (lower extravascular lung water and higher P/F ratio) in the GDT therapy group. This is most probably due to the marked reduction in fluid requirements in the GDT group. This is the first study that addresses the impact of GDT on postoperative respiratory profile. Our findings suggest that GDFT is would be beneficial not only in high-risk cardiovascular patients but also in patients with compromised respiratory status. All our patients had prolonged surgery (above 3 hours); this special population would be more sensitive to fluid overload.

Although the patients in the GDT group received less intraoperative fluids, they had higher MAP compared to the control group. This better hemodynamic profile is explained by the accurate evaluation of volume status using SVV. Monitoring of SVV allowed early detection and correction of hypovolemia; and allowed providing the appropriate volume of fluids in the appropriate time. However, we should also clarify that although MAP was lower in the control group, MAP was still in within the acceptable limits.

Our protocol in the study group was based on a restrictive fluid rate (2 mL/Kg/hour) with supplementary boluses to correct hypovolemia. The fluid restriction had been increasingly recommended in the operating room [7], [17] especially in major surgery [18]. In a meta-analysis, school et al. had reported that restrictive fluid therapy is associated with less perioperative complications compared to liberal fluid therapy [17]. We used SVV as an index of volume status. SVV was previously used for guiding

fluid therapy in the operating room [4], [7], [8] as well as the intensive care unit [19]. In our study, we chose a special population undergoing prolonged (above 3-hour duration), major abdominal surgery.

We used electrical cardiometry for measurement of SVV. Electrical cardiometry has the advantage of being non-invasive, simple, user-friendly, and does not need expensive disposables. Electrical cardiometry was previously evaluated in the operating room in human patients [15], [20]; as well as animals [12].

The collective evidence about the value of GDT in the operating room is controversial; This is most probably to the high heterogeneity in the meta-analyses which investigated GDT. GDT is a term with a broad spectrum of targets such as SV, CO, oxygen delivery, and heart-lung interaction parameters. Specifically, most of the previously reported fluid protocols which optimised dynamic targets (including SVV) showed good outcomes [3], [9], [10]. In a study conducted by Feng et al., they suggested that the use of perioperative GDT might facilitate recovery in patients undergoing noncardiac surgery combined with the application of alpha-1 adrenergic agonists [21]. A meta-analysis had reported that GDT did not improve postoperative outcomes after major abdominal surgery; however, the studies included in this meta-analysis compared GDT to standard care in the context of enhanced recovery after surgery setting [1].

Our study had some limitations. It is a single-centred study. Most of our patients were elective and not emergency patients. Although the lung ultrasound was performed by a blinded physician, we were not able to blind the anaesthetist responsible for patient management.

In conclusion, using SVV for guiding fluid therapy in prolonged, major abdominal operations were associated with higher MAP, less intraoperative fluid administration, lower extravascular lung water and better oxygenation compared to standard care.

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Clinical Science

The Role of Presepsin in Patients with Acute Surgical Diseases

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Keywords: Intra-abdominal hypertension; Compartment syndrome; Presepsin; sCD14: D-dimer

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Abstract

AIM: The purpose of this study was to determine the level of significance of markers in the development of intra-abdominal hypertension in patients with acute surgical diseases of the abdominal cavity.

METHODS: The authors surveyed 100 patients who were monitored at the Regional Clinical Hospital, Karaganda. The criterion for inclusion in the study was the informed consent of patients to participate in the study, the presence of acute surgical pathology, and the monitoring of intra-abdominal pressure over time. The exclusion criteria for patients from the study is the presence of sub and decompensation of associated diseases: trauma (hematoma of the bladder), bladder tumour and impaired integrity of the pelvic ring. The design of the study was by the legislation of the Republic of Kazakhstan, international ethical norms and normative documents of research organizations, approved by the ethics committee of the Karaganda State Medical University.

RESULTS: According to the world scientific literature, there are 4 indicators that change their value in response to increases in pressure in the abdominal cavity: fibrinogen and prothrombin index (the main indicators of the coagulogram); marker of blood clots D-dimer; early marker of translocation of bacterial flora into the bloodstream sCD14 (presepsin).

CONCLUSION: The authors concluded that the obtained data indicate that an increase in intra-abdominal pressure in acute surgical diseases of the abdominal cavity causes hypercoagulation and an increase in presepsin. Monitoring IAP with simultaneous measurement of the level of presepsin significantly improves the stratification of critical patients in need of emergency surgery.

Introduction

Intraabdominal hypertension (IAH, intraabdominal pressure, IAP) is one of the most dangerous complications of the abdominal catastrophe in patients with acute surgical diseases. The main reason for the lethality from abdominal compartment syndrome is the translocation of microorganisms with the development of abdominal sepsis. Surgical abdominal sepsis takes 30% from all it cases [1] and is the principal cause of the death rate in the surgical departments of intensive therapy (IT) [2], [3]. The multivariable character of development of acute surgical pathology of abdominal organs, a complication of pathogenesis, creates problems for determination of diagnostics criteria and an adequate surgical correction. The traditional biomarkers of systemic inflammatory response syndrome (SIRS) or abdominal sepsis are not always sufficiently informative for early diagnosis and monitoring of systemic infection.

Currently focuses on the study of presepsin and D-Dimer for an in-depth understanding of SIRS, abdominal sepsis and severe sepsis.

Presepsin is the circulating concentration of protein in the blood increases rapidly with the

development of systemic infections, sepsis, severe sepsis and septic shock, it was first described in 2005 by a group of researchers from the Medical University, Iwate, Japan [4].

Presepsin is a membrane glycoprotein with a molecular weight of 55 kDa. The mCD14 is expressed on the surface of monocytes/macrophages, neutrophils, chondrocytes, the cells, dendritic cells and other mature myeloid cells [5], [6].

The mCD14 is a receptor, which "learns" the signal of the infecting bacteria presence and turns the nonspecific immunity system and inflammatory process on [7], [8].

The mCD14 may independently communicate with LPS and turns activation signal of macrophages, a special lipopolysaccharide-binding protein on (LSB, LBP lipopolysaccharide-binding protein), improves the effectiveness of such a binding in the 100-1000 times.

In vivo, at low LPS (low numbers of bacteria that can grow quickly) LSB in advance "strengthens" signal for activation of the inflammatory response [9].

In addition to endotoxin of gram-negative bacteria, LSB specifically binds to cell wall components: gram-positive bacteria — lipoteichoic acids, peptidoglycans [10]; mycobacteria — lipoproteins, lipomannans [11]; Mycoplasma — lipopeptides [12]; spirochete — glycolipids and lipoproteins [13] and fungi [14]. The spectrum of microorganisms, activating monocytes/macrophages through interaction with mCD14 are very wide. The mCD14 receptor associated with the LSB-LPS complex is activated and transmits a signal to the TLR4 co-receptor located next to the membrane and related to the so-called Toll-like receptors (Toll-like receptor), which activate non-specific immunity. After activation of macrophages mCD14 is disconnected from the membrane, goes into circulation and becomes soluble sCD14 (s-soluble). sCD14 function-is induction of inflammation in endothelial and other cells that do not have mCD14 and do not respond to endotoxins. It is assumed that the circulating sCD14-marker of monocyte response to the action of LPS; increased blood sCD14 levels are associated with the severity of inflammation and septic shock [15].

Presepsin is an early biomarker of sepsis development, which reliability is 100%, confirmed by hemocultures, diagnoses sepsis is defined before manifestation of clinical symptoms and predicts treatment outcomes, with dynamic monitoring, reflects the real severity of sepsis, changes rapidly depending on the effectiveness of therapy, predicts recurrence of sepsis after remission, when clinical characteristics temporarily normalize [16], [17], [18], [19], [20], [21], [22].

The purpose of the study was to assess the relationship of the level of presepine with the development of intraabdominal hypertension in patients with acute surgical deseases.

Material and Methods

The present study is based on clinical observations of 100 patients with various acute surgical diseases who were hospitalised and operated in the regional clinical hospital of Karaganda in 2017. Among them 52 (52%) men and 48 (48%) women. The number of men as a whole exceeded the number of women by an average of 1.08 times.

The design of this study was approved by the ethical Commission at Karaganda state medical University.

The age of patients ranged from 20 to 80 years (mean age — 46.66 years). At the same time, the age composition was dominated by young patients: younger than 25 years – (7.3%), 25-44 years – (38.1%), 45-60 years – (29.3%), older than 60 years – (25.3%) patients. It was found that the prevalence of diseases of the abdominal cavity is high in the groups of patients 25-44 and 45-60 years.

Clinical and laboratory-instrumental examination of patients with acute surgical pathology before surgery allowed to distribute patients according by nosology (the structure of patients):

- acute appendicitis 16 (16%),

- acute intestinal obstruction 37 (37%) patients,

- pancreonecrosis 5 (5%),

- perforated ulcer 22 (22%) patients,

- acute cholecystitis (mechanical jaundice) 15 (15%) patients,

- varicose veins of the lower extremities (control group) 5 (5%) patients.

Criteria for inclusion of patients in the study were the presence of acute surgical pathology: acute intestinal obstruction, acute appendicitis, pancreatic necrosis, perforated ulcer, acute cholecystitis (mechanical jaundice),

Control group is the patients with varicose veins of the lower extremities; the age of patients 20-80 years of age; disease duration more than 24 hours.

Group of patients:

1. group-patients with IAD within normal limits (0-4 mmHg).

2. group-patients with IAD 5-15 mmHg art.

3. group-patients with IAD 16-25 mmHg art.

4. group-patients with IAD 26-35 and more mmHg.

A common criterion for excluding patients from the study (for all groups) is the presence of sub- and decompensation (remission) of comorbidities,

injury (hematoma) and swelling of the bladder. Concomitant pathology in the studied patients was in remission, which was confirmed by anamnesis and clinical and laboratory.

Measurement of IAP was carried out using the device "Triton-electronics", invasive portable electronic meter Autonomous Central venous pressure and other low pressures in various cavities of the human body.

25 or 50 ml of warm sterile isotonic sodium chloride solution was injected into the emptied bladder with a syringe without a needle. The urinary catheter was connected to the device "Triton-electronics". The zero value was set at the level of the pubic junction.

Currently, this method is the "gold standard" for indirect measurement of intraabdominal pressure. All patients underwent 3 measurements of intraabdominal pressure-before surgery, as well as 6 and 24 hours after surgery.

As a result of the IAP measurement of all patients before surgery were divided into 4 groups depending on the degree of IPG:

0 group-IAP 0-11 mm Hg, 6 patients (6%),

group 1-IAP 12-15 mm Hg, 9 patients (9%),

group 2-IAP 16-20 mm Hg, 26 patients (26%),

group 3-IAP 21-25 mm Hg, 23 patients (23%),

group 4-IAP more than 25 mm Hg, 36 patients (24%).

Results

According to the world scientific literature, there are 4 indicators that change their value in response to increased abdominal pressure: fibrinogen and prothrombin index (PTI) (the main indicators of coagulation); a marker of thrombosis D-dimer; an early marker of bacterial flora translocation into blood flow sCD14 (presepsin).

Statistical processing of the results of the study was carried out by methods of variation statistics with the calculation of each indicator of the median and standard deviation (sd). The significance of differences in the groups was determined using nonparametric methods of statistical evaluation: peer-to-peer analysis of variance (ANOVA). Differences were considered statistically significant at $p < 0.05$.

Table 1 shows the median and standard deviation (SD) of these markers by groups.

Table 1: Average and spread of markers value by groups

IAP до	Count	Prothrombin index		Fibrinogen		sCD14		D-dimer	
		Median	SD	Median	SD	Median	SD	Median	SD
< 12	6	90.9	30.1	3.5	0.782	246	162	5.88	13.5
12-15	9	90.1	9.41	4.68	1.15	570	186	24.6	11.2
16-20	26	87.9	18.1	4.65	2.22	651	384	35.3	33.2
21-25	23	78.6	24.3	5.1	1.65	656	336	36.5	35
> 25	36	78.7	26.4	5.94	2.82	800	572	28.2	18.1

The median of the prothrombin index in the blood plasma decreased with an increase in intra-abdominal pressure (IAP). Although the spread of this indicator in each group is quite large, it is larger than the difference of averages between any groups. This difference is not statistically significant. The figure below clearly shows that the values of this indicator are the same in all groups.

The median of the prothrombin index in the control group is 90.9%, which is within the known norm of PTI 80-110%.

IAP 12-20 mmHg (group 1 and 2)-normal values.

IAP 21-25 and more mmHg (group 3 and 4) leads to decrease of PTI compared to normal values (there are statistically significant differences compared to the control group and group 1 and 2, in the direction of hypercoagulation, which possibly associated with a massive flow of tissue thromboplastin into the bloodstream (Figure 1).

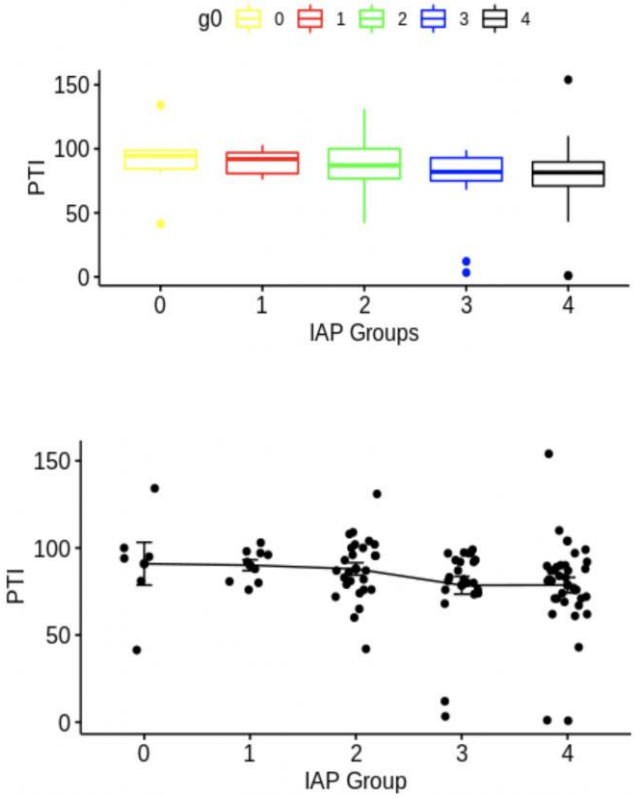

Figure 1: Dependence of the content of PTI in plasma on the level of IAP

The values of fibrinogen, CD14 and D-dimer

in the control group are less than in other groups.

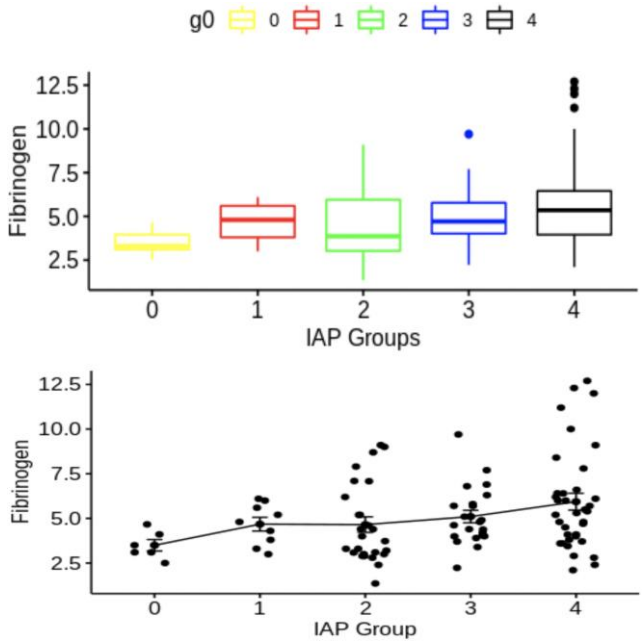

Figure 2: Dependence of the content of fibrinogen in plasma on the level of IAP

The content of fibrinogen in the blood plasma increases in proportion to the increase in pressure in the abdominal cavity. There is a statistically significant difference in the groups with IAH and control group (0 groups). Thus, the average concentration of fibrinogen in the control group is 3.5 g/l, which is within the known norm of fibrinogen 2.0 – 4.0 g/l (Figure 2).

IAP 12-20 mmHg (group 1 and 2) causes a slight increase in the concentration of fibrinogen compared to normal values (also lies within normal values).

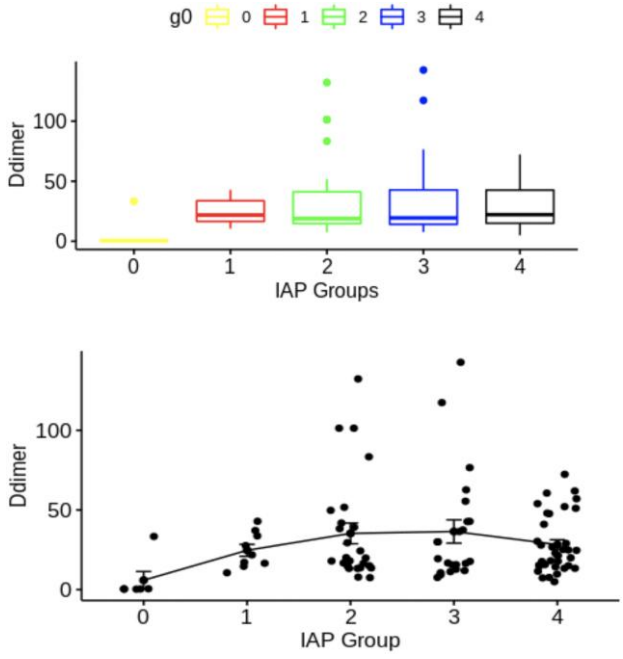

Figure 3: Dependence of the content of D-dimer in plasma on the level of IAP

IAP 21-25 mmHg (group 3) causes an increase in fibrinogen concentration compared to normal values (there are statistically significant differences compared to the control group and group 1 and 2.

Statistically significant changes in fibrinogen level are observed at IAH 25 mmHg and more (group 4) in the direction of hypercoagulation, which possibly associated with organ dysfunction and consumption coagulopathy.

The average concentration of D-dimer in the control group is 5.8 ng/ml.

IAP 12-15 mmHg (group 1) causes a significant increase in the concentration of D-dimer compared with the control group in the direction of hypercoagulation 4 times compared with normal values (Figure 3).

IAP 16-25 and above mmHg (group 2, 3 and 4) causes a pronounced statistically significant increase in the concentration of D-dimer in the direction of hypercoagulation by 5-6 times compared to normal values, which confirms the experimental data; thus, the determination of the d-dimer level is relevant in the development of IAH, since even with IAP 12-15 mmHg, there is a clear statistically significant dynamics to increase this indicator.

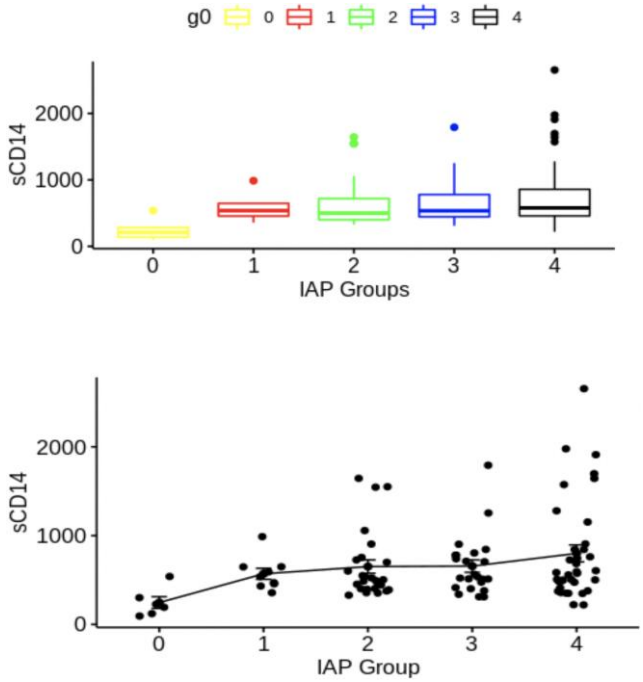

Figure 4: Dependence of the content of sCD14 in plasma on the level of IAP

The average concentration of the sCD14 biomarker of the control group is 246 ng/ml (Figure 4).

IAH 12-15 mmHg (group 1) causes statistically significant changes in the concentration of

sCD14 compared with the control group.

IAH 16-25 mmHg (group 2 and 3) causes an increase of the concentration of the biomarker sCD14 compared with normal values by 30% (there are statistically significant differences compared with the control group and group 1), which confirms the previously conducted experimental studies on the beginning of the enterogenous translocation of the bacterial flora into the bloodstream IAP level;

With IAH 25 or more mm Hg (group 4) there is acute increasing of the concentration of sCD14 almost 4 times, which probably indicates the development of a “preseptic” state.

In conclusion, the literature data and our results are allowed us to formulate a working hypothesis about the change in the level of presepsin, fibrinogen, d-dimer, which increase sharply in response to an increase in IAP, which leads, respectively, to hypercoagulation with further organ dysfunction, consumption coagulopathy.

Having estimated the level of presepsin with the development of intraabdominal hypertension, there is a clear positive correlation with the high sensitivity, specificity (a high level of statistical significance $p < 0.01$ is determined).

Thus, it can be proposed to use the determination of presepsin level in patients with acute surgical pathology of the abdominal cavity as a routine method along with the determination of fibrinogen, PTI, D-dimer to assess the stratification of the risk of abdominal sepsis, prognosis of the course and outcome of the disease, as well as timely surgical treatment.

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Comparison of Contrast Enhanced Low-Dose Dobutamine Stress Echocardiography with 99mTc-Sestamibi Single-Photon Emission Computed Tomography in Assessment of Myocardial Viability

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Keywords: Coronary artery disease; Myocardial perfusion scan; LV endocardial visualisation; Myocardial ischemia; LV dysfunction

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Abstract

INTRODUCTION: Dobutamine stress echocardiography (DSE) and myocardial perfusion scan are the commonly used modalities to detect viable myocardium. DSE is comparatively cheaper and widely available but has a lower sensitivity.

AIM: We aimed to compare contrast-enhanced low-dose dobutamine echocardiography (LDDE) and gated 99mTc-sestamibi myocardial perfusion scan (MPS) for the degree of agreement in the detection of myocardial viability.

METHODS: We studied 850 left ventricular segments from 50 patients (42 men, mean age 55.5 years), with coronary artery disease and left ventricular systolic dysfunction (ejection fraction < 40%), using contrast-enhanced LDDE and 99mTc-Sestamibi gated SPECT. Segments were assessed for the presence of viability by both techniques and head to head comparisons were made.

RESULTS: Adequate visualisation increased from 80% in unenhanced segments to 96% in contrast-enhanced segments. Of the total 850 segments studied, 290 segments (34.1%) had abnormal contraction (dysfunctional). Among these, 138 were hypokinetic (16.2% of total), 144 were severely hypokinetic or akinetic (16.9% of total), and 8 segments were dyskinetic or aneurismal (0.9% of total). Among 151 segments considered viable by technetium, 137 (90.7%) showed contractile improvement with dobutamine; in contrast, only 8 of the 139 segments (5.7%) considered nonviable by technetium had a positive dobutamine response. The per cent of agreement between technetium uptake and a positive response to dobutamine was 78.6% with kappa = 0.63, suggestive of a substantial degree of agreement between the two modalities.

CONCLUSION: Use of contrast-enhanced LDDE significantly increased the adequate endocardial border visualisation. Furthermore, this study showed a strong degree of agreement between the modalities in the detection of viable segments. So, contrast-enhanced LDDE appears to be a safe and comparable alternative to MPS in myocardial viability assessment.

Introduction

Left ventricular dysfunction associated with coronary artery disease (CAD) is a common cause of morbidity and mortality. However, reports show that > 50% of such patients may have viable myocardium [1]. Revascularization improves left ventricular ejection fraction (LVEF), heart failure symptoms and prognosis predominantly in patients with viable myocardium [2], [3], [4], [5], [6], [7], [8]. Though some recent studies provided contrary results, they had several significant limitations and confounding factors [9], [10].

Based on robust evidence on viability assessment, American Heart Association (AHA) has given class IIa recommendation for non-invasive imaging in patients with heart failure who have known CAD and no angina [11]. Guidelines from other societies also recommend detection of viability in the diagnostic workup of patients with CAD and severe

ventricular dysfunction [12], [13].

Most widely available and commonly used methods for this purpose are dobutamine stress echocardiography (DSE) and SPECT. Tc-99m sestamibi is the most widely used tracer in SPECT. The main limitations of SPECT include, higher cost compared to echocardiography, limited spatial resolution, poor availability, potential difficulty in interpreting results in patients with balanced myocardial ischemia (3-vessel disease), and risk of radiation. Higher cost and limited availability are the most important limitations in resource-poor countries like India. Results of DSE are immediately available, can be done bedside in sicker patients significantly supporting rapid patient management. An obvious advantage is the availability of ancillary information about chamber sizes and function, valves, pericardial effusion, aortic root disease. Additional parameters of viability can also be assessed like strain imaging, Doppler studies, and end-diastolic wall thickness (EDWT). However, in patients with chronic ischemic ventricular dysfunction, DSE has higher specificity (78% vs 65%) but a slightly lower sensitivity (81% vs 83%) compared to SPECT [14], [15], [16].

Recent advances in echocardiographic imaging have, however, significantly increased the sensitivity and interobserver agreement for the detection of coronary artery disease. These include harmonic imaging and LV opacification with contrast agents. Contrast-enhanced imaging, enable better visualisation of the endocardial border and a more reliable assessment of contractile function even in patients difficult to image with fundamental imaging. Therefore, contrast-enhanced imaging has been regularly used in DSE for myocardial detection ischemia in cases with ≥ 2 poorly visualised segments as recommended by various guidelines [17], [18], [19]. However, contrast enhancement has not been used in the setting of viability assessment by low dose dobutamine echocardiography (LDDE). Hence combining contrast with LDDE to increase sensitivity for viability assessment appears an attractive option. However, there is no head to head trial comparing the sensitivity of contrast-enhanced dobutamine stress echocardiography against myocardial perfusion SPECT. This study aimed to assess the degree of agreement between the two methods in detecting viability in dysfunctional myocardial segments.

Material and Methods

Study population

This was a single centre, double-blinded, a comparative study between DSE and Tc-SPECT. The study period was from May 2014 to December 2016, carried out in the department of cardiology, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, India. Fifty consecutive patients with chronic coronary artery disease and impaired left ventricular systolic function (ejection fraction, EF < 40% at rest) without chest pain were included in the study. The study was conducted according to the Helsinki Declaration and the Good Clinical Practice Guidelines. Written informed consent was obtained from all patients. Ethical approval for the study protocol was obtained from the Ethics Committee of JIPMER.

Study design

The patients included in the study first underwent low dose dobutamine stress echocardiography (LDSE) with proper safety precautions in the cardiology department. As per protocol, the contrast agent was administered to patients both before and after stress, for better visualisation of wall motion abnormality. Dobutamine infusion was then continued to a high dose to attain peak stress. At peak stress, technetium was injected, and the patient was shifted for stress SPECT in the nuclear medicine department of the institute. Rest SPECT was done on a separate day. Two-day rest/stress protocol was used for SPECT study [20], [21], [22]. Both procedures were done in short interval maximum one week apart. 17-segment heart model, as recommended by ACC/AHA, was used for defect localisation and comparison between two modalities [23]. The cardiologist doing the LDSE was blinded to SPECT results and vice-versa.

Dobutamine stress echocardiography protocol [17], [18], [19]

Beta-blockers, CCBs and nitrates were stopped at least 2 days before DSE. The subjects were kept fasting for at least 4 hours. 2 ml of SonoVue ultrasound contrast (Sulphur Hexafluoride microbubbles, Bracco, Switzerland) was used for LV opacification at baseline and after dobutamine stress in all patients. The contrast was used with harmonic imaging and low MI settings (Mechanical Index, 0.15 to 0.3). Four views were acquired in all patients: apical four chamber and two chamber views, parasternal short axis and long axis (or long apical axis) views. The frame rate was kept > 25 frames per second. The initial infusion of dobutamine was 2.5 µg/kg/min and gradually increased to 5, 7.5, 10, and 20 µg /kg/min, as required. Each dose was maintained for up to five minutes. An increase in heart rate by 10% was taken as end-point for completion of low dose dobutamine protocol. After completing the low dose protocol higher dosages of dobutamine (30 and 40 mcg/kg/min) was given to achieve peak stress so that stress SPECT could be done. All patients were continuously monitored for at least 30 min along with all emergency equipment.

All image acquisition and analysis were done by Philips IE33 system (*Phillips* Medical Systems, Andover, MA, USA), using 5 MHz probes. Off-line visual assessment of endocardial excursion and wall thickening was used for analysis. Function in each segment is graded at rest and with stress as normal, hypokinetic, akinetic/severely hypokinetic, dyskinetic, or aneurismal. Segments were considered dysfunctional when the wall motion score was 2 or more. Viability was considered to be present in a dysfunctional segment when there was an improvement in function by at least one grade, except improvement from dyskinesia to akinesia.

Myocardial perfusion scan protocol [20], [21], [22]

The stress SPECT was done on the same day of DSE, by using the ongoing dobutamine infusion to achieve peak stress. The radiotracer (Tc-99m) was injected intravenously at 1 minute into peak stress, and dobutamine infusion was continued for 2 minutes after the radiotracer injection. 30 min after injection of 99mTc-sestamibi, the patients were required to eat a fatty snack to accelerate hepatobiliary excretion of the radiotracer. For stress imaging, 555 MBq-1.11 GBq (15 – 30 mCi) was injected at peak stress. Gated SPECT was then performed from 30-60 minutes after injection. On a separate day rest, myocardial perfusion scan was done. For rest imaging, 555 MBq – 1.11 GBq (15-30 mCi) was injected at rest. 5 mg of sublingual nitrate was taken by patients 10 minutes before 9mTc-sestamibi injection. Gated SPECT was then performed within 45-60 min after injection.

The images were displayed as short axis, vertical and horizontal axes sections. Uptake was measured semiquantitatively based on the visual interpretation of colour scale. Segments were divided into 5 categories: 0 = normal perfusion, 1 = slightly reduced tracer uptake (> 75%), 2 = moderately reduced tracer uptake (50% t o75%), 3 = severely reduced tracer uptake (30% to 50%), and 4 = absent tracer uptake (< 30%). As a general rule, segments with rest score of 0 (normal perfusion) and 1 (slight reduction in counts) are considered viable. Segments with rest score of 2 (moderately decreased perfusion) are considered to represent a combination of viable and nonviable myocardium. In dysfunctional segments, perfusion was considered preserved when activity was 50% or more (score 0, 1 or 2).

Statistical analysis

Continuous variables are expressed as mean ± SD and categorical variables as the absolute values and percentages. A chi-squared test was used to compare the statistical parameters of this technique. The correlation between the two tests for the assessment of myocardial segment viability was expressed as per cent agreement and value of Kappa (k). A p-value < 0.05 was considered statistically significant. Statistical analysis was performed using an SPSS software version 18.0.

Results

In total, 50 patients constituted the final study population. Mean age was 55.5 ± 10.7 years and 42 were males (84%). The mean LVEF by echocardiography, calculated by Simpson's method, was 32 ± 4.6% (range = 21-40). The mean LVEF calculated by gated-SPECT was 29.5 ± 4.6% (range = 20-42). Twenty-seven patients had diabetes (54%), 21 were hypertensive (42%), 20 were a smoker (40%), and dyslipidaemia was seen in 19 patients (38%). Coronary artery disease was defined as > 50% diameter stenosis. Single vessel disease was seen in 13 patients (26%), double vessel disease in 18 patients (36%), triple vessel disease in 21 patients (42%), and left main disease was found in 9 patients (18%), (Table 1).

Table 1: Baseline characteristics of the study population

Variables	
Age 55.5 ± 10.7 years	(range = 25-76 years)
Male 42 (84)	
LVEF 32 ± 4.6%	(range = 21-40)
CV risk factors	
Diabetes	27 (54%
Hypertension 21 (42)	
Smoker	20 (40)
Dyslipidemia	19 (38)
Past ACS 24 (48)	
Severity of CAD	
SVD	13 (26)
DVD	18 (36)
TVD	21 (42)
M disease 9 (18)	

Values shown represent numbers (percentages) and mean ± SD; LVEF, Left ventricular ejection fraction; CV, Cardiovascular; ACS, Acute coronary syndrome; CAD, Coronary artery disease; SVD, Single-vessel disease; DVD, Double-vessel disease; TVD, Triple-vessel disease; LM, Left-main.

Low-Dose Dobutamine Stress Echocardiography (LDDE)

A total of 850 segments from 50 patients were analysed, based on the 17 segment model of heart both LDDE and Tc-SPECT (Figure 1). Out of 850 segments, 170 segments were inadequately visualised at rest in unenhanced DSE.

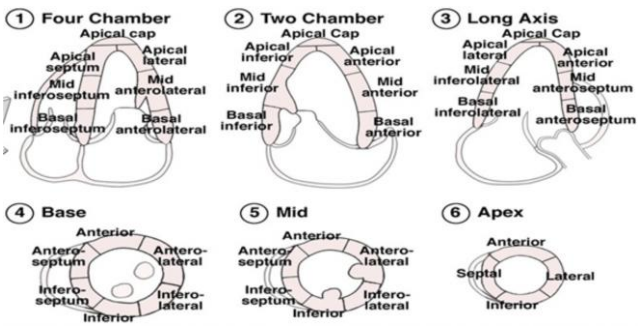

Figure 1: A 17-segment model of heart recommended by AHA

Adequate visualisation increased from 80% in unenhanced segments to 96%, in contrast, enhanced segments. Overall 4 patients (8%) had uninterpretable DSE in unenhanced images, with the use of contrast all studies became interpretable (Figure 2). Regional contractile function, as assessed by resting two-dimensional echocardiography, demonstrated normal contraction in 560 segments (65.8%) and abnormal contraction (dysfunctional) in 290 segments (34.1%). Of 290 dysfunctional segments, 138 were hypokinetic (16.2% of total), 144 were severely hypokinetic or akinetic (16.9% of total), and 8 segments were dyskinetic or aneurismal (0.9% of total) (Figure 2).

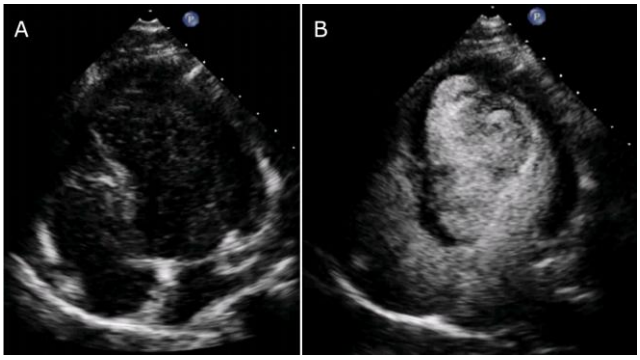

Figure 2: Representative still images showing significant improvement in visualisation of the endocardial border by use of contrast agent; A) without contrast; B) with SonoVue contrast

Dysfunctional segments were evaluated for the presence of contractile reserve, defined as an improvement of wall motion score by 1 grade or more. Of the 290 dysfunctional segments, 134 (46.2%) were viable and 156 (53.8%) nonviable by LDDE. Viability was found in 108/138 (78.2%) of hypokinetic and 26/144 (18%) severely hypokinetic/akinetic regions. All 8 dyskinetic/aneurismal segments were nonviable (Figure 3).

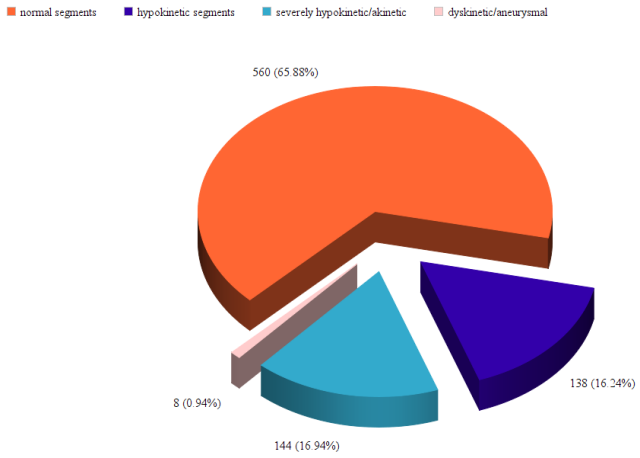

Figure 3: Distribution of regional contractile function assessed by echocardiography at rest

99mTc-Sestamibi gated SPECT

Significant LV dilatation was seen in 13 patients (26%) and transient ischemic dilatation found in 5 patients (10%). Of the 290 dysfunctional segments, 151 (52%) were viable and 139 (48%) nonviable. Results of resting two-dimensional echocardiography and MPS showed that 119/138 (86%) of hypokinetic, 28/144 (19.4%) of severely hypokinetic and 4/8 (50%) of aneurismal regions were viable. Of the non-viable region, perfusion was grade 3 in 70% patients and grade 4 in 30% of patients. Of the 151 viable segments by rest MPS, 129 segments showed ischemia on stress (85.4%).

The relation between LDDE and SPECT

The number of segments with regional wall motion abnormalities (RWMA) that were considered viable by technetium was greater than the number of segments showing a contractile improvement in response to dobutamine (52% versus 46.2%). However, the difference was not significant ($p = 0.31$). A significant relation was observed between technetium uptake and response to dobutamine. Among 151 segments considered viable by technetium, 137 (90.7%) showed contractile improvement with dobutamine; in contrast, only 8 of the 139 segments (5.7%) considered nonviable by technetium had a positive dobutamine response.

On the contrary, we observed that 1.7% (5/290) of dysfunctional segments showed contractile reserve according to DSE, but did not show technetium uptake. The rate of agreement between technetium uptake and a positive response to dobutamine was 78.6%. The value of kappa for the degree of agreement between the two modalities was found to be 0.63. This corresponds to a substantial degree of agreement between the two tests.

Discussion

In patients with severe coronary artery disease and a low LVEF, the evaluation of viability in regions with chronic contractile dysfunction has been shown to predict improvement of function after revascularization [2], [3], [4], [5], [6], [7], [8], [24], [25]. The current study was done to compare the most widely available and commonly used modalities, i.e. dobutamine stress echocardiography and 99mTc-sestamibi imaging for the assessment of viable myocardium.

A head-to-head comparison of the individual segments in this study showed a 78.6% agreement between contrast-enhanced low-dose dobutamine stress echocardiography and ECG gated myocardial SPECT. Various direct comparisons of nuclear imaging and LDDE have reported a fair degree of agreement between the techniques. Panza et al. reported an agreement between 201Tl imaging and

low dose dobutamine echocardiography of 68% [26]. The disagreement in their study was also mainly related to segments with viability on nuclear imaging but without functional improvement on echocardiography. The largest head-to-head comparison was made by Bax et al., which included 114 patients who underwent resting perfusion imaging with 99mTc-tetrofosmin and low-dose dobutamine echocardiography. The agreement rate reported between the techniques was 72% [27].

Moreover, a recent study to has reported with an agreement rate of 77% between LDDE and gated-SPECT with low dose dobutamine infusion [28]. However, Bax et al., compared LDDE with 99mTc-tetrofosmin and Panja et al., compared with thallium. In our settings (India), there is poor availability of thallium and 99mTc-tetrofosmin is not available, whereas, our study is more relevant in Indian setting because we have shown a substantial degree of agreement using 99m-technetium.

It has been seen that poor-quality images may occur in up to 30% of patients due to challenges imposed by excessive cardiac motion due to hyperventilation and tachycardia and in up to 10% due to patient-related factors like obesity and lung disease. Moreover, suboptimal studies result in increased interobserver variability and less reproducibility. Several recent studies have assessed whether better endocardial border visualisation by left ventricular opacification improves the accuracy of stress echocardiography for diagnosis of coronary artery disease. In the OPTIMIZE trial, which included 108 patients, the use of a contrast agent improved the percentage of segments adequately visualised at baseline (from 72 ± 24% to 95 ± 8%) [29]. Similar results were found in this study. Out of 850 segments, 170 segments were inadequately visualised at rest in unenhanced DSE. With the use of contrast, adequate visualisation increased from 80% in unenhanced segments to 96% contrast-enhanced segments. Overall 4 patients (8%) had uninterpretable DSE in unenhanced images, with the use of contrast all studies became interpretable.

Discordance was observed between the two methods in both hypokinetic and akinetic segments. Put in other words, for the same dysfunctional segments gated SPECT showed viability more often than LDDE. Similar results were reported by Panza et al., and Bax et al. who found that detection of viability by nuclear scan was more frequent than DSE. This difference is considered to be due to the difference in mechanisms of viability detection by the two methods. A higher degree of myocyte functional integrity is required for contractile reserve than radiotracer uptake by the cells [30]. We observed that 1.7% (5/290) of dysfunctional segments showed contractile reserve according to DSE, but did not show technetium uptake. Similarly, Panza et al., reported 2% (6/311) of dysfunctional segments without perfusion, but they thought it to be due to the error inherent in the comparison of the two techniques. However, this is more likely attributable to areas with non-transmural myocardial infarction, as described by Armstrong [31].

In this study, we reported the safety, and diagnostic accuracy of contrast-enhanced dobutamine stress echocardiography in a population of patients with known coronary artery disease. In agreement with the earlier reports, the technique was well tolerated by all patients, and in no case did the study have to be discontinued because of occurrence of serious side effects due to contrast (SonoVue) or dobutamine [32]. The echocardiographic approaches are attractive because there is no ionising radiation, is safe, widely available, low cost, shorter imaging time, portable, immediate availability of the results, additional parameters of viability (strain, Doppler, EDWT), availability of ancillary information about cardiac structures. None of the previous head to head trial used contrast-enhanced LDDE for comparison. This is extremely important for resource developing countries like India where echocardiography is more readily available and a significantly lower cost as compared to SPECT. This study demonstrated a good agreement between contrast-enhanced low dose dobutamine stress echocardiography and Tc-SPECT for the myocardial detection viability. Therefore, contrast-enhanced LDDE can be used for viability detection with a good level of confidence.

In conclusion, the use of contrast agent significantly increases the adequate endocardial border visualisation and increases interpretability of images. Our study demonstrated a substantial degree of agreement between contrast-enhanced low dose dobutamine stress echocardiography and Tc-99m SPECT for the detection of contractile reserve in infarcted areas. So, contrast-enhanced LDDE appears to be a safe and comparable alternative to MPS in myocardial viability assessment.

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Values and Correlations between C-Reactive Protein and Apolipoprotein B after Treatment with Methotrexate at Patients with Rheumatoid Arthritis

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Keywords: Rheumatoid arthritis (RA); Methotrexate (MTX); C-reactive protein (CRP); Apolipoprotein B (Apo B)

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Abstract

BACKGROUND: Patients with rheumatoid arthritis (RA) are at increased risk of cardiovascular disease (CVD). Lipid changes related to inflammation have been described in RA. Methotrexate (MTX) treatment is effective in controlling inflammation and decreasing the CRP (C-reactive protein) values.

AIM: To examine the disease activity, CRP and Apo B values in the detection of new patients with active and untreated RA, and impact of MTX therapy on their levels after 6 months and one year of treatment, and the correlation between their values in this period.

METHODS: 80 patients with active and newly discovered RA patients who meet the American Rheumatology Association (ARA) 1987 revised criteria were treated with disease-modifying anti-inflammatory drugs (DMARDs) according to the protocol for treatment.

RESULTS: After a year of therapy RA patients achieved significant decrease in the DAS28 (disease activity score) ($p < 0.01$ and $p < 0.001$), and CRP values ($p < 0.001$). Levels of Apo B values at the 12 months were nonsignificantly higher compared to the results obtained at the beginning of the study ($p < 0.001$). After 6 and 12 months there was a weak nonsignificant negative correlation about the values of CRP and Apo B at baseline and after 12 months ($r = –0.15$ and $r = -0.12$ $p > 0.05$).

CONCLUSION: Use of MTX therapy at RA patients had a reduced effect on disease activity and inflammation, but the nonsignificance effect on the values of Apo B lipoproteins.

Introduction

Rheumatoid arthritis (RA), a chronic inflammatory joint disease of unknown aetiology, affects approximately one per cent of the general population. Estimated standardised mortality ratio's associated with RA range from 1.3 to 3.0. This increased mortality is largely attributable to CVD, particularly coronary atherosclerosis. The cardiovascular morbidity found in RA patients appears to be increased by twofold or more compared to the general population (age and sex-matched) [1], [2], [3], [4], [5].

Autoimmunity and inflammation play a major role in the development of atherosclerotic plaque formation in many rheumatological conditions including RA. The mechanisms underlying these changes include the interplay of inflammation and autoantibody formation. Thus treatment options to reduce CVD risk amongst these conditions share a common theme, with the use of DMARDs paramount to all [3], [5], [6].

Atherosclerosis is an inflammatory condition, with high inflammatory level implicated for developing CVD. Inflammatory markers such as IL-6, CRP and fibrinogen are associated with a high frequency of cardiovascular events. In particular, CRP has received

large attention due to its ability to independently predict cardiovascular events in the general population, which may in part, be due to its ability to directly contribute to the onset of CVD [7], [8], [9].

In RA patients, inflammatory markers such as the CRP and erythrocyte sedimentation rate are elevated and remain greater even in periods of low disease activity when compared to the general population. In patients with inflammatory arthritis who were followed up for 10 years, CRP levels independently predicted CVD mortality. The similarities between the inflammatory process of RA and atherosclerosis are remarkable. In both diseases, concentrations of IL-6, CRP and TNF-α are elevated, and both have similar patterns of activation for T-cells and macrophages [8], 10], [11].

Apolipoproteins are found on the surface of lipoproteins and regulate lipid metabolism. The apolipoproteins that are of clinical interest are apo B and apolipoprotein A1 (Apo A1). Apo B is found on LDL particles and is responsible for the clearance of LDL cholesterol through the LDL receptor pathway [12].

Apolipoprotein-related Mortality Risk (AMORIS) study investigated the use of apo B, Apo A1, and the apo B: apo A1 ratio at predicting fatal myocardial infarction (MI). The study followed 75 553 Swedish men and women from 1985 to 1996. The authors found that apo B and the apo B: apo A1 ratio were both strongly predictive of increased risk of fatal MI in both men and women. Furthermore, they found that apo B was a stronger predictor of the risk of fatal MI than LDL cholesterol. However, apo B, although a strong predictor of coronary heart disease, is not currently recommended as the primary target of therapy and there is not enough evidence to justify apo B replacing LDL cholesterol as the preferred target of therapy by National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Cholesterol in Adults (NCEP/ATP III) guidelines [1], [13], [14], [15].

Traditional synthetic DMARDs, such as MTX, Sulfasalazine and Hydroxychloroquine, have a protective role against CV risk. The mechanisms by which DMARD use influences CV risk are poorly understood, but lend support to the hypothesis that reducing inflammation is important in reducing CV risk. Of the traditional DMARDs, MTX is the most widely used and is known as the anchor drug in RA, yet the mechanisms underlying its anti-inflammatory properties are not fully understood [17].

Given the high level of systemic inflammatory burden that characterises RA, which is regarded as a key CV risk factor, alongside an increased prevalence of traditional risk factors, EULAR recommendations highlight the importance of adequate disease control to lower CV risk. The vulnerability of the carotid plaque is influenced by RA disease activity, and remission may alleviate this threat. Therefore, effective CV risk management will likely comprise not only adequate treatment of conventional risk factors but also tight and sustained disease activity control [6], [7], [18].

The purpose of this study is to examine the disease activity, CRP and Apo B values in the detection of new patients with active and untreated RA, and impact of MTX therapy on their levels at the same patients after 6 months and one year of treatment, and correlation between their values in this period.

Material and Methods

Inclusion criteria for patients

Eighty consecutive, unselected patients who were referred to the outpatient rheumatology clinic at the Clinical Center in Skopje were investigated. All patients fulfilled the American College of Rheumatology (ACR) 1987 criteria for RA, had an early disease with disease duration of less than one year without prior use of DMARDs and or systemic steroids [24].

Exclusion criteria for patients

Smokers or patients suffering from conditions that affect the lipid profile, such as diabetes mellitus, hypothyroidism, liver or kidney disease, Cushing's syndrome, Carcinoma, obesity (body mass index > 30) and a history of familial dyslipidemia, were excluded. Also, patients receiving medications affecting lipid metabolisms, such as lipid-lowering drugs, beta-blockers, oral contraceptives, estrogen, progestin, thyroxin and vitamin E, were excluded from the study [9].

Thirty healthy, non-smoking volunteers also participated in the study and were used as a control group and fulfilled the same exclusion criteria reported for the patient group. None of the subjects participating in the control group had a history of CVD. The control group was proportionally matched for age and sex to the patient group. All controls reported no significant changes in their body weight for at least three months before entry to the study. All patients and controls gave informed consent, and the study protocol was approved by the Institutional Ethics Committee [21].

Study design

Patients were treated with methotrexate (MTX; 0.2-0.6 mg/kg/week; mean ± standard deviation 15.5 ± 1.3). Disease activity was assessed by measuring the disease activity for 28 joint indices

score (DAS-28), while the clinical response was evaluated according to the ACR 50% response criteria. All patients were followed up every month for the first three months, and every three months after that. During the follow-up period, a questionnaire concerning changes in dietary habits was carefully fulfilled by all patients. The body weight was also measured appropriately in each visit.

Blood sampling and laboratory monitoring

Overnight fasting blood samples were obtained at baseline, 6 and after 12 months follow-up from both untreated RA patients and the control group. Serum apolipoprotein B was measured by immune-nephelometry with the aid of a Behring Nephelometer BN100 and reagents (antibodies and calibrators) from Behring Diagnostics GmbH (Liederbach, Germany). C-reactive protein (CRP) was measured by nephelometry.

Statistical analysis

Statistical analysis was performed using Statistica software, ver 7.1. Due to the distribution which was not normal (according to Kolmogorov-Smirnov test), the variable differences were tested using non-parametric tests (Wilcoxon Matched Pair Test or Friedman ANOVA test – Chi-Square). The correlation between parameters was analysed using the Pearson correlation coefficient. Significance was set up at $p < 0.05$.

Results

Patients and control groups who participated in our study were allocated several parameters such as: age, sex BMI (Body mass index), duration of illness, MHAQ, morning stiffness, the affected joints, swollen joints, VAS index, global doctor assessment, sedimentation rate of erythrocytes, CRP, and rheumatoid factor (RF). These parameters show disease activity in early studies (Table 1).

Table 1: Patients and controls characteristics

	RA (n = 80)	Controls (n = 30)
Age (year)	45.7 ± 9.8	45.2 ± 9.8
Sex: M/F	80 F	6/24
BMI (kg/m²)	22.3 ± 2.6	21.8 ± 2.2
Duration of disease (month)	6.2 ± 16.6	
MHAQ (1-4)	1.5 ± 0.5	
Morning stiffness	111.4 ± 133.2	
Affected Joints	7.8 ± 7.1	
Swollen joints	5.2 ± 3.7	
VAS (0-10)	7.0 ± 2.1	
Global doctors' assessment	4.5 ± 2.3	
Sedimentation rate(mm/h)	45.5 ± 30.3	
CRP (mg/1)	21.69 ± 29.4	
RF (positive/negative)	64/16	

BMI: Body Mass Index; MHAQ: Question modified to improve health VAS: visual analog (pain) score; CRP: C-reactive protein; RF: Rheumatoid factor.

During the study, 80 patients with newly discovered active and untreated RA were treated with Methotrexate. These 80 patients were selected according to those who have responded to the therapy. The patients who did not respond to the therapy (7 in number) were excluded from the study.

Figure 1 shows the DAS28 (Disease Activity Score in 28 joints) index at the beginning, after 6 and 12 months of therapy.

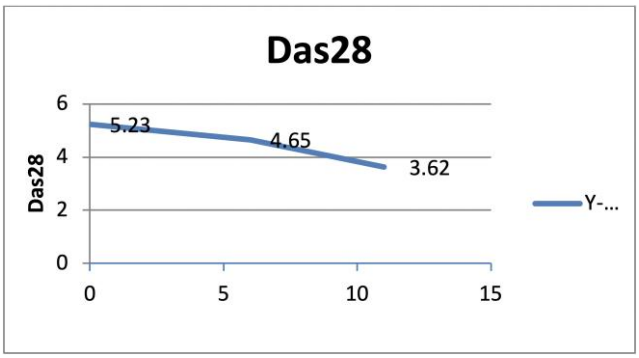

Figure 1: Disease activity score 28 (DAS28) at baseline, at 6, and 12 months during the treatment

At DAS28 score after 6 months for $p < 0.01$ there are significant differences in relation beginning, 6 and 12 months, the difference is significant ($p < 0.001$), and also in the relation beginning/12 month there is also a significant difference.

Table 2: Descriptive statistics / RA patients

	Valid N	Mean	Confidence -95,00%	Confidence +95,00%	Minimum	Maximum	Std.Dev.
CRP 0 m.	80	21.69	17.93	25.44	1.80	63.00	15.98
CRP 6 m.	80	20.51	12.00	29.01	1.00	307.00	36.19
CRP 12 m.	80	12.31	9.50	15.11	0.00	62.00	11.95
Apo B 0m.	80	1.79	1.67	1.92	0.70	3.30	0.53
Apo B 6 m.	80	1.79	1.67	1.92	0.60	3.50	0.54
Apo B 12 m.	80	1.83	1.71	1.95	0.70	3.50	0.52

CRP levels at baseline, at 6 months, and 12 months.

As for CRP, there was a significant consecutive decrease from baseline, both at 6 and at 12 months (for both variables, between each of time-points; Friedman ANOVA; $p < 0.001$) (Table 3).

Table 3: CRP levels at baseline, at 6 months, and 12 month

	Average Rank	Sum of Ranks	Mean	Std.Dev.
CRP 0 m.	2.44	175.50	21.69	15.98
CRP 6 m.	2.08	150.00	20.51	36.19
CRP 12 m.	1.48	106.50	12.31	11.95

Friedman ANOVA; Chi Sqr. (N = 80, df = 2) = 36.07, p = 0.000

For $Z = 5.16$ and $p < 0.001$ ($p = 0.000$) average values of CRP after 12 months ($x = 12.31$ mg/l) of therapy are significantly lower according to the values of CRP at the beginning ($x = 21.69$ mg//l) (Table 4).

Table 4: CRP / CRP beginning & CRP 12 m

Wilcoxon Matched Pairs Test				
	Valid	T	Z	p-level
CRP 0m. & CRP 12 m	80	361.50	5.16	0.000

Apolipoprotein B (Apo B) levels at baseline, at 6 months, and at 12 months.

As for Apo B, there was no significant consecutive difference from baseline, both at 6 and at 12 months (for both variables, between each of time-points; Friedman ANOVA; $p < 0.001$) (Table 5).

Table 5: Apo B levels at baseline, at 6 months, and 12 months

	Average Rank	Sum of Ranks	Mean	Std.Dev.
Apo B 0 m.	1.90	136.50	1.79	0.53
Apo B 6 m.	1.97	141.50	1.79	0.54
Apo B 12 m.	2.14	154.00	1.83	0.52

Friedman ANOVA; Chi Sqr. (N = 80, df = 2) = 2.80, p = 0.25.

For $Z = 0.94$ and $p > 0.05$ ($p = 0.35$) average values of Apo B after 12 months ($x = 1.83$ g/l) of therapy were nonsignificantly higher according to the values of Apo B at the beginning ($x = 1.79$ g/l) (Table 6).

Table 6: Apo B / Apo B beginning & Apo B 12 m.

Wilcoxon Matched Pairs Test				
	Valid	T	Z	p-level
Apo B 0 m. & Apo B 12 m.	80	871.00	0.94	0.35

Correlation in relation to CRP/Apo B.

Comparing the results of CRP and Apo B at baseline, after 6 and 12 months we found a weak nonsignificant negative correlation about the values of CRP and Apo B at baseline and after 12 months ($r = -0.15$ and $r = -0.12$, $p > 0.05$). In other words, the elevation of CRP by 1 mg/l was accompanied by a decrease of Apo B values by 0.005 g/l (Figure 2).

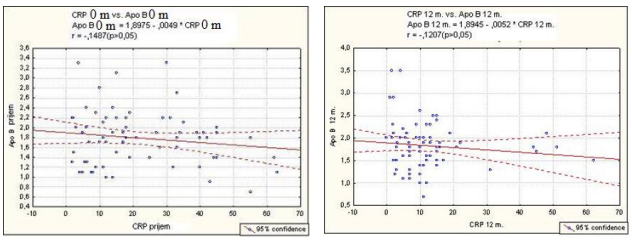

Figure 2: Correlation of CRP and Apo B levels at baseline (left); Correlation of CRP and Apo B levels at 12 months (right)

As for the relationship at 6 months, we found a moderately poor and weak insignificant correlation ($p > 0.05$).

Discussion

Our findings support the view that present chronic inflammation at RA patients affects the endothelium, and that, in association with atherosclerosis, it may be the mechanism that at least partly explains the increased mortality and morbidity occurring in patients with RA. Our goal was to determine the disease activity, values of CRP and Apo B lipoproteins in patients with active RA and DMARD-naïve RA patients before treatment and after 6 and 12 months of treatment with DMARDs, respectively with MTX. The patients with active RA had nonsignificantly increased levels of Apo B after 6 and 12 months of treatment with MTX. On the other hand, the values of acute phase reactant-CRP were lower after 6 months and one year of treatment. This suggests that moderate increasing levels of Apo B lipoproteins were accompanied by a decrease of inflammation at the end of the study.

Several studies have suggested that CRP has direct effects on the vessel wall promoting atherosclerosis. An association between high-grade, chronic CRP elevation and subclinical atherosclerosis in patients with RA has been reported. Additionally, a higher risk of CV events in patients with RA with chronic inflammation expressed by persistently increased CRP serum levels has been found, although high-sensitive C-reactive protein is shown to have a strong relationship with recurrent events of CVD in several randomised clinical trials [21], [22], [23].

The clinical importance of dyslipidemia concerning CV events or death in RA is unclear. Even though there is observational data that suggests that there is no significant difference between RA and non-RA subjects in the risk imparted by hyperlipidemia [21], it could be possible that non-traditional CVD risk factors, or other lipid parameters besides those found in cholesterol profiles, explain a greater proportion of CVD risk than in non-RA patients [24]. It has been proposed that the non-fasting apoB/apoA1 ratio was superior to any of the cholesterol ratios for estimation of the risk of acute myocardial infarction [25]. Some have suggested that the elevation in lipids after RA therapy might be offset by a reduction of more atherogenic molecules [26] such as apo B and apoA-I or total cholesterol.

Importance of apo B is because of apo B presence in very low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), large buoyant LDL and small dense LDL (sd-LDL), with one molecule of Apo B in each of these atherogenic particles. Therefore, total apo B reflects the total number of potentially atherogenic particles and in the AMORIS study was find that apo B has a stronger relationship with risk of fatal MI and other CV events than does non-HDL C [12], [15], [16]. Also, the report of the Uppsala Longitudinal Study of Adult Men (ULSAM), about these risk values correspond well with those found in the AMORIS study [27]. Same conclusions have been confirmed by many other studies (The EPIC-Norfolk study, Nurses' Health study, THROMBO study, GRIPS and Caerphilly studies, etc.) [12].

Katherine P. Liao report for a nonsignificant increase of apo B values and no significant correlation between change in CRP and apo B levels ($r = 0.14$, $P = 0.20$) and the atherogenic indices after treatment of RA patients [28]. At Sana P. study both Apo A1 and

Apo B are significantly lower in cases than in controls. Elevated Apo B levels indicate an increased risk of cardiovascular disease, but in their study, Apo B was 23% decreased in cases when compared to controls [29]. In a similar study, Magarò M et al. have also shown Apo A1 and Apo B to be significantly lower in RA patients. They have also shown reduced levels of albumin in these patients which perhaps indicate a reduced rate of synthesis of proteins by the liver reflected in the decreased levels of the apoproteins [30]. A study by Eva Hurt Camejo et al., showed an Apo B decrease by 7 %, while other lipoproteins were in the normal range in the RA patients and similar to those in the controls [31].

According to the use of antirheumatic drugs in RA, tumour necrosis factor inhibitors and MTX are associated with a decreased risk of all CVEs while corticosteroids and NSAIDs are associated with an increased risk. Targeting inflammation with tumour necrosis factor inhibitors or methotrexate may have positive cardiovascular effects in RA [32]. In about 10 comparative trials, a combination of MTX and a TNF-alpha antagonist was more effective than MTX monotherapy on functional status and symptoms, especially in initially severe RA. In practice, MTX is the first-line antirheumatic drug and [33] MTX does not have any effect on the lipid profile of RA and Westlake et al., suggested that MTX use is associated with a reduced risk of CVD events in patients with RA. This may be important early in the disease course. The mechanism for this possible benefit cannot be fully determined from the current literature but, is likely to be multi-factorial. As disease control continues to improve in RA, future studies need to address the impact of MTX and other synthetic and biologics DMARDs on CVD, which remains the leading cause of death and a significant comorbidity in these patients [34] As we have presented in our study MTX was the initial treatment as a monotherapy but also chosen by most rheumatologists, which is in accordance with the EULAR and ACR guidelines [35].

Therefore, the expert opinion is: identifying the RA phenotype at greatest risk of CVD, understanding the interplay of increased traditional risk factors, common inflammatory processes and RA-specific factors, and personalised use of DMARDs according to disease phenotype and comorbidity to reduce this risk are key areas for future research [32].

Limitations of Study: The sample size was small to allow for a generalisation of the results. The long-term effects of the treatment on lipids and disease activity can be deciphered only through further follow up.

In conclusion, we examined the impact of Methotrexate on disease activity, values of C-reactive protein and the Apolipoprotein B at patients with Rheumatoid Arthritis. Eighty patients with active and newly discovered RA after a year of therapy achieved a significant decrease in the DAS28 (disease activity score) and CRP values. Changes in Apo B levels between the start and the end of the study were with nonsignificant differences. Levels of Apo B at the 12 months were nonsignificantly higher compared to the results obtained at the beginning of the study. Use of MTX therapy at RA patients had a reduced effect on disease activity and inflammation, but nonsignificance effect on the values of Apo B lipoproteins.

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Clinical Science

Efficacy of Albendazole and Mebendazole With or Without Levamisole for Ascariasis and Trichuriasis

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Abstract

BACKGROUND: Helminthiasis in school-aged children potentially causing physical growth and intellectual development retardation. Trichuriasis was the most common type of helminthiasis in children.

AIM: To investigated the efficacy and side effects of albendazole, albendazole combined with levamisole and mebendazole combined with levamisole for trichuriasis and ascariasis.

METHODS: This study was conducted as a double-blind, randomised clinical trial by comparing the efficacy and side effects of albendazole, albendazole combined with levamisole and mebendazole combined with levamisole for trichuriasis. The sample of this study were 180 elementary school students at Deli Serdang Regency State Elementary School, Medan, Indonesia. The study was conducted from April to June 2015.

RESULT: The cure rate of helminthiasis on the 7th day was 81.7% after albendazole therapy, 88.3% after albendazole levamisole therapy, and 83.3% after mebendazole combined with levamisole therapy ($p = 0.577$). Cure rate on the 14th day was 88.3%, 95%, and 91.7% for albendazole, albendazole combined with levamisole, and mebendazole combined with levamisole therapy, respectively ($p = 0.418$). On the 21th day, the cure rate was 88.3%, 96.7%, and 91.7% ($p = 0.230$). Combination of albendazole and levamisole showed the highest cure rate, despite the statistically insignificant difference for all groups ($p > 0.05$). Combination of albendazole combined with levamisole showed better cure rate for mild trichuriasis (95.8%) than albendazole therapy (46.2%) and mebendazole combined with levamisole (83.3%), ($p < 0.05$).

CONCLUSION: Single-dose albendazole, a combination of albendazole and levamisole, and a combination of mebendazole and levamisole had similar efficacy in reducing egg count in helminthiasis. Combination of albendazole and levamisole showed better cure rate for mild trichuriasis and mixed infections. Side effects were similar in all treatment groups.

Introduction

Intestinal worm infection is still a major public health problem in Indonesia, especially in rural areas. In Indonesia and other developing countries, Ascaris lumbricoides, Trichuris trichiura, and hookworm are the most common intestinal parasites [1], [2]. World Health Organization (WHO) estimated that at least two billion people or nearly one-third of the world's population had been infected with soil-transmitted helminths (STH) or helminthiasis [3]. About 300 million infected people suffered from severe illness, and about 400 million school-aged children worldwide had been infected [4], [5].

Many species of worms had been reported to cause infection in Indonesia. Trichuris trichiura, was resided in the human caecum, was the most common cause of helminthiasis and one of the most important intestinal nematodes in human [6], [7]. School-age children were frequently infected with helminthiasis, which potentially causing diarrhoea, nutritional deficiency, anaemia, growth disorders, and intellectual disturbance [8], [9].

Public health program to control helminthiasis was largely dependent on the administration of anti-helminthic drugs for elementary-school children [10].

Theoretically, there were several broad-spectrum anti-helminthic drugs such as albendazole, levamisole, mebendazole, and pyrantel pamoate with variable advantages and disadvantages [11], [12], [13], [14]. Benzimidazole group was the most common anti-helminthic used in a public health setting [15], [16], [17].

We conducted a clinical trial to compare the efficacy and side effects of albendazole, albendazole with levamisole and mebendazole with levamisole therapy against trichuriasis and ascariasis in elementary-school children to determine the most effective regimen.

Material and Methods

This study was a double-blind, randomised clinical trial comparing the efficacy and side effects of albendazole, albendazole combined with levamisole and mebendazole combined with levamisole for trichuriasis. The sample of this study were 180 elementary-school students at Deli Serdang Regency State Elementary School, Medan, Indonesia. The study was conducted from April to June 2015. Study steps consisted of preparation, data collection, processing, data analysis, and data improvement.

Written informed consent was conducted by all subjects' parents or guardian two weeks before the scheduled intervention date. A container was given to each subject to keep their stool sample at home. The collected stool samples were qualitatively and quantitatively examined by the Kato-Katz method at Parasitology Laboratory of Medical Faculty, University of Sumatera Utara, Medan. Stool sample with a positive finding of trichuriasis was randomised as the study subjects into the three treatment groups. Stool sample examination for evaluation was done on day 7, day 14 and 21 after treatment.

Each subject in the first group was given one tablet of 400 mg albendazole. Second group was given one tablet of 400 mg albendazole and 2 tablets of 25 mg levamisole (for children weighed 10 – 20 kg), or one tablet of 400 mg albendazole and 4 tablets of 25 mg levamisole (for children weighed 21 – 40 kg), or one tablet of 400 mg albendazole and 6 tablets of 25 mg levamisole (for children weighed > 40 kg). The third group was given one tablet of 500 mg mebendazole and levamisole dose is the same as the second group. For the following 14 days, parents were instructed to record any side effect after treatment. Comparison of recovery rate between the intervention groups was analysed using Chi-square test or Wilcoxon test as suitable. The p-value of less than 0.05 was considered significant.

Results

There were 807 Deli Serdang elementary students; after the exclusion, we found 185 students with helminthiasis based on stool examination. Five of them had started therapy before the study. Therefore, they were excluded. The remaining 180 students were study subjects who undergone randomisation into three intervention groups (60 students in each group). The characteristics of the subjects can be seen in Table 1.

Table 1: Characteristics of Subjects

Characteristics	First Group Albendazole	Second Group Albendazole + Levamisole	Third Group Mebendazole + Levamisole
	(N = 60)	(N = 60)	(N = 60)
Age, years (mean ± SD)	9.2 ± 1.734	8.9 ± 1.540	9.1 ± 1.567
Sex, n (%)			
Male, n (%)	35 (58.3)	28 (46.7)	31 (51.7)
Female, n (%)	25 (41.7)	32 (53.3)	29 (48.3)
Weight, kg	27.2 ± 7.957	27.1 ± 6.374	27.9 ± 7.363
Height, cm (mean ± SD)	129.5 ± 13.944	132.0 ± 18.516	128.4 ± 12.785
Wight/height (mean ± SD)	97.1 ± 6.574	95.7 ± 13.014	94.1 ± 17.757
Parental occupation			
Entrepreneur	38 (16.7)	14 (23.3)	16 (26.6)
Farmer	11 (18.3)	45 (75)	38 (63.3)
Civil servant	1 (1.6)	-	1 (1.6)
Others	10 (16.6)	1 (1.6)	5 (8.3)
Parental education, n (%)			
Uneducated	1 (0.83)	7 (5.83)	13 (10.83)
Elementary school	22 (18.33)	36 (30)	25 (20.83)
Junior high school	46 (38.33)	38 (31.67)	42 (35)
Senior high school	3 (35.83)	39 (32.5)	38 (31.67)
University	8 (6.67)	-	2 (1.67)
Helminthiasis (%)			
A. lumbricoides	47 (78.3)	34 (56.7)	11 (18.3)
T. Trichur	6 (10)	19 (31.0)	12 (20)
A. lumbricoides + T. trichura	7 (11.7)	7 (11.7)	36 (60)
A. lumbricoides + T. trichura + Enterobiuss	-	-	1 (1.7)

The most common helminthiasis was infection by Ascaris lumbricoides, followed by mixed infection by Ascaris lumbricoides with Trichuris trichuria, Trichuris trichuria only, and mixed infection by Ascaris lumbricoides with Trichuris trichuria and Enterobius vermicularis as seen in Table 2.

Table 2: Prevalence of helminthiasis

Helminthiasis Etiology	Numbers (%)
Ascaris lumbricoides	92 (51.11)
Trichuris trichiura	37 (20.55)
A. lumbricoides + T. trichiura	50 (27.78)
A. lumbricoides + T. trichiura + E. vermicularis	1 (0.56)

Subjects with mild A. lumbricoides infection were 50 children (83.3%) in the first group, 39 students (65%) in the second group, and 46 students (76.7%) in the third group. Subjects with mild T. trichuria infection were 13 children (21.7%) in the first group, 24 children (40%) in the second group, and 24 children (40%) in the third group.

Table 3: Basic Characteristics of Research Based on Intensity of Infection

Characteristics	First Group Albendazole (N = 60)	Second Group Albendazole + Levamisole (N = 60)	Third Group Mebendazole + Levamisole (N = 60)
The intensity of infection, n (%)			
Ascaris lumbricoides			
Mild	50 (83.3)	39 (65)	46 (76.7)
Moderate	4 (6.7)	2 (65)	1 (1.7)
Trichuris trichiura			
Mild	13 (21.7)	24 (40)	24 (40)
Moderate	-	2 (3.3)	1 (1.7)

The Statistical analysis did not show a significant reduction in egg count on the 7th day after therapy in all groups, whereas there was a significant reduction in the 14th and 21st day in all groups. Determination of egg reduction rate can be seen in Table 4.

Table 4: Egg Reduction Rate on Day 7th, 14th, 21th

Parasites	Antihelmintics regiment	Total eggs per gram			
		Mean (SD) 7th day	Mean (SD) 14th day	Mean (SD) 21st day	P value
A. lumbricoides	Albendazole	34.00 ± 165.64	-	-	0.651
	Albendazole + Levamisole	14.40 ± 111.54	-	-	
	Mebendazole + Levamisole	34.80 ± 126.25	-	-	
T. trichiura	Albendazole	33.60 ± 95.91	24.80 ± 81.23	20.40 ± 74.96	0.247
	Albendazole + Levamisole	20.00 ± 89.38	12.40 ± 73.47	7.60 ± 47.89	
	Mebendazole + Levamisole	40.00 ± 122.66	18.80 ± 68.03	8.80 ± 29.63	

The cure rate of helminthiasis on the 7th day was 81.7% after albendazole therapy, 88.3% after albendazole + levamisole therapy, and 83.3% after mebendazole + levamisole therapy (p = 0.577). Cure rate on the 14th day was 88.3%, 95%, and 91.7% for albendazole, albendazole + levamisole, and mebendazole + levamisole therapy, respectively (p = 0.418). On the 21th day, the cure rate was 88.3%, 96.7%, and 91.7% (p = 0.230). Combination of albendazole and levamisole showed the highest cure rate, despite the statistically insignificant difference for all groups (p > 0.05). Determination of cure rates analysis can be seen in Table 5.

Table 5: The Cure Rates Analysis On Day 7th, 14th, 21th

Recovery					
Therapy	Recovered		Not Recovered		P
	n	%	n	%	
Albendazole (Day-7)	49	81.7	11	18.3	0.577
Albendazol + Levamisole (Day-7)	53	88.3	7	11.7	
Mebendazol + Levamisole (Day-7)	50	83.3	10	16.7	
Albendazole (Day-14)	53	88.3	7	11.7	0.418
Albendazole + Levamisole (Day-14)	57	95.0	3	5.0	
Mebendazole + Levamisole (Day-14)	55	91.7	5	8.3	
Albendazole (Day-21)	53	88.3	7	11.7	0.230
Albendazol + Levamisole (Day-21)	58	96.7	2	3.3	
Mebendazol + Levamisole (Day-21)	55	91.7	5	8.3	

Combination of albendazole combined with levamisole showed better cure rate for mild trichuriasis (95.8%) than albendazole therapy (46.2%) and mebendazole + levamisole (83.3%), p = 0.00. Determination of cure rate mild helminthiasis analysis can be seen in Table 6 and Table 7.

Table 6: The Cure Rate of Mild Helminthiasis

Parasite(s)	Treatment	Recovery				
		Cured		Not Cured		P
		n	%	n	%	
A. lumbricoides	Albendazole	46	92	4	8	0.176
	Albendazole + Levamisole	39	100	-	-	
	Mebendazole + Levamisole	42	91.3	4	8.7	
T. trichiura	Albendazole	6	46.2	7	53.8	0.01
	Albendazole + Levamisole	23	95.8	1	4.2	
	Mebendazole + Levamisole	20	83.3	4	16.7	

The side effects during the treatment process in each group had been observed. Side effect observed in the albendazole group were 13.3%, Albendazole combined with Levamisole was 26.7%, and Mebendazole combined with Levamisole were 20 %.

Table 7: Cure Rate of Each Intervention Group

Parasite(s)	Intervention	Cure Rate (%)	P Value
A. lumbricoides	Albendazole	100	-
	Albendazole + Levamisole	100	
	Mebendazole + Levamisole	100	
T. trichiura	Albendazole	66.7	0.136
	Albendazole + Levamisole	94.7	
	Mebendazole + Levamisole	92.3	
A. lumbricoides + T. trichiura	Albendazole	28.6	0.079
	Albendazole + Levamisole	85.7	
	Mebendazole-Levamisole	66.7	

Observation data of side effect can be seen in Table 8

Table 8: Side Effects analysis

Side Effect	Albendazole n (%)	Albendazole + Levamisole n (%)	Mebendazole + Levamisole n (%)
None	52 (86.7)	44 (73.3)	48 (80.0)
Yes	8 (13.3)	16 (26.7)	12 (20.0)

Discussion

Helminthiasis has still been a major health problem in Indonesia. A. lumbricoides, T. trichiura and hookworm (N. americanus and A. duodenale) were the most common aetiology. WHO data on 2012 reported a high prevalence of helminthiasis in North Sumatera, i.e. 80% of school-aged children [18], [19].

Statistical analysis did not show a significant reduction in egg count on the 7th day after therapy in all groups, whereas there was a significant reduction in the 14th and 21st day in all groups. Even after the reduction in egg number at 14th and 21st day, we still found several T. trichuria eggs in subjects' stool, indicating the difficulty in eradicating trichuriasis as mentioned in the literature [20]. A study by Saputri in 2010 found significant egg reduction rate in single-dose mebendazole and mebendazole with levamisole therapy for A. lumbricoides and T. trichuria infections [21]. The contradictive result was found by Sihite et al., (2014) and Knopp et al., (2010) study, which found no significant difference in the treatment with mebendazole, albendazole, and mebendazole with levamisole [22], [23].

Based on Table 5, the combination of albendazole and levamisole showed the highest cure rate, despite the statistically insignificant difference for all groups (p > 0.05). Therefore, this finding indicated the similar efficacy of albendazole, albendazole + levamisole, and mebendazole + levamisole therapy.

Based on Table 6, the combination of albendazole with levamisole showed better cure rate for mild trichuriasis (95.8%) than albendazole therapy (46.2%) and mebendazole combined with levamisole (83.3%), p = 0.01. We hypothesised that levamisole had enhanced efficacy than albendazole and mebendazole for mild trichuriasis. Previous studies

showed that a single dose of albendazole or mebendazole had 28% and 36% recovery rate, respectively [16], [23]. For mixed infection, albendazole combined with levamisole was more effective (cure rate 85.7%) than single albendazole (28.65%) or mebendazole combined with levamisole (66.7%), p = 0.079 that showed on Table 7. A study by Sihite et al., (2014) found no significant difference in the recovery of helminthiasis with mebendazole + levamisole or single mebendazole therapy [22]. Another study found no significant differences in the recovery rate of helminthiasis between mebendazole with or without levamisole therapy [21]. The most common side effects in all groups were nausea and diarrhoea. No serious side effects were observed in this study, and mild side effects had recovered by their own. Table 8 shows that there was no difference in side effects between intervention groups.

It can be concluded that single-dose albendazole, a combination of albendazole and levamisole, and the combination of mebendazole and levamisole had similar efficacy in reducing egg count in helminthiasis. Combination of albendazole and levamisole showed better cure rate for mild trichuriasis and mixed infections. Side effects were similar in all treatment groups.

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Sexual Function in Iranian Female Multiple Sclerosis Patients

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Keywords: Sexual function; Multiple sclerosis; Female Sexual Function Index (FSFI); Beck Depression Inventory (BDI); Expanded Disability Status Scale (EDSS)

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Abstract

BACKGROUND: One of the typical complaints in females with multiple sclerosis (MS) is Sexual dysfunction (SD).

AIM: This study aimed to compare the sexual function of women with and without MS and to recognise factors that possibly related to sexual dysfunction of women with MS.

MATERIAL AND METHODS: Sexual function of 64 women with MS as a case study group were compared to a group of control comprised of 64 women. Female Sexual Function Inventory (FSFI) and Beck Depression Inventory (BDI) were used accordingly to assess sexual function and severity of depression of case and control groups. Functional status of MS Patients was assessed by the Expanded Disability Status Scale (EDSS). The data were analysed using chi-square, independent Samples t, Pearson's correlation coefficients, and multiple linear regression tests.

RESULTS: There were no differences in the Total FSFI and 4 FSFI subscale scores (i.e. sexual desire, arousal, lubrication and satisfaction) between women with MS and controls. The only significant difference between the two groups was the dimension of orgasm ($p = 0.016$). Multivariate analysis demonstrated that only BDI and FSFI total scores have significantly related ($B = -0.436$, $P < 0.001$). In women with MS, a significant negative correlation was found between FSFI and EDSS scores ($rho = -0.35$, $P = 0.032$), as well as between FSFI scores and disease duration ($rho = -0.25$, $P = 0.01$).

CONCLUSION: Depression was associated to sexual dysfunction in women. It could be advantageous to evaluate and treat depression in women with MS who suffer from sexual dysfunction.

Introduction

Multiple Sclerosis (MS) is a chronic disease caused by autoimmune and inflammatory reactions in which nerve fibres are demyelinated in the central nervous system [1]. Young people with the ages of 20 to 40 are most endangered to MS, the ages with most sexual activity. Although MS occurs in both sexes, its occurrence in females is 2 to 3 times more than in males [2]. Disability of MS among adults is common [3]. It can negatively affect the physical and mental well-being of affected people [4], [5]. The patients with MS most often suffer from sexual dysfunction (SD). The relationship between MS and sexual dysfunction is so complex [6], [7]. It seems that there is a complex interaction between social, biological and psychological factors which strongly affected by 'emotions and social competence' of individuals [8]. Sexual function is a delicate issue in every culture, including Iran.

In most cases, MS patients are unwilling to speak about their sexual problem. As a result, their problem remains under-diagnosis. Sexual dysfunction caused by MS is more often in women, and its prevalence varies from 40% to 80%. According to some studies, the most common symptoms of sexual dysfunction in women with MS are loss of genital sensations, diminished libido, anorgasmia or hyporgasmia, and reduction of vaginal lubrication [6], [9].

The quality of life is affected by the sexual function. Thus early diagnosis and intervention of the disorder are essential in patients with MS [10].

It is estimated that the prevalence rate of MS is 45 per 100,000 people in Iran. Within 70% of MS patients in Iran are women aged between 20 and 40 [10]. Due to the religious and culture of Iranian people, women tend to conceal their sexual feelings and experiences, leading their sexual dysfunction to remain unidentified [11]. Sexual dysfunction can lead to divorce and family failure [12]. There are several studies that investigated the rate of sexual dysfunction in Iranian females with MS. Ghajarzadeh (2013) was the first who evaluate sexual function in Iranian women with MS and reported that the rate of sexual dysfunction is 66%. According to Merghati-Khoei study, about 87.1% of Iranian women with MS suffer from sexual problems [14]. Finally, Mohammadi reported that 55.3% of Iranian women with MS has sufficient criteria to be classified as sexual dysfunction [15].

Better identifying the MS patients with sexual dysfunction as well as determining the associated factors requires a better understanding of the prevalence and nature of MS to provide therapeutic strategies for these patients [16].

This study aimed to assess sexual dysfunction of women with MS and to compare it with a control group of healthy women. Also, possible factors associated with sexual dysfunction, including depression and demographic variables (age, education, employment, marriage duration) are examined in this study.

Material and Methods

Design and participants of the study

This comparative case-control analytical study was performed from June 2015 to August 2016 on MS women who were admitted in hospitals affiliated by Islamic Azad University of Mashhad, Iran. The criteria for patients to be included in this study were: aged between 18 and 50 years, being married, definite MS according to MC Donald criteria [17], and having at least once sexual intercourse in the last 4 weeks. The patients with the following conditions were excluded from the study: sexually inactive or postmenopausal women, those having had any exacerbations of MS during the last 6 months, women with known cases of psychiatric disorders, those were under-medicated with medicines which could affect sexual activity like anti-depressants and beta-blockers, and women with other concomitant diseases. Accordingly, 64 women were selected as the case group using consecutive sampling method. The control group included 64 healthy women in the same ageing range with the case group that were selected from the relatives of patients.

Sample size

From one hand, according to the Ghajarzadeh et al., [13], the mean values of general sexual function score were 23.2 ± 7.1 and 26.8 ± 5.2 in the case and control groups, respectively, and from the other hand, considering the coefficient of confidence of 95% and the test power of 90%, the least size of 64 were considered for each group.

Questionnaires

To collect demographic data including age, marriage duration, employment and educational status, a specific checklist was designed. Also, valid FSFI questionnaire and a Persian version of Beck depression inventory (BDI) were used in this study to collect required data.

FSFI is a self-report questionnaire with 19-item that is used to evaluate the sexual function of women. In addition of a total score of sexual function [18], it provides scores of sexual function on six domains as follows: desire (2 items, questions 1and 2), arousal (4 items, questions 3, 4, 5 and 6), lubrication (4 items, questions 7, 8, 9 and 10), orgasm (3 items, questions 11, 12 and 13), satisfaction (3 items, questions 14, 15 and 16), and pain (3 items, questions 17, 18, 19). According to Wiegel et al., [19], to distinguish between women with and without sexual dysfunction, an FSFI total score of 26.55 is optimal. Mohammadi et al., have well documented the Persian version of FSFI and found that the cut-off point for FSFI questionnaires is 28 with the sensitivity of 83% and specificity of 82%. Accordingly, the FSFI score of 28 or less is considered to show the sexual dysfunction of Iranian women [20].

The BDI consisted of 21 questions about the feelings of respondents over the last week. Each item had a score between 0 and 3 that, respondents expressed their depression accordingly. The scores of all items are added together and Individuals are classified as follows: those with a total scores between 0 and 9 were identified as not depressed, scores between 10 and 18 as mild to moderate depression, scores between 19 and 29 as moderate to severe depression, and scores between 30 and 63 as severe depression [21]. The psychometric characteristics of the Iranian version of BDI are well documented in the literature [22].

Expanded Disability Status Scale (EDSS) was evaluated after a neurological examination of the patients, with ranging from 0 to 10 [23].

Analysis

Data analysis was performed using SPSS software V.18.0 (SPSS Inc., Chicago, IL, USA). Sample characteristics were examined using descriptive statistics such as frequency and mean (SD). The data had a normal distribution, so Independent Samples t-Test was used to compare quantitative variables in two groups of case and control. Qualitative variables in the two groups were compared using the chi-square test, and the relationship between two quantitative variables was investigated using the Pearson test. Afterwards, all variables were combined into a multiple linear regression model. The coefficient of confidence of 95% was considered in the analysis.

Ethics

The study had been conducted by the Helsinki declaration and approved by the local ethics committee. The confidentiality of participants' information and their answers to questions were assured and written informed consent of all participants was taken.

Results

The numbers of patients with MS and healthy participants in the study was 64 in each group. Ninety four per cent (60 patients) of MS patients suffer from relapsing-remitting MS, and other 6 per cent (4 patients) had secondary progressive MS. The results of the comparison between cases and control group are presented in Table 1. As it could be seen from this table, the median of EDSS is 2 with a range from 0 to 6 and the mean duration of the disease is equal to 52.5 months (ranging from 6 to 84.5).

Table 1: Comparison of personal properties in cases and controls groups

Quantitative variables		Cases Mean (SD)	Controls Mean (SD)	Statistic p-value
Age (years)		35.25 (8.07)	32.83 (8.07)	t=1.697 *P* =0.092
Marriage duration (years)		13.17 (8.77)	10.58 (8.60)	T = 1.683 *P* =0.095
Qualitative variables		Cases No. (%)	Controls No. (%)	Statistic *P* -value
Employment	Non-employed	60 (93.8%)	17(26.6%)	Pearson Chi-Square = 60.268
	Employed	4(6.2%)	47(73.4%)	*P* = 0.000
Education	Primary - secondary	43 (67.2%)	19(29.7%)	Pearson Chi-Square = 18.018
	Higher	21(39.8%)	45(70.3%)	*P* = 0.000

It could be seen from the table 1 that mean age (p = 0.092) and marriage duration (p = 0.095) of two groups are not significantly different. However, there is a significant difference in employment (p < 0.001) and educational level (p < 0.001) of the two groups. Table 2 shows the comparison of BDI score and total score of sexual dysfunction and its subscales between the case and control groups.

Table 2: Mean BDI, the total score of sexual dysfunction and its subscales in case and control groups

	MS Patients Mean (SD)	Controls Mean (SD)	statistics	*P* -value
BDI	18.92 (10.81)	9.93 (7.46)	T = 5.467	*P* < 0.001*
Total score of sexual dysfunction	22.86 (5.36)	24.39 (4.75)	T = -1.714	*P* = 0.089
Desire	3.26 (1.11)	3.50 (1.02)	T = -1.297	*P* = 0.197
Arousal	3.43 (1.22)	3.81 (1.12)	T = -1.814	*P* = 0.072
Lubrication	5.76 (1.40)	6.20 (1.41)	T = -1.730	*P* = 0.086
Orgasm	3.81 (1.26)	4.29 (0.96)	T = -1.730	*P* = 0.016**
Satisfaction	3.71 (1.39)	3.85 (0.94)	T = -0.683	*P* = 0.496
Dyspareunia	2.67 (1.01)	2.75 (0.98)	T = -0.426	*P* = 0.671

* Significant at *P* < 0.001; ** Significant at *P* < 0.01.

According to Table 2, the total score of sexual function is not significantly different between the two groups. Among the items of the questionnaire, orgasm is the only item that has a significant difference in the two groups (p = 0.016), and the value of mean score for orgasm is better in control group in comparison with the patient group. The obtained score of depression in the case group is significantly higher than the score in the control group (p = 0.000).

Univariate analysis was firstly conducted to investigate the relationship between variables of age, marriage duration, depression, education and employment using total score of sexual function. The results are presented in Table 3. From the results shown in Table 3, it could be seen that age and depression are only items with a significant relationship in terms of the total score of sexual function.

Table 3: Univariate analysis results and risk factors for sexual dysfunction

Independent Variables	Statistic	*P*-value
Multiple sclerosis a	T = -1.714	0.089
Depression inventory of Beck b	R = -.411	*P* < 0.001**
Age b	R = -.0181	0.041*
Education a	T = -1.563	0.121
Employment a	T = -1.027	0.306
Marriage duration b	R = -0.167	0.06

Dependent Variable: Total score of FSFI; a conducted using independent sample t-test; b conducted using Pearson's correlation test; * Significant at *P* < 0.05; ** Significant at *P* < 0.001.

Afterwards, all variables used in univariate analysis were combined into a multiple linear regression model. The results are indicated in Table 4. It is seen from this table that, there is only a significant relationship between the BDI and FSFI total scores.

Table 4: The results of multivariable linear regression analysis and risk factors for sexual dysfunction

Variables	B	SE	Beta	t	*P*-value
Multiple sclerosis	0.156	1.207	0.015	.129	0.897
Beck depression inventory	-0.216	0.048	-0.436	-4.474	< 0.001*
Age	-0.035	0.103	-0.056	-0.341	0.737
Education	-0.851	1.193	-0.084	-0.713	0.477
Employment	-0.699	1.321	-0.067	-0.529	0.598
Marriage duration	-0.055	0.103	-0.094	-0.528	0.598

Dependent Variable: Total score of FSFI; * Significant at *P* < 0.001.

Univariate analyses of Pearson's and independent sample t-tests were used to examine the

relationship between sexual dysfunction subscales and independent variables. The obtained results are given in Table 5.

Table 5: The results of univariate analyses for the relationship between sexual dysfunction subscales and other variables

	BDI (*r*, *P* value)	Age (*r*, *P* value)	Marriage duration (*r*, *P* value)	Employment (t, *P* value)	Education (t, *P* value)
Desire	(-0.377,0.000***)a	(-0.184,0.037*)a	(-0.215,0.015*)a	(-1.255,0.212)b	(-1.573,0.118)b
Arousal	(-0.440,0.000***)a	(-0.173,0.051)a	(0.210,0.017*)a	(-1.579,0.117)b	(-2.085,0.039**)b
Lubrication	(-0.306, 0.000***)a	(-.0183,0.038*)a	(-0.120,0.176)a	(-1.067,0.288)b	(-0.975,0.332)b
Orgasm	(-0.453,0.000***)a	(-0.183,0.039*)a	(-0.180,0.042*)a	(-1.429,0.156)b	(-2.962,0.004**)b
Satisfaction	(-0.356,0.000***)a	(-0.137,0.124)a	(-0.195,0.027*)a	(-0.393,0.695)b	(-2.526,0.013**)b
Dyspareunia	(0.067,0.647)a	(0.044,0.621)a	(0.90,0.310)a	(-0.496,0.621)b	0.751,0.454)b

a: performed using Pearson's correlation test; b: performed using independent sample t test; * Significant at P < 0.05; ** Significant at P < 0.01; *** Significant at P < 0.001.

The results of multivariate analysis represented a significant relationship between depression and all other sexual function subscales (p < 0.001) except for dyspareunia.

Different scores in patients with and without sexual dysfunction are shown in Table 6. As could be seen in this table, there is a significant difference between patients who have a total FSFI score higher than 28 and those with a score lower than 28 in terms of mean BDI and all subscales of FSFI except for dyspareunia.

Table 6: Total FSFI scores of patients with and without sexual dysfunction

	Patients with FSFI < 28 N = 53 Mean (SD)	Patients with FSFI > 28 N = 11 Mean (SD)	Statistic	*P* values
BDI	20.9 (10.33)	9.45 (7.9)	T = 5.467	*P* = 0.001
Total sexual score	21.22 (4.25)	30.75 (2.16)	T = -1.714	*P* < 0.001
Desire	2.96 (0.89)	4.69 (0.96)	T = -1.297	*P* < 0.001
Arousal	3.08 (0.96)	5.1 (0.9)	T = -1.814	*P* < 0.001
Lubrication	5.45 (1.28)	7.27 (0.93)	T = -1.730	*P* < 0.001
Orgasm	3.50 (1.08)	5.3 (0.95)	T = -1.730	*P* < 0.001
Satisfaction	3.39 (1.24)	5.23 (1.06)	T = -0.683	*P* < 0.001
Dyspareunia	2.77 (1.05)	2.21 (0.63)	T = -0.426	*P* = 0.1

There are significant negative correlations between disease duration and FSFI scores (rho = -0.25, P = 0.01) and between EDSS and FSFI scores (rho = -0.35, P = 0.032) in MS patients. Also, a significant positive correlation could be seen between EDSS and BDI (rho = 0.31, P < 0.001) in the patients.

Around 12.5 per cent of patients in the case group (eight people) and 4.7 per cent in the control group (three people) answered “Yes” to this question that "Have physicians and medical staff ever asked about your sexual problems?", that in this regard didn't exist a significant difference between the two groups. (p = 0.26)

Discussion

Sexual dysfunction is one of the common side effects of multiple sclerosis (MS) that usually goes unreported by patients and do not address by clinicians [16]. The prevalence of sexual dysfunction in MS patients had been estimated to be between 40% and 80 % [24].

The aim of this study was to investigate the prevalence of sexual dysfunction in MS patients and to recognize factors that possibly related to sexual dysfunction of women with MS. considering a cut-off point of 28 on the FSFI in this study, high prevalence of sexual dysfunction (82.5%) was found that is consistent with the previous research. In his study, Ghajarzadeh (2013) considering a cut-off point of 26.55 for FSFI, reported that the prevalence of sexual dysfunction in Iranian MS women are 66% [13]. Mohammadi [15] considered a cut-off point of 28 for FSFI and found a high frequency of sexual dysfunction among Iranian women with MS (55.3%). In their study on the sexual problem of Iranian women with MS, Merghati-Khoei et al., [14] based on Multiple Sclerosis Intimacy and Sexuality Questionnaire-19 (MSISQ-19) reported that 87.1% of women with MS had a primary sexual problem.

To compare the sexual function of MS patients with healthy people, a control group consisting of 64 healthy women was selected in our study. As far as we know, the only research amongst Iranian studies on MS women that used a control group is Ghajarzadeh study [13].

Anyhow, regarding all subscales and total score of FSFI in our study, the mean scores for the case group were worse than the control. According to our study results, there are no statistical differences between the two groups except for orgasm. This is not consistent with Ghajarzadeh study, in which significant differences between the scores of two groups were found. This disagreement may be caused by different sample sizes used in the two studies. In the Ghajarzadeh study, the case group consisted of 100 patients and the control group including 50 women [13], while in our study, the numbers of people in both groups were 64. The members of the control group in our study were selected amongst patients` relatives, and this may be another reason for the difference between the two studies. Various socio-cultural spheres have different sexual behaviour [25]. As a result, individuals of a family because of they're common sociocultural have close sexual function. As mentioned previously, the orgasm was the only item that differed between the case and control groups. This is consistent with Merghati-Khoei et al., [14] who found that the most frequent symptom of primary sexual problems in MS women is delayed orgasm (75.7%). Ashtari et al., [10] in their study about sexual dysfunctions among Iranian women showed that orgasmic problems are the most common sexual dysfunction with 41.2% prevalence in Iranian women.

Marriage duration had no relationship with sexual function in this study. Contrarily, Merghati-Khoei et al. found an inverse relationship between sexual problems and marriage duration. They

attributed this inverse relationship to change in perceptions and problem discounting that exist as sexual frequency has declined over time [14].

There was also no significant relationship between age and sexual function in the present study. Although, univariate analysis identified age as a significant factor (Table 1), but its effect reduced when multiple linear regression analysis was performed. Similar results are reported by Ghajarzadeh [13] and Mohammadi [15]. Also, Khan et al., [24] using Sexual Frequency Scale found no relationship between age and sexual dysfunction. In the other hand, Merghati-Khoei has reported a significant positive relationship between age and sexual problems [14]. This could be attributed to the different questionnaire (MSISQ-19) as well as using the univariate analysis in Merghati-Khoei study.

One of the predominant psychological problems that can influence sexual dysfunction in MS patients is depression. Depression is more common in MS patients [25], [26], [27], so it seems logical to expect that these patients suffer from sexual dysfunction more than others. In the present study, the mean BDI score in MS patients was significantly higher than in the control group. In both the overall score and the subscale scores, mean BDI was the only predictor of FSFI score, except for dyspareunia. Similarly, there are many studies that reported a strong relationship between depression and sexual dysfunction [15], [30]. [31], [32] In their study, Ghajarzadeh et al., reported a negative and significant correlation between the total score of sexual dysfunction and BDI score in MS patients [13]. According to ELIXIR study, 76 per cent of depressed participants suffer from arousal problems, and the other 24 per cent had problems with erectile or lubrication [33]. Physiological and psychological factors can cause depression symptoms in MS patients that require close monitoring and selecting appropriate treatment options such as behavioural and pharmacological approaches) [34]. Furthermore, using antidepressants by depressed patients may worsen the sexual dysfunction in them, as studies have confirmed the relation between antidepressants and sexual dysfunction [35], [36].

The relationship between sexual dysfunction and employment and education wasn't significant in this study. This is not in line with some studies [14], [38], but is consistent with the results of Redelman study [35].

The results of our study showed a significant difference between patients with and without sexual dysfunction (patients with FSFI score of less and more than 28) in terms of mean total FSFI scores and its subscales. These results are in line with the findings of Ghazarzadeh et al., [13], and Lombardi et al., [39].

This study has some limitations. First, the collection of data and samples were only done in one outpatient centre. When generalising the results of this study to whole Iranian MS women, one should notice this limitation. Second, the information on the spouses of the understudy patients was not collected. Future studies must address this issue.

In conclusion, the results of this study showed a significant relationship between depression and sexual dysfunction in MS population. As a result, it is helpful to evaluate and treat depression in patients with MS who suffer from sexual dysfunction as a starting point for intervention. The ideal is that physicians make it a part of their daily assessment. However, many physicians and health care providers do not spend enough time or have not convenience to talk about sexual issues of their patients, and many of them do not have enough education about treatment of sexual dysfunction [34].

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Clinical Science

Correlation between Obesity and Lipid Profile in Type 2 Diabetes Mellitus Patients at the Endocrine and Metabolic Polyclinic in General Hospital Pirngadi Medan

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Abstract

BACKGROUND: Obesity is a multifactorial disease that is dangerous and is a factor in the emergence of serious diseases such as dyslipidemia, stroke, coronary heart disease and others. In Type 2 Diabetes Mellitus (T2DM) patients there is a disorder of lipid metabolism, namely dyslipidemia. Changes in lipid profile that occurred were an increase in total cholesterol levels, Low-Density Lipoprotein (LDL), and triglycerides, and decreased levels of high-density lipoprotein (HDL). The phenomenon of an increase in T2DM patients in Indonesia caused double mortality in recent decades.

AIM: This study was to determine the relationship between obesity and lipid profile in T2DM patients at Pirngadi Medan Hospital in 2018.

METHODS: This study was conducted in an observational analytic with a cross-sectional study approach. Fifteen obese patients with T2DM who were treated at the Endocrine and Metabolic Polyclinic in Pirngadi General Hospital Medan from January to December 2018 were recruited into the study sample.

RESULT: Based on the analysis using the results of a one-way correlative analytical test showing that there was a positive correlation between obesity and total cholesterol levels ($r = 0.209$; $p = 0.455$) and LDL levels ($r = 0.335$; $p = 0.222$) but not significant. There was a negative correlation between obesity and HDL levels ($r = -0.072$; $p = 0.798$) and triglyceride levels ($r = -0.025$; $p = 0.930$) but not significant. There was no significant relationship between obesity and blood glucose levels ($r = 0.463$; $p = 0.082$). This study concluded that there was no significant relationship between obese patients and lipid profiles in T2DM patients.

CONCLUSION: Obesity positively correlates with blood glucose level, but its correlation with a lipid profile is not reliable.

Introduction

Diabetes Mellitus (DM) is a group of metabolic diseases characterised by hyperglycemia due to defects insulin work in the liver and peripheral tissues, insulin secretion from pancreatic beta cells, or both [1]. Nutritional status influences the incidence of Type 2 DM (T2DM). High BMI (Body Mass Index) has a 2 times greater risk of developing T2DM compared to low BMI. The results showed that general obesity had a risk of 2.24 times while abdominal obesity had a risk of 2.44 times for the occurrence of DM [2].

In T2DM patients, abnormalities of lipid metabolism can be found in the form of dyslipidemia. Dyslipidemia is a disorder of lipid metabolism characterised by an increase or decrease in lipid fraction in the plasma. The main lipid fraction abnormalities include increases in total cholesterol, triglycerides, Low-Density Lipoprotein (LDL), and decreased High-Density Lipoprotein (HDL). Dyslipidemia caused by DM is secondary dyslipidemia [3], [4].

Since 1980 the incidence of obesity in the epidemic has begun to increase. Where currently, more than 30% of the US population is obese with a

Body Mass Index (BMI) more than 30 kg/m² and almost two-thirds are overweight (BMI between 25 and 29.9 kg/m²) [4], [5].

In Indonesia, the incidence of obesity continues to increase. Where in adult men there was an increase from 13.9% in 2007 to 19.7% in 2013? Whereas in adult women there was a very high increase reaching 18.1%, from 14.8% in 2007 to 32.9% in 2013 [6].

Obesity is a risk factor for developing insulin resistance and type 2 diabetes. In 2015, an estimated 2.3 billion adults will experience overweight, and 700 million of them will be obese. Significant relationship exists between per cent body fat and body weight in DM, besides that the prevalence of diseases associated with insulin resistance increases together with increasing BMI because an increase in adipose tissue is characterised by decreased HDL and increased triglycerides [7].

Obesity is a multifactorial disease that rises very sharply throughout the world which reaches dangerous levels and is a factor for the emergence of serious diseases including hypertension, stroke, dyslipidemia, coronary heart disease, and type 2 diabetes [8]. Based on the background described, the researchers were interested in examining the relationship between obesity and lipid profile in patients with type 2 diabetes.

Material and Methods

This study was conducted in an observational analytic study with a cross-sectional approach. In this study, no follow-up was carried out on the measurements taken. This study was conducted on a set of objects, within a certain period aimed at knowing the relationship of obesity and lipid profile in patients with type 2 diabetes mellitus. Fifteen obese patients with type 2 DM who were treated at the Endocrine and Metabolic Polyclinic in Pirngadi General Hospital Medan from January to December 2018 were recruited to become a research sample.

Inclusion criteria were m en and women who suffer from obesity (BMI > 25 kg/m² or obesity I and obesity II), adults over 25 years, and willing to give written permission after being given informed consent to take part in this research

Exclusion criteria were patients who are not cooperative, patients suffering chronic diseases, the patient's health condition is not possible to take part in this study, Patients are outside the city so they cannot take part in the study according to the set schedule

The variables examined in this study were the independent variable was Obesity (Obesity I and Obesity II). The dependent variables were lipid profiles (total cholesterol, HDL, LDL and triglycerides).

In this study, the patient's lipid profile data were obtained from the report of the attached laboratory examination results and the method of measurement by looking at the lipid profile data from the laboratory examination in units of mg/dl. Obesity is measured by the measurement body Mass Index (BMI) (body weight kg/body height m^2).

Statistical analysis

Data were analysed through statistical calculations to test hypotheses using the Pearson correlation test method. If the normality test was found that the data is not normally distributed, the hypothesis test could be done using the Spearman correlation test method. P value considered significant if it is less than 0.05

Results

The 15 diabetic patients who were divided into 4 men and 11 women in the Internal Medicine Department at the Endocrine and Metabolic Polyclinic in General Hospital Pirngadi Medan, the characteristics in Table 1.

Table 1: Basic characteristics of research subjects

Characteristics	Mean ± SD
Age (year)	56.733 ± 10.271
Body weight (kg)* (Md, Min, Max)	77.533 (75,65,106)
Body height (cm)	154.00 ± 7.156
Cholesterol total	230.2 ± 45.896
HDL	53.6 ± 13.69
LDL	128.6 ± 38.52
Triglycerides	240.86 ± 219.10
IMT	32.83 ± 3.05
Blood Glucose	209.06 ± 84.11
Systole	138 ± 14.735
Diastole	86.66 ± 9.75

Md: Median, Min: minimum, Max: maximum.

Based on the results obtained from the correlative analytic test found a positive correlation between obesity and Total Cholesterol levels (r = 0.209; p = 0.455) and LDL levels (r = 0.335; p = 0.222) but not significant. There was a negative correlation between obesity and HDL levels (r = -0.072; p = 0.798) and triglyceride levels (r = -0.025; p = 0.930) but not significant. There was no significant relationship between obesity and blood glucose levels (r = 0.463; p = 0.082), (Table 2).

Table 2: The Correlation between obesity and lipid profiles

Variable	r value	P value
Cholesterol total	0.209	0.455
HDL	-0.072	0.798
LDL	0.335	0.222
Triglycerides	-0.025	0.930
Glucose	0.463	0.082

P value considered significant if it is less than 0.05.

In Table 3 there was a positive relationship between obesity and systolic blood pressure (r = 0.213) but not diastole (r = -0.226).

Table 3: Correlation of obesity with blood pressure

Group	Obesity	
	r value	P value
Sistole	0.213	0.446
Diastole	-0.226	0.418

Discussion

In this study, 15 samples were obtained that met the inclusion and exclusion criteria. The sample consisted of 4 men (10%) and 11 women (90%). This finding is by the opinion of Brunner and Suddart (2002) who stated that women suffer from diabetes mellitus more than men. This is triggered by the presence of a greater percentage of body fat in women compared to men which is one of the factors that can reduce sensitivity to the workings of insulin in the muscles and liver [9].

Fluctuations in estrogen levels can affect blood glucose levels. When estrogen levels increase, the body can become resistant to insulin [10]. Irawan (2010) stated that menopause causes body fat distribution to be easily accumulated due to these hormonal processes so that women are at risk of suffering from T2DM [11].

The average BMI of the patient is 32.83. The average total cholesterol level was 230.2 gr/dl, 53.6 gr/dl HDL levels, 128.6 gr/dl LDL levels, average triglycerides 240.86 gr/dl and the average blood glucose level 209.06 mg/dl (Table 1).

To find out the relationship between obesity and lipid profile in patients with type 2 diabetes, a one-way correlative analytic test was performed. The correlative analytic test results between obesity and lipid profile (total cholesterol, HDL, LDL and triglyceride) and blood glucose in table 2. The results obtained showed a positive correlation between obesity levels and total cholesterol levels (r = 0.209; p = 0.455) and LDL (r = 0.335; p = 0.222). There was a positive relationship between the two but not significant. There was a negative correlation of HDL levels (r = -0.072; p = 0.798) and triglyceride levels (r = -0.025; p = 0.930) but not significant. With a p-value considered significant, it is less than 0.05. This is not by the theory that the most frequent picture of dyslipidemia in type 2 DM is a decrease in HDL levels and an increase in triglyceride levels by the theory [12].

In this study, there was a positive correlation between obesity and blood glucose levels (r = 0.463; p = 0.082). The results of this study are not much different from previous studies conducted by Jin Ook Chung, Dong Hyeok Cho, Dong Jin Chung, and Min Young Chung (2012) in the Associations among Body Mass Index, Insulin Resistance, and Pancreatic-Cell Function in Korean Patients with New-Onset Type 2 Diabetes. This study showed that there was a significant relationship between BMI and the occurrence of insulin resistance which caused an increase in fasting blood glucose levels, $p < 0.05$ [13].

The results of other studies were also conducted by Ninh T. Nguyen, Xuan-Mai T Nguyen, John Lane, and Ping Wang (2011) in Relationship Between Obesity and Diabetes in US Adult Population: Findings from the National Health and Nutrition Examination Survey, 1999-2006 showed a significant relationship between obesity and the occurrence of type 2 diabetes mellitus [14]. The results of this study meant that the greater body mass index value, the greater the fasting blood glucose value. The greater body mass index value means the patient leads to obesity. This was accordance with the theory of Suyono (2011), that the risk factors of type 2 diabetes mellitus are overweight/obesity factors which include lifestyle changes from traditional to the western lifestyle, overeating, and relaxed life (lack of motion) [15].

In the digestive system, food is broken down into the basic ingredients of the food itself. Carbohydrates become glucose, proteins become amino acids, and fats become fatty acids. The three food substances will be absorbed by the intestine and then enter the blood vessels and circulated throughout the body to be used by organs as fuel. To function as a fuel, food cells, especially glucose, must be metabolised first. In the metabolic process, insulin plays an important role, which is to enter glucose into cells, which can then be used as fuel [15], [16].

In normal circumstances, it means that insulin levels are enough and sensitive, insulin will be captured by insulin receptors that are on the cell surface, and then open the cell entrance, so that glucose can enter the cell and then burned into energy. As a result, blood glucose levels become normal [15]. This is different in the state of obesity, an increase in mRNA Lipopolysaccharides (LPS) - induced TNF-α factor (LITAF) and protein levels along with increased BMI indicate a parallel relationship between LITAF and metabolic disorders.

According to the study, LITAF is activated in obese patients and contributes to the development of obesity which induces inflammation and insulin resistance, since LITAF plays a role in the inflammatory process in regulating the expression of TNF-α, IL-6 and MCP-1 which results in insulin resistance, and TLR4. One of the LITAF receptors in macrophages can also be stimulated by free fatty acids which can cause an inflammatory process in obese patients. LITAF is a TNF-α description of the regulator that should play a role in the immune mechanism against infection. The LITAF gene is

located at 16p13.13 which is significantly present in lymph, lymph nodes, and peripheral blood leukocytes. TNF-α is a strong trigger for proinflammatory cytokines such as IL-6, MCP-1, leptin and PAI-1. It is very involved in the inflammatory process in obese patients. The increase in TNF-α observed in fat tissue in obese patients shows a direct relationship to the emergence of insulin resistance in obese patients [17]. The occurrence of insulin resistance causes glucose circulating in the blood to be unable to enter the cell, so the sugar level in the blood becomes higher than normal [15].

Hyperglycemia in people with diabetes mellitus is also closely related to fat metabolism. Fat has the main task of storing energy in the form of triglycerides through the process of lipogenesis which occurs in response to excess energy and mobilises energy through the process of lipolysis in response to energy shortages. Under normal circumstances, these two processes are strictly regulated [18].

The condition of obesity is caused by excessive intake of nutrients continuously causing fat deposits to become excessive. Deposits of fatty acids in the form of chemical compounds in the form of triacylglycerol contained in adiposity cells can protect the body from the toxic effects of fatty acids. Freeform fatty acids can circulate in blood vessels throughout the body and cause oxidative stress which we are familiar with lipo-toxicity. The emergence of lipo-toxicity effects caused by several free fatty acids released by the triacylglycerol to compensate for the destruction of excessive fat deposits affects the adipose and non-adipose tissue and plays a role in the pathophysiology of diseases in various organs such as the liver and pancreas. This release of free fatty acids from excessive triacylglycerol can also inhibit fat synthesis and reduce the clearance of triacylglycerol. This can increase the tendency of hypertriglyceridemia.

The release of free fatty acids by endothelial lipoprotein from triglycerides which increases in the increase of lipoprotein β causes lipo-toxicity which also interferes with the function of insulin receptors. The consequence of insulin resistance is hyperglycemia, which is compensated by glucose synthesis from the liver (gluconeogenesis), which contributes to aggravating hyperglycemia.

Free fatty acids also contribute to hyperglycemia by reducing glucose use from insulin-stimulated muscles. Lipotoxicity due to excess free fatty acids also decreases insulin secretion from pancreatic β cells, which ultimately β cells will experience fatigue [19]. The weakness of this study is a cross-sectional study that has limitations: 1. Not all confounding variables can be controlled properly so that better follow-up research is needed with appropriate research designs. 2. This study is based on local state hospitals so that to generalise to the population of type 2 diabetes mellitus with obesity globally should be wiser. 3. The number of samples is limited so that more samples are needed. Further research is needed with better research designs and with more and varied samples to prove the relationship between Obesity and lipid profiles in patients with type 2 DM.

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The Association between Asthma and Obesity in Children – Inflammatory and Mechanical Factors

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Abstract

BACKGROUND: Association of asthma and obesity has been demonstrated in numerous epidemiological studies. However, the underlying mechanisms of the association are not well understood. Both conditions are characterised by chronic tissue inflammation, which includes numerous different inflammatory markers, and possible atopy.

AIM: The study aimed to investigate the association between asthma and obesity in children and assess several of potential underlying mechanisms, including the parameters of systemic inflammation (CRP, fibrinogen) and the mechanical effect of obesity on the respiratory system through parameters of lung function. An additional aim was to examine the role of atopy in overweight children with asthma and to investigate the type of respiratory inflammation.

MATERIAL AND METHODS: This prospective study included 72 patients in the age group of 7-15 years, including 38 with high body mass index (BMI), 16 with asthma and normal BMI, and 18 with asthma and high BMI for sex and age. Non-specific inflammatory markers (fibrinogen, CRP), eosinophilia, and total serum IgE were investigated. The patients underwent a skin prick test (SPT) with standard inhalant allergen extracts, measurement of fractional exhaled nitric oxide Fe (NO), and an assessment of lung function.

RESULTS: In overweight groups of children we determined significantly higher values ($p < 0.001$) of both acute inflammatory reactants, CRP and fibrinogen, with no difference between children with and without asthma. There was a significant increase in eosinophilia, total IgE, and positive SPT in the asthmatic groups compared to the group of non-asthmatic patients ($p < 0.001$ for the three parameters). Compared to the group composed of overweight patients without asthma, the asthmatic patients had higher NO values ($p < 0.001$). No significant difference in the lung function parameters was found between the three groups ($p > 0.05$).

CONCLUSION: A positive association between asthma and obesity with inflammation as an underlying mechanism, eosinophilic one in asthmatic patients and non-eosinophilic one in overweight patients, was determined. It seems that the lung function parameters did not differ between asthmatic patients and overweight patients. No influence of atopy in the association between asthma and obesity was verified. Further analyses of specific inflammatory markers, for an in-depth evaluation of the mechanisms leading to the association of obesity and asthma, are warranted.

Introduction

Recently, a parallel epidemic increase in the prevalence of asthma and obesity in children has been observed. With more than 334 million patients worldwide, asthma is considered one of the most common chronic diseases. The International Study of Asthma and Allergies in Childhood (ISAAC) has found a wide variation in the prevalence of asthma in childhood [1]. Within this study, in the period between 2002-2004, a moderate low prevalence of asthma has been reported in the Republic of Macedonia that was due, at least partially, to under-diagnosis [2]. The increasing trend in the prevalence of asthma in both adults and children in the last decade has reached a

plateau in developed countries. However, the rise of obesity prevalence has been established recently in developing countries, including Macedonia [3]. Worldwide obesity has been nearly tripled since 1975 as a consequence of changes in diet and the decrease in overall physical activity. Obesity has become a major public health problem, especially in developed countries with more than 1.9 billion overweight people, 600 million of which were obese [4]. Within ISAAC, a moderately low prevalence of overweight children and the low prevalence of obese children have been reported in the Republic of Macedonia [5]. However, thirteen years later, in a new epidemiological study, a significant increasing trend in the prevalence of overweight/obese children has been identified [6].

The relationship between asthma and obesity has been demonstrated in numerous epidemiological studies, but underlying mechanisms of this association are not well understood. Obesity increases the prevalence of asthma and asthma-like symptoms, compromises the control of the disease, decreases the response to anti-inflammatory therapy, and impairs the quality of life in patients with asthma. The physical or mechanical effect of the adipose tissue on the respiratory system is manifested by a reduction in pulmonary volumes and respiratory compliance [7]. On the other hand, the mechanical effect of obesity upon the respiratory system can affect the contractile power of the smooth muscle of the bronchial trunk inducing bronchial hyperreactivity [8]. Both conditions are characterised by chronic tissue inflammation, which includes numerous, although different inflammatory markers which may increase the bronchial hyperreactivity in patients with asthma [9]. Obesity promotes a low-grade chronic inflammatory state. Both, cellular and humoral immunity are reprogrammed to function under excessive body weight [10]. The number of leucocytes and lymphocytes correlates with the degree of obesity, as well as with the monocyte/macrophage ratio. They secrete a large number of inflammatory cytokines such as tumour necrotic factor-alpha (TNF-α), interleukin-6 (IL-6), plasminogen activator (PAI-1), macrophage chemotactic protein (MCP-1).

Moreover, an increase in the level of acute-phase inflammatory reactants such as C-reactive protein (CRP), fibrinogen, and complement components, especially in abdominal obesity can be observed [11], [12]. On the other hand, mediators contribute to increased IgE production, subepithelial fibrosis, and remodelling of the bronchial trunk, which is the primary observation in the pathogenesis of asthma [13]. Adipocytes also produce numerous hormones, such as leptin and adiponectin, which can affect the respiratory system directly, because of the expression of leptin receptors in the lung [14] or through the cells of the immune system [15]. Atopy is an important factor in the development of childhood asthma, present in over 80% of children with asthma. Most of the studies, however, suggest that inflammation of the airway in obese asthmatic adults is non-eosinophilic, with significant neutrophilia in the induced sputum [16].

In this study we sought to investigate the association between asthma and obesity in children and to establish some of the potential underlying mechanisms with particular emphasis on the parameters of systemic inflammation (CRP, fibrinogen) and the mechanical effect of adipose tissue upon the respiratory system through the parameters of the lung function as well as to explore the role of atopy in overweight children with asthma and the type of respiratory inflammation.

Material and Methods

The study has been approved by The Ethics Committee at the Medical Faculty in Skopje. Written informed consent for inclusion in the study was obtained by the parents. The study was a prospective cross-sectional and included 72 children aged 7-15 years (11.08 ± 2.23), treated as outpatients or inpatients at the University Children's Clinic, Skopje, from March to October 2018. Patients were divided into three groups: Group 1 consisted of 38 children with a high body mass index (BMI); Group 2 consisted of 16 children with asthma and a normal BMI; Group 3 consisted of 18 patients with asthma and high BMI. Exclusion criteria included other respiratory, gastrointestinal, urogenital, and cardiovascular diseases, as well as obesity-associated with other than asthma disease or syndrome, and administration of systemic corticosteroid therapy three months before being included in the study. Medical history was taken from the parents and included questions about the familial history of diabetes mellitus and atopy, premature birth, breastfeeding in the first year of life and passive exposure to cigarette smoke at home. Each patient underwent through clinical check-up including auxology measurements. The following laboratory analyses were performed in each child: blood counts for eosinophils, systemic inflammatory markers (fibrinogen, CRP), and total serum IgE.

BMI was calculated by a standard formula and expressed as a weight (kg)/height(m)^2. The International cut-off points for BMI for overweight and obesity by sex between 2 and 18 years, defined to pass through a BMI of 25 kg/m^2 for overweight and 30 kg/m^2 for obesity at age 18, were used [17]. For abdominal obesity assessment, the waist circumference (WC) was measured in centimetres between the lower border of the ribcage and midline of the iliac crest, as well as the hip circumference (HC) in centimetres from the widest point of the hips, and then the waist-hip ratio (WHR) was calculated as previously described [18]. Reference values were

used by sex and age, defined above the 90th percentile for abdominal obesity [19].

Skin prick test (SP) was performed using commercial inhalant allergen extracts, i.e. a mixture of grass pollen, a mixture of trees pollen, Dermatophagoides pteronyssinus, Dermatophagoides farinae, cat epithelium, dog epithelium, mould, and cockroach. The test was performed at the volar side of the forearm where drops of commercial allergen extract Allergopharma (Reinbek, Germany) were gently pricked on the skin surface with a separate lancet. The distance between drops was not less than 2 cm. One mg/ml of histamine or saline were used as positive or negative control solutions. The test was interpreted 15 minutes after the administration of allergen extracts, in comparison with the histamine and the saline reaction. Atopy was defined as a positive response to 1 or more inhalant allergens [20].

The assessment of pulmonary function was performed with the spirometer Schiller SP-1 [21], [22]. Spirometric parameters FEV1, FVC, FEV1/FVC ratio or Tiffeneau index, peak expiratory flow (PEF), and forced expiratory flows in 25%, 50%, 75% of FVC (FEF25, FEF50, FEF75) were assessed. The results were expressed in per cent of predictive value for gender and age [23]. Spirometric measurements were repeated 15 minutes after administration of 200 mcg of salbutamol (albuterol) through a metered-dose inhaler and a Volumatic spacer device. An increase in FEV1 of 12% over baseline was considered as a significant bronchodilator response.

The assessment of eosinophilic airway inflammation was carried out by measuring fractional exhaled nitric oxide-Fe (NO) using NO analyser Niox Vero (Upsala, Sweden). After maximum inspiration, the patients exhaled air without prior retention at a speed of 50 m/s. Values < 20 ppb in childhood indicated non-eosinophilic airway inflammation, or a stable clinical condition [24].

Statistical Analyses

Data was statistically analysed in SPSS software package, version 22.0 for Windows (SPSS, Chicago, IL, USA). The qualitative series were processed by determining the coefficient of relations, proportions, and rates, and were shown as absolute and relative numbers. Quantitative series were analysed with measures of central tendency (average, median), as well as with dispersion measures (standard deviation, standard error).

The distribution of frequencies was analysed through the Shapiro-Wilk test. Pearson Chi-square test, Yates corrected, Fischer exact test, and Fisher Freeman Halton exact test was used to determine the association between certain attributive dichotomies. To test the significance of the difference between certain analysed numerical parameters, depending on the frequency distribution, Student's t-test, One Way ANOVA test, Mann Whitney U test and the Kruskal-Wallis H test were conducted. A two-sided analysis with a significance level of $p < 0.05$ was used to determine the statistical significance.

Results

The survey included a total of 72 children. According to the clinical diagnosis, 38 (52.8%) were overweight/obese without asthma, 16 (22.2%) were asthmatic with normal BMI, and 18 children (25%) were overweight/obese suffering from asthma. The average age of the children in the three groups was 11.4 ± 2.1, 11.0 ± 2.4 and 10.9 ± 2.1 years, respectively without a significant difference between the groups ($p = 0.835$).

Children with asthma in both groups, in the group with normal BMI and high BMI, had a significantly higher frequency of familial atopy compared to the group with high BMI without asthma ($p = 0.034$). There was no significant association between the groups regarding sex ($p = 0.859$), the familial history of diabetes mellitus ($p = 0.139$), premature birth ($p = 0.111$), breastfeeding in the first year of life ($p = 0.769$) and passive exposure to cigarette smoke at home ($p = 0.265$), (Table 1).

Table 1: Demographic characteristics of the patients

Parameters	Groups of children according to the clinical diagnosis			P
	Overweight/ obesity N = 38	Asthma N = 16	Overweight/ obesity and asthma N = 18	
Sex (male)	27 (71.05%)	12 (75%)	14 (77.78%)	** p = 0.859
Age	11.37 ± 2.19	11.00 ± 2.42	10.89 ± 2.09	***X^2 (2) = 0.359; p = 0.835
Fam. atopy	19 (50%)	13 (81.25%)	14 (77.78%)	** p = 0.0338*
Fam. history of diabetes	15 (39.47%)	2 (12.50%)	5 (27.78%)	** p = 0.139
Premature birth	9 (23.68%)	1 (6.25%)	1 (5.56%)	**p = 0.111
Smokers at home	27 (71.05%)	9 (56.25%)	9 (50%)	† 2.652; df = 2; p = 0.265
Breastfeeding period	4 (10.53%)	2 (12.5%)	1 (5.56%)	** p = 0.769
non breastfed	2 (5.25%)	0 (0%)	0 (%)	
> 1 ≤ 4 months	10 (26.32%)	2 (12.5%)	3 (16.67%)	
> 4 ≤ 6 months	22 (57.89%)	12.75%)	14 (77.78%)	
> 6 ≤ 12 months				

* p < 0.05; ** Fisher Freeman Halton exact test; *** Kruskal-Wallis H test; †Pearson Chi-square.

There was a significant difference between the patients from the three groups regarding the average value of the following non-specific inflammatory markers: a) eosinophilia-elevated values above normal (> 4%) were detected in children with asthma both with normal and with high BMI compared to the overweight children without asthma ($p = 0.001$); b) CRP values were higher in the overweight groups ($p = 0.004$); c) fibrinogen levels were higher in the overweight groups ($p = 0.001$); d) IgE levels were higher in the patients with asthma ($p = 0.001$), (Table 2).

Table 2: Analysis of non-specific inflammatory markers and other parameters analysed according to the clinical diagnosis

Parameters	Groups of children according to the clinical diagnosis			P
	Overweight/obesity n = 38	Asthma n = 16	Overweight/obesity and asthma n = 18	
WBC	8.52 ± 2.32	7.31 ± 1.98	8.25 ± 2.02	** χ^2 (2) = 3.955; p = 0.138
Eo	2.60 ± 2.35	6.46 ± 4.77	5.75 ± 2.79	** χ^2 (2) = 17.992; p < 0.001*
CRP	3.92 ± 3.54	1.47 ± 2.56	3.93 ± 6.31	** χ^2 (2) = 11.304; p = 0.004*
Fibrinogen	3.04 ± 0.46	2.52 ± 0.40	2.78 ± 0.41	*** F = 8.357; p = 0.001*
IgE	119.75 ± 189.11	374.02 ± 494.97	380.17 ± 333.39	** χ^2 (2) = 19.566; p < 0.001*
FEV1	103.79 ± 10.59	97.21 ± 10.74	102.21 ± 12.36	** χ^2 (2) = 3.221; p = 0.120
FVC	98.74 ± 9.89	95.99 ± 8.59	99.52 ± 8.47	*** F = 0.6735; p = 0.513
FEV1/FVC	99.01 ± 7.89	95.90 ± 8.92	96.47 ± 6.73	** χ^2 (2) = 3.191; p = 0.203
PEF	85.24 ± 11.79	87.82 ± 27.28	82.18 ± 14.18	** χ^2 (2) = 2.942; p = 0.230
FEF25	81.26 ± 22.41	71.04 ± 27.06	73.69 ± 21.38	*** F = 1.298; p = 0.280
FEV50	49.13 ± 13.99	55.76 ± 15.73	49.31 ± 17.02	*** F = 1.163; p = 0.319
FEV75	94.72 ± 17.14	81.74 ± 21.64	87.18 ± 17.73	** χ^2 (2) = 8.172; p = 0.017*
FeNO (>20 ppb)	15.59 ± 9.93	43.83 ± 29.27	39.07 ± 25.01	** χ^2 (2) = 16.738; p < 0.001*
BMI	30.38 ± 5.57	18.59 ± 2.01	28.83 ± 5.24	*** F = 33.249; p < 0.001*
WC	95.75 ± 19.69	39.06 ± 31.39	90.61 ± 11.09	** χ^2 (2) = 36.427; p < 0.001*
WHR	0.94 ± 0.10	0.86 ± 0.07	0.94 ± 0.12	** χ^2 (2) = 4.278; p = 0.118
SPT (positive)	4 (10.53%)	13 (81.25%)	16 (88.89%)	Yates correcting = 41.768; df = 4; p = 0.000001**
Bronchodilator test (positive)	7 (18.92%)	14 (87.50%)	16 (100%)	/

FeNo = fractional exhaled nitric oxide; BMI = body mass index; WC = waist circumference; WHR = waist-hip ratio; * p < 0.05; ** Kruskal-Wallis H test; *** One Way ANOVA test.

Regarding the spirometric parameters, there was a significance only about FEV75, which was the lowest in the asthma group (p = 0.017). Regarding all other parameters, there was no significant difference between the three examined groups. High FeNO values had the children from the asthma groups (p = 0.001). There was a significant association between the parameter of personal atopy-SPT and asthma diagnosis (p = 0.001). Positive bronchodilator test was detected in 14 children with asthma (87.50%), in 18 children (100%) in an overweight/obese group with asthma, and 7 children (18.92%) in the group with high BMI.

Discussion

The positive association between asthma and obesity has been reported in many studies. Recently, a meta-analysis related to the BMI and asthma association has been published, which has confirmed that being overweight or obese increase the risk of asthma by 1.64 and 1.92, respectively [25]. Most of the studies included in this meta-analysis have supported a female-specific association. However, others have suggested no gender difference [12]. In our study, there was no significant difference regarding gender distribution between the groups.

Children with high BMI have been found to have elevated values of the acute-phase inflammation reactants, CRP and fibrinogen, not depending on asthma. Most studies have reported an elevated CRP level in obese children and adults [15], [16]. Visser et al., have pointed out that CRP increases with BMI. Regarding the level of fibrinogen, published studies are inconsistent [26]. Canöz and Hafez have determined an elevated level of fibrinogen in overweight subjects, especially in the presence of abdominal obesity [16], [27]. Buyukozturk has not established a correlation between obesity, asthma, and fibrinogen level [28]. In our study, there was no difference between asthmatic children with normal or high BMI regarding eosinophilia, total IgE antibodies, and positive SPT. For these markers, significantly higher values were found in asthma groups compared to the overweight/obese group.

Additionally, asthma groups had significantly higher FeNO values and greater occurrence of familial atopy, which all support the eosinophilic allergic basis of asthma, most common in childhood. FeNO is considered as a marker of eosinophilic airway inflammation. Thus it is high in atopic asthma. On the other hand, asthma in obese adults is usually non-allergic, with neutrophilia in induced sputum [20]. The results of this study support the positive association between asthma and obesity with inflammation as a common underlying mechanism, eosinophilic one in asthma and non-eosinophilic one in obesity. Atopy was not established as an underlying mechanism. Contrary to our results, Rastogi has not established systemic and airway inflammation in overweight children with asthma, investigating FeNO, eosinophils in induced sputum, CRP, and IL-6 [29].

In addition to BMI in the assessment of overweight and obesity, some authors like Appleton and Kronander have included other parameters, such as WC, which better correlates with abdominal obesity in children with asthma [30], [31]. Bustos et al., have confirmed the association between asthma and BMI, but not regarding WC. We assessed the nutritional status by examining BMI, WC, and WHR in the three study groups and found a positive association of the overweight/obese groups either with BMI or with WC. Abdominal fat and visceral fatty tissue are considered the main source of inflammatory markers that makes obesity a "pro-inflammatory condition" [32].

Given the goal of our study to examine the mechanical effect of obesity on lung function, the children with asthma were stable with normal lung function, i.e. not in the stage of exacerbation when the functional parameters would have been reduced. Additionally, all patients with asthma received anti-inflammatory therapy with inhaled corticosteroids. Although the values of the investigated pulmonary functional parameters were within the limits of the reference among all three groups of children, we did not find a significant difference in the mean values between them. Only a significantly lower FEV75 value

was found in the group of children with asthma, which is a parameter for estimating the airflow in the small airways. This is understandable because asthma is a chronic inflammatory disease predominantly affecting small airways. In obesity, due to increased intra-abdominal pressure by adipose tissue on the diaphragm, a pressure on lung and chest is also increased, reducing its elasticity and thus leading to a decrease in residual volume (RV) and forced respiratory capacity (FRC). A large longitudinal study involving children and adults with asthma has reported a significant reduction in FVC in obese adults but not in children [33]. In children with BMI above 85th percentile, a significant reduction in FEV1/FVC ratio has been demonstrated [34]. However, there are some published studies in overweight children with asthma that have reported normal static volumes, but poor control of asthma due to the impaired perception of dyspnoea, which also occurs because of reduced elasticity of the chest [35], [36]. The absence of a significant difference in pulmonary function parameters between asthmatic children and overweight children might be in favour of the influence of mechanical factors in their association.

Bronchial hyperreactivity (BHR), a major feature of asthma, was tested with a bronchodilator test which was positive in over 87% of the asthma groups, but also 26% of the overweight children. There are studies suggesting BHR in obese adults, although they do not include children. Due to obesity, the lumen of the airway is reduced, which leads to disturbed function of the smooth muscle and over time leads to bronchospasm and BHR [37]. Another study has suggested that there was no difference in BHR, by bronchoprovocation test, between asthmatic overweight children and children with normal BMI [38]. Van Leeuwen et al. have confirmed that after weight reduction in children with asthma, the severity of exercise-induced airflow obstruction decreased and the quality of life improved [39].

The present study has some limitations. The number of children in the three examined groups was not similar in the time of the analyses, where the group composed of overweight patients was larger than the other two groups. Additionally, to avoid bias because of the small sample, obese patients were included in the overweight group.

A positive association between asthma and obesity with inflammation as an underlying mechanism was identified, eosinophilic inflammation in asthmatic patients and non-eosinophilic inflammation in overweight patients. It appears that the parameters of lung function did not differ in asthma and obesity. The role of atopy in the association between asthma and obesity was not established. Further investigations are required, including specific inflammatory markers for a more detailed clarification of the underlying mechanisms.

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Refractive Status in Children with Laser-Treated Retinopathy of Prematurity: Our Experience in Bulgaria

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Abstract

BACKGROUND: With the establishment of laser photocoagulation as a standard treatment modality for prethreshold retinopathy of prematurity (ROP), a dramatic reduction of cases with ROP blindness and severe visual impairment have been reported. In the same time, high refractive errors, a common complication in ROP cases and especially in ROP treated infants, have become the main cause of visual and often severe visual impairment.

AIM: The purpose of our study was to analyse the long-term refractive status in children at 3.5 years after laser-treatment for type 1 prethreshold ROP.

PATIENTS AND METHODS: A retrospective, one centre study of refractive status of 18 children with laser-treated type 1 prethreshold ROP was conducted. The refraction after cycloplegia with 1% cyclopentolate was measured at a mean age of 3.56 years (SD ± 0.34). Hyperopia was subdivided into two groups – low hyperopia (SE < +5.0 D) and high hyperopia (SE ≥ +5.0 D). Myopia was classified as myopia (SE ≥ –0.25D) and high myopia (SE ≥ -5.0 D). Astigmatism was divided into low astigmatism (plus CD ≥ +1.0 D) and high astigmatism (plus CD ≥ +2.0 D).

RESULTS: Thirty-three eyes of 18 children were recruited in the study. Three eyes were excluded because of unfavourable anatomical results. The mean gestational age at birth was 27.3 weeks (24-31 weeks, SD ± 1.78), and the mean birth weight – 928.9 g (550-1500 g, SD ± 252.8). The mean spherical equivalent for the whole group was -1.82 D and ranged from -9.00 D to +4.50 D (SD ± 3.48). Hyperopia was observed in 12 (36.4%) eyes. Myopic refraction had 21 (63.6%) eyes. Astigmatism was detected in 18 (54.5%) eyes. Anisometropia had 3 (16.7%) children. Six children (33.4%) had strabismus (4 esotropia; 2 exotropia).

CONCLUSION: High per cent of treated infants for vision-threatening ROP have visual significant refractive errors and strabismus that can cause serious visual impairment if not treated properly and on time.

Introduction

Retinopathy of prematurity (ROP) is a leading cause of avoidable childhood blindness and severe visual impairment worldwide [1]. Significant advances in neonatology and perinatal medicine in our country in recent years lead to the survival of more and more premature babies born with low and extremely low birth weight, making us contemporaries of the third ROP epidemic. According to the Bulgarian ROP screening guidelines, every prematurely born baby with birth weight (BW) less than 1500 grams and gestational age (GA) below 32 weeks must be screened, and all babies with type 1 prethreshold ROP must be treated preferably with diode laser photocoagulation [2]. Significant refractive errors and anisometropia are frequently associated findings in ROP patients, especially in treated children [3], [4], [5], [6]. Higher prevalence of high myopia in children treated with cryotherapy for threshold ROP is first discussed in CRYO-ROP study [4]. Later, Early Treatment for Retinopathy of Prematurity (ETROP) study reveals a higher prevalence of refractive errors (myopia, astigmatism) in treated premature babies than in spontaneously regressed ROP infants and mature children, nevertheless better anatomical and functional results in children treated for prethreshold ROP with laser coagulation compared to children treated for threshold ROP with cryoablation or laser therapy [5], [6].

The purpose of our study was to analyze the long-term refractive status in children at 3.5 years after laser-treatment for type 1 prethreshold ROP.

Patients and Methods

Patients

A retrospective, one centre study of refractive status of 18 children with laser-treated type 1 prethreshold ROP was conducted. All infants were treated at Pediatric Eye Department, Eye Clinic, University Hospital "Alexandrovska", Medical University, Sofia, Bulgaria for the period August 2011 – December 2013. All children were born prematurely with birth weight less than 1500 grams and gestational age below 32 weeks. Retinal changes before and regularly after treatment were documented with the RetCam imaging system (Clarity Medical Systems Inc., Pleasanton, CA, USA). All children were treated with transpupillary diode laser photocoagulation (Iridex Oculight SLx Tri-Mode 810nm Diode Laser®) by the same qualified pediatric ophthalmologist. The indications for treatment were prethreshold type 1 ROP (zone I, any stage with plus disease; stage 3 ROP in zone I with or without plus disease; stage 3 ROP in zone II with the plus disease) and aggressive posterior ROP (AP-ROP). The laser was applied on the avascular retina without treatment of the present ridge or epiretinal fibrovascular proliferation. Eyes with unfavourable structural outcomes (posterior retinal detachment; retinal fold involving the macula; retrolental fibrous tissue) [4] were excluded from the study.

Methods

Refraction was measured by cycloplegic retinoscopy with spot retinoscope. A cycloplegia with 1% cyclopentolate and regimen of 3 installations in 15 minutes, and examination in 30 minutes after the third drop was performed. A conversion to the spherical equivalent (SE) was made for every eye for statistic reasons [7].

Hyperopia was subdivided into two groups – low hyperopia (SE < +5.0 D) and high hyperopia (SE ≥ +5.0 D). Myopia and astigmatism were defined using the ETROP trial definitions [5], [6] – myopia (SE ≥ -0,25 D) and high myopia (SE ≥ –5.0 D); astigmatism (plus cylindrical degree (CD) ≥ +1.0 D) and high astigmatism (CD ≥ +2.0 D). Anisometropia was defined as a difference equal or more than 1.0 D for hyperopia and equal or more than 2.0 D for myopia.

The data were analysed using the IBM SPSS 20 software. For statistical purposes of the study, each eye of every infant was used independently.

Results

Eighteen children, but 33 eyes were conducted in the study. Three eyes were excluded for unfavourable anatomical results – total retinal detachment (1 eye) and retinal folds involving the macula (2 eyes).

The mean age at the time of examination was 3.56 years (range from 3 to 4 years; SD ± 0.34). Sex distribution was almost equal – 10 (55.6%) boys and 8 (44.4%) girls. The mean gestational age at birth was 27.3 weeks (24-31 weeks, SD ± 1.78), and the mean birth weight – 928.9 g (550-1500 g, SD ± 252.8). With extremely low birth weight (under 1000 g) were 13 (72.2%) children and with very low birth weight (1000-1500 g) – 5 (27.8%) children. Zone 2 ROP was observed in 23 (69.7%) eyes; Zone 1 ROP – 5 (15.2%) eyes and AP-ROP – 5 (15.2%) eyes.

Table 1: BW and GA characteristics in different studies

Study/author	Year	Mean BW (g)	Mean GA (weeks)
Present study	2017	928.9 ± 252.8	27.3 ±1.78
Stoica F et al [7]	2016	1363.4 ± 304.7	29.4 ± 1.96
Nguyen PH et al [12]	2015	1426.4	29.8
Katoch D et al [13]	2011	1121.7 ± 254.8	28.9 ± 2.03
Roohipoor R et al [14]	2015	1441 ± 491	28.6 ± 3.2
Axer-Siegel R et al [9]	2008	833 ± 250.3	26 ± 1.9
ETROP [5,6]	$2004^{(2011;2013)}$	703	25
Yoon JM et al [11]	2017	646 ± 143	24.3 ± 1.1

The mean spherical equivalent for the whole group was -1.82 D and ranged from -9.00 D to +4.50 D (SD 3.48). Hyperopia was observed in 12 (36.4%) eyes – 10 (30.3%) eyes with low hyperopia and 2 (6.1%) eyes with high hyperopia more than +5.00 D SE. Myopic refraction was observed in 21 (63.6%) eyes – myopia in 14 (42.4%) eyes and high myopia in 7 (21.1%) eyes. Astigmatism was observed in 18 (54.5%) eyes.

Low astigmatism was measured in 12 (36.4%) eyes, and 6 (18.2%) eyes had high astigmatism. Anisometropia was observed in 3 (16.7%) children. Six (33.4%) children had strabismus (4 esotropia; 2 exotropia). Three of the strabismic infants were with unfavourable structural results.

Table 2: Myopia prevalence in different studies

Study/author	Myopia (%)	High Myopia (%)
Present study	63.6	21.1
Nguen PH et al [12]	59.0	32.0
Stoica F et al. [7]	70.8	30.2
Axer-Siegel R et al. [9]	55.2	23.9
Katoch D et al. [13]	26.1	1.4
Kaur S et al. [26]	75	26.3
Yang CS et al [25]	77.0	16.7

Discussion

Bulgaria is a small country in South East Europe with a population of about 7 million people and the delivery rate of 9.2/1000. About 10.0% of all babies are prematurely born with birth weight less than 2500g. Mandatory ROP screening is conducted in almost all neonatal intensive care units of every baby born before 32 gestational weeks and with birth weight less than 1500 g [2], [8]. In different eye centres, different treatment modalities are used – cryotherapy, intravitreal anti-VEGF medications and diode laser photocoagulation [8]. Pediatric Unit of Eye Clinic, University Hospital "Alexandrovska", Department of Ophthalmology, Medical University, Sofia is the biggest centre in Bulgaria and here for a period of 5 years (August 2011 – December 2016) we had 54 children (102 eyes) treated for type 1 prethreshold ROP with diode laser photocoagulation.

According to our ROP guidelines for screening and treatment every prematurely born baby with BW < 1500 grams and GA < 32 weeks must be screened, and if type 1 prethreshold ROP is detected, it must be treated [2]. Different countries have different ROP criteria, according to their social and economic development and neonatal intensive unit care. High-income economies are focused mainly on babies with BW less than 1250 g [5], [9], while other countries have higher criteria – BW < 2000g and/or GA < 34 weeks [7], [10]. In our study, ROP treated children were with a mean birth weight of 928.9 g (SD ± 252.8g) and meant gestational age of 27.3 weeks (SD ± 1.78w). They are higher than those reported by studies where ROP screening guidelines were BW < 1250 g [5], [9] and lower than these discussed by many other authors with higher screening criteria [7], [11], [12], [13], [14].

Laser photocoagulation of the avascular retina is the standard treatment modality for ROP and most countries worldwide have been adopted the ETROP study treatment criteria [5] and CRYO-ROP study criteria for unfavourable structural outcomes [4]. In our study we had unfavourable anatomical results in 3 (8.3%) eyes showing the high effectiveness of type 1 prethreshold ROP laser treatment compared to eyes treated at threshold [4], [5], [15].

We had a very high incidence of strabismus (33.4%), but half of the cases were in children with eyes with unfavourable structural results. If we exclude these 3 cases, the strabismus rate just in children with the favourable bilateral outcome will become 20.0%. These results are similar to the squint rate of ETROP study [16] and lower than data reported by Stoica et al., (46.15%) and Sahni et al., (50%) [7], [17]. Very low strabismus rate was found by Katoch et al., (8.3%) and Nguyen et al., (10%) [12], [13]. These big differences between different studies can be mainly explained with the different follow-up time, but all show that esotropia is the main type of strabismus. In our study, anisometropic amblyopia was the main risk factor for the treatable strabismic cases with our anisometropia prevalence of 16.7%. Nevertheless, this prevalence was very low compared to the results of Stoica et al., with their reported rate of 55.7% [7].

High prevalence of refractive errors, mainly myopia and high myopia are main functional disturbances not only in threshold [15], [18] but in prethreshold ROP laser treated infants [5], [6], [19], [20]. In our study the mean spherical equivalent for the whole group was -1.82 D, which is similar to the results of Kuo et al., (-1.71) [21], Lolas et al., (-1.75 D) [22] and Nguyen et al., (-2.87 D) [12]. Higher SE values than ours were reported in many other studies. Hwang CK et al. reported SE of -5.4 D [23]; Dhawan A et al., had SE of -4.71D [15]; Stoica F et al., found mean SE value of -4.12 D [7].

The most common refraction in our group was myopic. Shortsightedness had 63.6% of the eyes and 21.1% of the eyes were with high myopia more than -5 D. Myopia is very common in children with laser-treated ROP (higher than those that can be found in mature children or premature children with no ROP or spontaneously regressed ROP) and vary significantly from 14% [24] to 77% [25].

In our study hyperopia was observed in 36.4% eyes. Hyperopic rate varies significantly in different studies from 20% [26] to 86% [24], mainly depending on the follow-up duration of the study.

Astigmatism had 54.6% of children, and 18.2% had high astigmatism. Our astigmatic prevalence is similar to that reported by Marinov et al., after 7 years follow-up period – 59.0% [3] and lower than that reported by many authors [7,10] and especially by Yang et al., [25] with their rate of 98% astigmatism rate.

Our study has several limitations. The main limitation is the sample size – 33 eyes of 18 children. The group was small limiting the power of the findings, but have its objective explanations: 1) relatively small number of premature babies and premature babies with ROP that must be treated because of the small population and negative demographic situation in our country; 2) one centre study; 3) the limited infant age of examination – just children between 3 and 4 years. Other limitations of this study are the lack of a control group, short follow-up period and retrospective character.

In conclusion, diode laser photocoagulation is the established treatment modality for prethreshold ROP in Bulgaria in recent years with better anatomical and functional results than cryotherapy [2]. Nevertheless, a high per cent of treated infants have visually significant refractive errors and strabismus that can cause serious visual impairment if not treated properly and on time. This reveals the need for obligatory long-term follow-up examinations of all

prematurely born babies and especially ROP treated infants.

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Multi-Modal Analgesic Technique for Pain Control in Patients Undergoing Diagnostic Gynecological Laparoscopy: Randomized Controlled Clinical Trial

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Keywords: Diagnostic laparoscopy; Multi-modal analgesia; Intraperitoneal lidocaine; Pulmonary recruitment

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BACKGROUND: Advancement in minimally invasive laparoscopic surgeries make it one of the best choices for both the surgeon and the patient. The anesthesiologist had to improve the techniques used to control post-operative pain.

AIM: In this study, we hyposethized that multi-modal analgesic technique which is a combination of two simple techniques (intraperitoneal lidocaine and pulmonary recruitment) allow better result than using only one of them.

PATIENTS AND METHOD: This randomised controlled, double-blind study was conducted in Kasr-Alainy hospital, faculty of medicine, Cairo University, Egypt from September 2017 till February 2018. Fifty female patients, scheduled for diagnostic gynecologic laparoscopy were included in the study. Patients were randomly allocated using random computer allocation with numbered closed opaque envelopes into four study group. GM (n = 12): Patients received pulmonary recruitment maneuver and intra-peritoneal Lidocaine, GL (n = 13): Patients received intra-peritoneal Lidocaine, GP (n = 13): Patients received Pulmonary Recruitment Maneuver, GC (n = 12): Patients received passive exsufflation through the port site. In the ward, patients were asked to fulfil a questionnaire about pain severity using (VAS) at 1, 3, 6-hour post-operative both the patients and the anesthesiologist that assess the (VAS) were blind of the patient group

RESULTS: Regarding pain score between groups VAS 1 (the primary outcome) was lowest in GM {4.5 (3-5)} in comparison with other groups (P value = 0.015), while VAS 3 & VAS 6 wasn't statistically significant between groups. Regarding Time of first rescue analgesia; GM {3 (1.75-4)} showed the longest time in between groups (P-value = 0.042). As regard nausea and vomiting; there was no statistically significant difference in in-between groups.

CONCLUSION: Application of Multi-modal analgesic technique allows better analgesia for a longer duration than the use of the sole technique for control of abdominal pain in patients undergoing diagnostic gynaecological laparoscopy.

Abstract

Introduction

The advancement of laparoscopy and minimal access surgeries has greatly influenced the evolution of anaesthetic techniques. However, postoperative pain intensity may be significant, with up to 40% of patients being unsatisfied with routine analgesia and up to 80% requiring rescue opioids during their hospital stay. Pain relief after diagnostic laparoscopy, being a day case, is an issue of great practical importance [1], [2].

Pain after laparoscopy arises from three main sources: the incision site (50% to 70%), the pneumoperitoneum (20% to 30%) (in association with peritoneal and diaphragmatic stretching, ischemia, and acidosis), and the procedure site (10% to 20%). Pain can also be referred from the sub-diaphragmatic region as shoulder pain, which is often mild in intensity and can remain for 24 hours [3], [4].

Local anaesthetics (LA) that are infiltrated pre-incisional can only eliminate incision postoperative pain. In contrast, intraperitoneal local anaesthetic installation is a good method for providing post laparoscopy pain relief. Several mechanisms of action of intraperitoneal local anaesthetic have been proposed either by a sensory neural block of peritoneal pain receptors, through a block of vagal nerve afferent that transmit visceral stimulation or

through the anti-inflammatory analgesic effect of LA. Other opinions refer to the analgesic effect was due to systemic absorption of LA through the peritoneum. Intraperitoneal local anaesthetic should not be administrated to patients who have allergy for LA [4], [5].

The Pulmonary Recruitment Maneuver (PRM) is a simple manoeuvre that can reduce post laparoscopic shoulder and upper abdominal pain. Various mechanisms of action of PRM were proposed, but all were focused on mechanical removal of residual carbon dioxide (CO_2). As CO2 produce phrenic nerve irritation moreover the accumulated residual CO2 that persist between the liver and the diaphragm irritate both diaphragm and the peritoneum. PRM should not be done in patients have increased intracranial pressure or right-side heart failure [5], [6].

The Multi-modal analgesic technique applied through a combination of two simple and safe methods (intraperitoneal local anaesthetic and pulmonary recruitment) as analgesia for post laparoscopic pain has not been discussed before. Therefore, this study aimed to investigate the effect of Multi-modal analgesic technique on postoperative pain following diagnostic gynecologic laparoscopy to gain the benefit of both techniques, compared to intraperitoneal instillation of lidocaine alone and pulmonary recruitment manoeuvre alone.

Patients and Method

This randomised controlled, double-blind study was conducted in Kasr-Alainy hospital, faculty of medicine, Cairo University, Cairo, Egypt (one centre) from September 2017 till February 2018. After obtaining approval from the Institutional Research Committee by code, N-61-2017 registered the study at clinical trials.gov by trial number: NCT03241602.

Informed written consent was taken from 48 female patients, aged 18-45 years, ASA I or II, scheduled for diagnostic gynecologic laparoscopy.

Patients were randomly allocated to either of four study groups,12 per group, using random computer allocation with numbered closed opaque envelopes.

- GM (n = 12): Patients received Multi-modal technique by applying pulmonary recruitment manoeuvre and intra-peritoneal Lidocaine.

- GL (n = 12): Patients received only intra-peritoneal Lidocaine.

- GP (n = 12): Patients received only Pulmonary Recruitment Maneuver.

- GC (control) (n = 12): Patients had passive exsufflation through the port site and did not receive intra-peritoneal lidocaine nor pulmonary recruitment.

Patients younger than 18 or above 45 years old, patients with (ASA) physical status ≥ III, and who were allergic or hypersensitive to amide-type local anaesthetics were excluded. Also, patients with pre-existing chronic pain disorders, or with history of alcohol or drug abuse, including opioids or tranquillisers for > 1 week preoperatively, were excluded. If the laparoscopy included any interventional procedure or was converted to an open procedure, the patient was also excluded from the study.

Patients attended the pre-anaesthesia room 1 hour before the procedure. A twenty-gauge intravenous cannula was inserted peripherally, and each patient was pre-medicated with intravenous Midazolam 0.02 mg/kg, Ranitidine 50 mg and 10 mg Metoclopramide .

In the operative room, standard monitoring (electrocardiography, pulse oximetry, capnography and non-invasive blood pressure measurement) was applied to the patient. Anaesthesia was induced with propofol 2 mg kg^{-1}, Fentanyl 1 mcg kg^{-1}, Atracurium 0.5 mg kg^{-1} and the trachea were intubated after bag-mask ventilation for 3 minutes. Anaesthesia was maintained with isoflurane 1-2%, and muscular relaxation was maintained with Atracurium 0.1 mg kg-1 every 15 minutes, and mechanical ventilation (volume control mode) started to keep end-tidal CO_2 at normal values. Depth of anaesthesia was adjusted according to clinical signs.

Laparoscopy was done using CO2 as a distension medium. Veress needle was introduced at first through the lower border of the umbilicus, and a water test was done to ensure intra-peritoneal placement. Then, reaching proper distension pressure was ensured by the disappearance of dullness over the lower border of the liver — the pressure adjusted to be about 15 mmHg. The patient was placed in a Trendelenburg position to provide optimum conditions for the laparoscopic view. A 10 mm laparoscopic trocar was introduced with 45 degrees towards the pelvis, and zero cameras were introduced through the cannula-trocar. The second puncture could be done through the right or left iliac fossa.

In groups GM and GL, Lidocaine (1.75 ml kg-1 of 2% lidocaine (3.5 mg kg-1) was splashed under the right diaphragmatic area by the surgeon early in the procedure.

At the end of the procedure, the patient was placed back from the Trendelenburg position. In groups GM and GP, the pulmonary recruitment manoeuvre was done, and it consisted of five manual pulmonary inflations with a maximum pressure of 40 cm H_2O. The anesthesiologist holds the fifth positive pressure inflation for approximately 5 seconds. In group GC, CO_2 was removed by passive exsufflation

through the port site, and gentle abdominal pressure was applied to evacuate the residual gas.

In all groups, after the end of the surgery, the surgeon will infiltrate the incisions with 2 ml of 0.5% bupivacaine. Residual neuromuscular block was antagonised with atropine 0.02 mg kg^{-1} and neostigmine 0.05 mg kg^{-1}, extubation was done according to extubation criteria.

In the recovery room, patient was asked by anesthesiologist who were both unaware of the intra-operative analgesia technique used for post-operative pain to assess Pain intensity using Visual Analogue Scale (VAS) that was described to the patients before the surgery (it is a straight horizontal line its length is 100 mm. The ends of the line defined as the extreme limits of pain as 0 is no pain, and 100 is the worst pain). The patient asked for analgesia was controlled by intravenous infusion of 1000 mg Acetaminophen. Unsatisfied Patients 30 minutes after acetaminophen received intra-venous Pethidine 1 mg kg^{-1}.

Then, the patient was discharged to the ward according to the standard criteria.

In the ward, patients were asked to fulfil a questionnaire about pain severity using Visual Analogue Score (VAS) at 1, 3 and 6 hours postoperative. The Primary outcome of the study was the Visual Analogue Scale (VAS) at 1-hour post-operative. Secondary outcomes were Visual Analogue Scale (VAS) at 3, 6-hour post-operative, Blood pressure (systolic and diastolic), a Heart rate that recorded hourly for the first 6 hours' post-operative. Time of first rescue analgesic and Incidence of nausea and vomiting were also recorded

Sample size

A previous study that compared intra-peritoneal Lidocaine instillation versus placebo reported 1hour postoperative VAS to be 2.2 vs 4.7 with standard deviation (1.7) [7]. The sample size was calculated using one-way analysis of variance (ANOVA). Taking the power of the study of 90% and an alpha error of 0.5, a minimum number of 10 patients will be needed for each group. This number will be increased to 12 patients per group to compensate for possible dropouts.

Statistical analysis

Data were presented as means (stander deviation SD), medians (quartiles), frequencies (%) as appropriate. The analysis was done using one-way ANOVA for single measures, mixed model ANOVA for repeated measures, Kruskal Wallis for categorical variables. A P value of ≤ 0.05 was considered significant.

Results

Fifty female patients undergoing diagnostic gynaecological laparoscopy were randomised between four groups. In both groups, GL and GP 13 patient were allocated while 12 patients were analysed as one patient in GL excluded (the patient has the additional intraoperative procedure) and one patient in GP excluded (the patient had an additional abdominal drain). In GM, GC 12 patient was allocated and analysed (Figure 1).

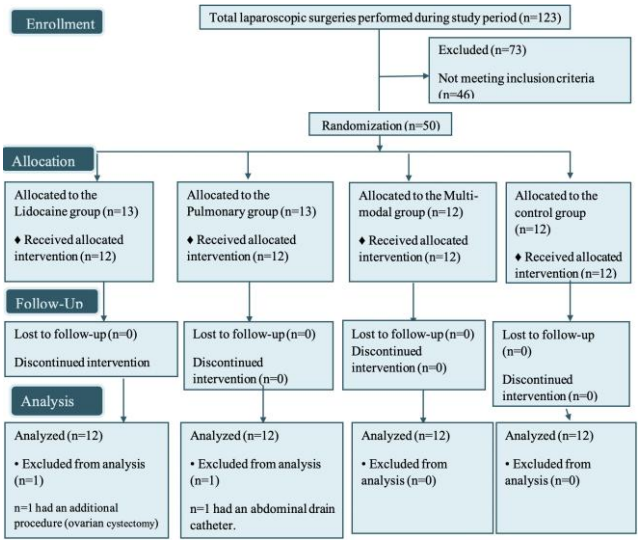

Figure 1: Flow diagram for the patients in the trial

Demographic data (Age, BMI) were comparable between groups Table (1).

Table 1: Demographic data (Age and Body Mass Index BMI) in the four groups

	Pulmonary Group GP (n = 12)	Lidocaine Group GL (n = 12)	Multimodal Group GM (n = 12)	Control Group GC (n = 12)	P value
Age (years)	23.42 ± 2.87	23.08 ± 2.15	23.42 ± 3.15	24.25 ± 2.67	0.755
BMI ($kg.m^{-2}$)	29.19 ± 2.60	29.05 ± 2.26	29.37 ± 2.05	29.23 ± 2.28	0.990

Categorical data were expressed as Mean ± Standard Deviations.

Regarding the score of pain, Visual Analog Scale (VAS) was used (primary outcome). Pain complained by patients was abdominal pain. There was no incidence of shoulder pain during the assessment in the first 6 hours postoperative.

VAS 1 was lowest in GM in comparison with the other groups (P-value = 0.015). GM showed a statistically significant decrease when compared with GP, GC (P-value 0.013, 0.005) respectively.

Regarding VAS 3 & VAS 6 in all groups, the results were not statistically significant Table (2).

Table 2: Pain score using Visual Analog Scale (VAS) in four groups

	Pulmonary Group GP (n = 12)	Lidocaine Group GL (n =12)	Multimodal Group GM (n =12)	Control Group GC (n =12)	P value
Visual Analog Scale after first hour (VAS 1)	5.5 (5 -7)	5 (4 -6)	4.5 (3-5) *#	7 (5 -7)	0.015**
Visual Analog Scale after third hour (VAS 3)	3 (2.5-5)	2.5 (0-5)	3 (1 -3.5)	5 (3 -5)	0.248
Visual Analog Scale after sixth hour (VAS 6)	0 (0 -3)	0 (0-0)	0 (0 -3)	3 (0 - 5)	0.089

Categorical data were expressed as Median and range; * statistically significant in comparison with G_C; # statistically significant in comparison with G_P; ** statistically significant in the comparison between the four groups.

Regarding Time of first rescue analgesia; GM showed the longest time in between groups, and that was statistically significant (P value = 0.042). GP shows less time in comparison to GM and was statistically significant (P value = 0.04) (Table 3).

Table 3: Time of first rescue analgesia between four groups

	Pulmonary Group GP (n = 12)	Lidocaine Group GL (n = 12)	Multi modal Group GM (n =12)	Control Group GC (n =12)	P value
Time of first rescue analgesia (hours)	1.75 (1 -2)	2.25 (1.5-2.75)	3 (1.75-4) #	1.25 (1-2)	0.042 ***

Categorical data were expressed as Median and range; *** statistically significant in comparison to the four groups; # statistically significant in comparison to GP.

Regarding the comparison of vital signs over time in each group, Systolic Blood Pressure (SBP) in GM was statistically, but not clinically, significant in the comparison between SBP 1 – SBP 6.

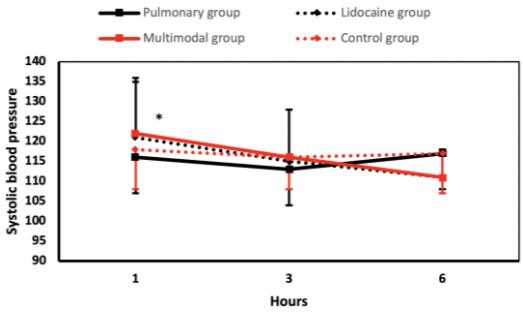

Figure 2: Systolic Blood Pressure measurement over postoperative first 6 hours. Data expressed as means ± Standard deviation

There was a decrease in Diastolic Blood Pressure DBP over time. In GP and GL there was a minimal decrease in both Systolic Blood Pressure SBP and Diastolic Blood Pressure DBP (Figure 2, and 3).

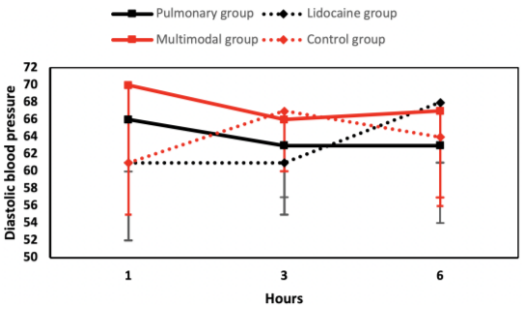

Figure 3: Diastolic Blood Pressure Measurement over postoperative first 6 hours. Data expressed as means ± Standard deviation

There were marginally significant values of decreasing heart rate from the 1st to the 3rd hour in GP (P-value = 0.053) and GM (P-value = 0.056). No significant differences in vital signs were found between groups in the 1st hour with the incidence of tachycardia around 40% (Figure 4).

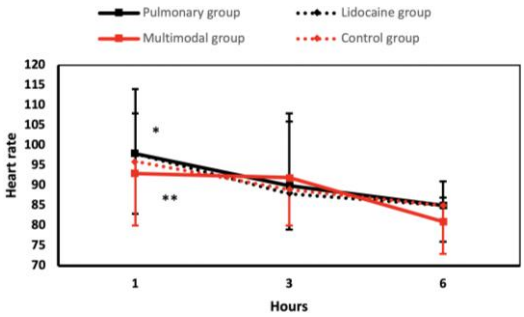

Figure 4: Heart Rate measurement over postoperative first 6 hours. Data expressed as means ± Standard deviation

As regard nausea and vomiting; There were 2 patients in GP, 1 patient in GM and 3 patients in GC who complained of nausea but that was not statistically significant. There was no incidence of vomiting in all groups (Table 4).

Table 4: incidence of nausea and vomiting in the four groups

		Pulmonary Group GP (n = 12)	Lidocaine Group GL (n =12)	Multi modal Group GM (n = 12)	Control Group GC (n = 12)	P value
Nausea	n (%)	2 (16.7%)	0 (0%)	1 (8.3%)	3 (25.0%)	0.476
Vomiting	n (%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	---

Numerical data were presented as number (percentage).

Discussion

Pain after laparoscopic surgery is still considered significant, despite being markedly less than the pain after open surgery. This study was designed to find a safe, simple, and effective method to decrease pain after laparoscopy. Diagnostic gynecologic laparoscopy was chosen as our study model procedure because it is less invasive and less time-consuming than the more advanced laparoscopic procedures.

In this study, we use two simple techniques to control postoperative pain: pulmonary recruitment and intraperitoneal lidocaine. We use 1.75 ml kg^{-1} (3.5 mg kg-1) lidocaine for intraperitoneal injection according to study done by study Yang SY, et al., [8] comparing Efficacy of intraperitoneal and intravenous lidocaine on pain relief. The study showed that 1.75 ml kg^{-1} (3.5 mg kg^{-1}) lidocaine dose for intraperitoneal (i.p) injection has almost the same efficacy as intravenous lidocaine for postoperative analgesia in laparoscopic

surgeries. Therefore, in this study, we hypothesise that this dose would be both effective and safe for the patients.

In our study, there was no shoulder pain detected, and for assessment of abdominal pain, Visual Analogue Scale (VAS) was used. The VAS of Multi-modal group after one hour, which showed the best result in all groups. No difference was found between the intervention groups in assessment in the 3rd and 6th hour.

In the lidocaine group, the VAS 1 was 5 (4-6), which was better than that of the pulmonary group. Many studies were done to show the efficacy of i.p. Lidocaine in gynaecological surgeries and general abdominal surgeries. In consistency with our results, a study was done by M. Parsanezhad et al., [9] and study done by W. Elsherbiny et al., [10] use intraperitoneal instillation of lidocaine for pain control after diagnostic gynecologic laparoscopy.

Regarding the pulmonary group, in our study, during the assessment of abdominal pain, VAS 1 was 5.5 (5-7), and VAS 3 was 3 (2.5-5). In consistency with our results, a study was done by S. Sharami et al., [11] showed a shoulder pain score of 1.28 ± 1.7, recorded 4 hours postoperatively.

In contrast with our study, a study was done by H. Liu et al., [12] investigated the effect of combining local anaesthetic infiltration of ropivacaine with pulmonary recruitment manoeuvre on postoperative pain following diagnostic hysteroscopy and laparoscopy. The postoperative pain score was significantly lower in this study: 1st hour, 1.6 ± 1.3; a 3rd hour, 0.5 ± 0.8. They added 20 ml of 0.5% ropivacaine injected preincisionally before placing the trocars.

In the following studies [7], [13], [14], [15] that record the effect of intraperitoneal lidocaine or pulmonary recruitment in abdominal pain after laparoscopic surgeries, the patients had received an oral, intravenous or intraperitoneal analgesic drug from the beginning of the procedure with either pulmonary recruitment or intraperitoneal lidocaine. While in Multi-modal analgesic technique, the patients received two simple and safe manoeuvres. The intraperitoneal local anaesthetic blocks the nociceptors involved in phrenic irritation by CO_2 and diaphragmatic and peritoneal stretching. The pulmonary recruitment manoeuvre washes the CO_2 content from under the diaphragm, so it decreases the local effect and washes CO_2 from the abdomen, decreasing its systemic absorption.

Regarding the need for analgesia, in this study, the time of first rescue analgesia was the longest in GM compared to the other groups. That proves the efficacy of the combination of the two simple techniques. That also allows early ambulation of the patient and early discharge from the hospital.

Concerning the vital signs, we found marginally significant values of decreasing heart rate from the 1st to the 3rd hour in the pulmonary group and the combined group. There were a small number of studies that showed the importance of vital signs to assess the effect of analgesic techniques.

M. Khan [16] showed that there was no significant difference in the mean heart rate and blood pressure at any time between the groups, with the incidence of tachycardia being 5% at 0 hours and 2% at 4 hours in the lidocaine group.

Regarding nausea, there was no significant incidence in the lidocaine group (0%), GP (16.7%), and GM (8.3%). Vomiting was not reported in any patient over the first 6 hours.

In consistency with our results, a study did H. Liu et al., [12] used the PRM manoeuvre to wash CO_2. Nausea and vomiting were reported in 26.7% of patients. However, intravenous propofol and remifentanil were used for maintenance of anaesthesia, and no inhalational agents were used. Propofol infusion was associated with less postoperative nausea and vomiting.

A study was done by H. Tsai et al., [17] on the analgesic effect of PRM after laparoscopic surgeries for the benign gynaecological lesion. They reported nausea and vomiting in 50.9% of patients. This high incidence may be due to the more invasive procedure than our selected diagnostic one as the surgical site adds to the complexity of pain, in contrast, other studies reported a higher incidence of nausea and vomiting.

In this study, we hypostatize that multimodal technique offer to the patient the benefits of the two simple techniques pulmonary recruitment and intraperitoneal lidocaine, the results showed the effectiveness of the multimodal technique.

Limitations of the study

In this study we include only female patients due to the type of procedure; we did not Record the total consumption of postoperative analgesics.

In conclusion, application of Multi-modal analgesic technique allows better analgesia for a longer duration than the use of the sole technique for control of abdominal pain in patients undergoing diagnostic gynaecological laparoscopy**.**

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Clinical Science

Serum Vascular Endothelial Growth Factor in Egyptian Obese Women with Insulin Resistance

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Keywords: Vascular Endothelial Growth Factor; Insulin resistance; Obesity; Metabolic profile; Serum lipids

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Abstract

BACKGROUND: Obesity is a major factor in the development of several sub-clinical anomalies. Insulin resistance (IR) is associated with obesity. Vascular endothelial growth factor (VEGF) plays a significant role in inflammation and vascular neogenesis. However the precise relationships of its levels with clinical, lipid, and metabolic profiles are unknown.

AIM: This study aimed to examine the association between serum VEGF concentrations with IR risk and metabolic and lipid parameters in obese women.

METHODS: Serum VEGF, metabolic biomarkers and anthropometry were measured in 83 obese women with IR and 50 healthy women. Fat distributions in the abdominal, subcutaneous and visceral area were assessed. Homeostasis model assessment for insulin resistance index (HOMA-IR) was calculated. For analytical purposes, VEGF levels were categorised into three tertiles groups.

RESULTS: Obese women with IR showed significantly higher levels of serum VEGF as compared with the control group. Moreover, obese women in the highest VEGF tertile had significantly higher values of obesity indices, visceral fat index, abnormal lipid levels and HOMA-IR compared to with those in the lower tertile.

CONCLUSION: Elevated VEGF levels are associated with IR and high visceral fat index in obese women which in turn increased the risk for metabolic complications.

Introduction

Obesity is a major health issue and considered as an epidemic disease that is still rising all over the world. It leads to and can be accompanied by other diseases; predominantly type 2 diabetes mellitus (T2DM) and cardiovascular complications [1]. However, it is not completely clear how obesity leads to atherosclerosis and cardiovascular diseases. Obesity involves an increase in adipose tissue mass. Therefore, the angiogenesis is required to supply the adipose tissue with sufficient oxygen and nutrients [2]. However, several studies suggested that the expansion of the vascular network does not provide sufficient oxygen to all adipocytes causing local hypoxia [3], [4]. The insufficient blood flow in the adipose tissue has been supposed to provoke insulin resistance through effects on inflammation, adipokine expression, and/or adipocyte differentiation [5]. In obese patients, it is hard to predict the development of diabetes as some of the obese individuals could be metabolically healthy, and several studies have found negative IR status and no complications in severely obese patients [6]. Therefore, the relationship between T2DM and obesity could not be determined by the absolute amount of the stored fat [7].

Vascular endothelial growth factor (VEGF) is responsible for most of the angiogenic actions in adipose tissue [8], in addition to its pathological role in the vascular disease processes [2]. However, its expression was found to be not specific to endothelial cells [9]. Insulin has a vascular-specific action in the endothelium as it is regulating the expression of VEGF, at physiological concentrations insulin can increase the expression of VEGF [10]. Few studies

have explored the relationship between adipose tissue angiogenic capacity, obesity and IR [11]. It is known that the development of adipose tissue necessitates adipogenesis and angiogenesis. The major angiogenic pathway is through the action of the VEGF factor on the VEGF receptor-2 [12]. It has been previously demonstrated that VEGF is responsible for lots of the angiogenic activity of adipose tissue [13].

Higher circulating levels of VEGF have been found in overweight and obese cases [14]. A positive correlation has been observed between concentrations of VEGF and body mass index (BMI) regardless of the insulin sensitivity [15]. However, according to a population-based cross-sectional study, it has been observed that the VEGF has a small effect on the development of atherosclerosis [16].

Therefore, in the present study, the relationship between VEGF and IR in obese women was examined to assess its pathological significance in the acceleration of metabolic complications. This could be of great importance in the management of metabolic diseases and understanding of the pathophysiological alterations in obese women.

Subjects and Methods

Subjects

Eighty-three obese women with IR aged between 18 and 35 years were included in the present study in addition to 50 age-matched healthy non-obese controls. Cases and controls were chosen between October 2016 and January 2018 from obesity Clinic at National Research Centre, Egypt.

Clinical and biochemical assessment

Full medical history and clinical examination for all patients and controls have been done. Anthropometric measurements including body mass index (BMI), waist circumference (WC) and hip circumference (HC) have been measured as previously described [17]. Consequently, the Waist-to-Hip-Ratio (WHR) was calculated as WC divided by HC. The sum of skinfolds (sum SF) was calculated for each subject and control from the skin-fold thickness measured at the biceps, triceps, subscapular, supra-iliac, and abdominal areas using Holtain calliper.

Blood pressure was measured using calibrated sphygmomanometer and brachial inflation cuff (HEM-7200 M3, Omron Healthcare, Kyoto, Japan). Systolic and diastolic blood pressures (SBP and DBP) were measured twice, and then the average is used for analysis. Visceral adiposity index (VAI) was calculated as follows [18]:

$$\text{VAI} = \left(\frac{\text{WC}}{36.58+(1.89\times\text{BMI})}\right) \times \left(\frac{\text{TG}}{0.81}\right) \times \left(\frac{1.52}{\text{HDL}}\right)$$

Laboratory measurements

Blood samples were collected after fasting for a minimum of 12 h. Fasting plasma glucose and serum lipids comprising total cholesterol, high-density lipoprotein cholesterol (HDL-C), triglycerides (TG) have been measured. Low-density lipoprotein cholesterol (LDL-C) was calculated according to the equation LDL-C = Total cholesterol - Triglycerides/5+ HDL-C) [19]. Serum insulin concentration was analyzed by chemiluminescent immunoassay (Immulite2000, Siemens, Germany. Insulin resistance has been estimated by the Homeostasis Model Insulin Resistance (HOMA-IR) as previously described [20]. The Human Vascular Endothelial Growth Factor (VEGF) levels were measured using a solid-phase enzyme-linked immune sorbent assay sandwich ELISA kit developed by NOVA NO. 18, Keyuan Road, DaXing Industry Zone, Beijing, China.

Statistical analysis

All the statistical analyses have been performed using SPSS version 16.0 software. We explored the distribution of the variables using the Kolmogorov-Smirnov test of normality. While means ± SDs were used to describe the normally distributed data, logarithmic transformation was performed to all skewed variables. Triglycerides and serum VEGF levels have shown skewness necessitating using median and tertile ranges. For analytical purposes, VEGF levels were categorised into three groups according to tertiles. A one-way analysis of variance was performed for the comparison of more than two groups. The unpaired t-test or the Mann-Whitney U test was used, as appropriate, to evaluate differences between the two groups of continuous variables. Regression analysis was used to investigate the association between VEGF levels and obesity indices. Two-tailed $P < 0.05$ was considered statistically significant.

Results

Table 1 shows the clinical and biochemical characteristics of both obese and healthy women. Significantly higher concentrations of serum VEGF have been observed in obese cases with IR compared to controls. Obese-IR patients also showed significantly higher levels of BMI, WC, WHR, and total cholesterol, TG, LDL-C and HOMA-IR relative to controls ($p < 0.05$).

Table 1: Clinical and biochemical characteristics of obese women with IR and healthy controls

	Controls N = 50 Mean ± SD	Patients N = 83 Mean ± SD
Age (years)	27.0 1± 3.09	28.14± 4.37
BMI (kg/m^2)	22.47 ± 2.36	33.41± 6.06**
WC (cm)	82.9 ± 7.4	90.4 ± 9.9**
WHR	0.78 ± 0.08	0.83 ± .06*
Sum SF	100.5 ± 22. 9	150.1 ± 21.1**
VAI	3.57 ± 2.52	9.57 ± 2.52**
SBP (mmHg)	98.75 ± 15.86	103.33 ± 17.575
DBP (mmHg)	64. 8 ± 5.4	67.46 ± 8.79
HOMA-IR	2.86 ± 1.2	5.86 ± 2.70**
Total cholesterol (mg/dl)	163.06 ± 46.5	201.17 ± 54.8**
TG (mg/dl)	79.61 ± 33.53	104.45 ± 40.5**
HDL-C (mg/dl)	44.39 ± 10.777	45.77 ± 10.69
LDL-C (mg/dl)	106.47 ± 44.561	153.78 ± 50.8*
VEGF (pg/ml)	80.68 ± 17.64	156.64 ± 14.35*

BMI: body mass index; WC: waist circumference; Sum SF: sum of skin folds; SBP: systolic blood pressure; DBP: diastolic blood pressure; VAI: visceral adiposity index; TG: triglycerides; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; HOMA-IR: homeostasis model assessment-insulin resistance; VEGF: vascular endothelial growth factor; *p < 0.05; ** p < 0.001.

The clinical and biochemical parameters of obese-IR women were further analysed by tertiles of serum VEGF levels (Table 2). The concentration of VEGF in the Lower tertile was ≤ 79.04, while the intermediate tertile ranged from 79.05-143.26 and the higher tertile was ≥ 143.27. Obese subjects in the higher tertiles showed significantly higher values of WHR, the sum of skinfolds, visceral adiposity index (VAI), total cholesterol, TG and LDL-C, and HOMA-IR compared to those in the lower tertile.

Table 2: Clinical and anthropometric parameters of obese women with IR, divided according to tertiles of circulating VEGF concentrations

Characteristic	Lower tertile VEGF ≤ 79.04 pg/ml	Intermediate tertile VEGF between 79.05 and 143.26 pg/ml	Higher tertile VEGF ≥ 143.27 pg/ml
WHR	0.8 ± 0.06	0.8 ± 0.07	0.9 ± 0.05*
Sum SF	131.4 ± 10.9	133.7 ± 11.6	149.2 ± 12.0*
VAI	5.57 ± 2.52	6.71 ± 4.89	10.28 ± .5.01**
SBP (mmHg)	110.3 ± 11.4	118.4 ± 9.3	125.4 ± 9.3
DBP (mmHg)	74.3 ± 7.4	75.3 ± 6.8	89.9 ± 8.2
HOMA-IR	3.5 ± 1.2	4.4 ± .9	5.2 ± .7*
Total cholesterol (mg/dl)	142 ± 25.5	155.9 ± 22.1	199.9 ± 20.9*
TG (mg/dl)	142. 5± 26.7	153.6 ± 25.9	196.6 ± 26.7*
HDL-C (mg/dl)	46.5 ± 26.2	44.8 ± 23.8	44. 6 ± 24.4
LDL-C (mg/dl)	128.6 ± 21.8	1 40± 20.7	157. 4± 21.9*

WHR: waist-hip ratio; Sum SF: sum of skin folds; VAI: visceral adiposity index; SBP: systolic blood pressure; DBP: diastolic blood pressure; HOMA-IR: homeostasis model assessment-insulin resistance; TG: triglycerides; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; *p < 0.05; ** p < 0.001 indicates a significant increase.

Regression analysis showed a positive association between VEGF serum concentration and VAI (Figure 1).

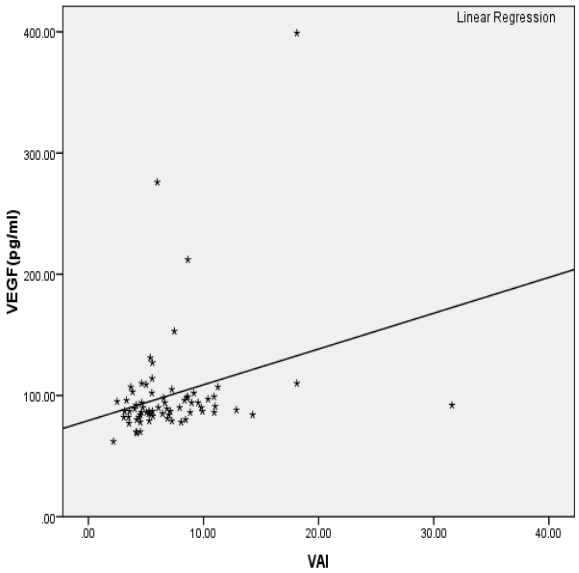

Figure 1: Relation between visceral adiposity index and serum vascular endothelial growth factor in obese women with IR

Discussion

Vascular endothelial growth factor (VEGF) is engaged in vessel formation in both the normal and pathological conditions. It is a key factor involved in adipose tissue angiogenesis. The fats that accumulate intra-abdominally might be a source of many risk factor syndromes via insulin resistance.

Lots of studies suggested that the vascular network expansion during obesity does not give enough oxygen supply to all adipocytes. Consequently, local hypoxia occurs [21], [22], [23]. In the present study, significantly increased levels of serum VEGF have been observed in obese IR women relative to the control group ($p < 0.05$). Furthermore, obese IR patients that have the highest VEGF levels showed also higher values of the waist to hip ratio, visceral fat index, lipid and HOMA-IR levels compared to other obese IR with lower VEGF values. A significant correlation between the VEGF concentrations and visceral fat and WHR have also been found. Our findings are in agreement with another study [24] that reported the presence of correlation between VEGF levels and visceral fat accumulation. The authors also performed stepwise regression analysis and demonstrated that although the visceral fat is an independent factor, it is the most important factor in the determination of serum VEGF levels. Furthermore, the reduction in the body weight and the subsequent decline in the visceral fat area caused a decrement in the VEGF concentrations. Subsequently, it was suggested that serum VEGF concentration is regulated by the adipose tissue secretions particularly, the area of the visceral fat [24].

The vascular endothelium plays a substantial role in the regulation of vasomotor functions, maintenance of vessel walls, and anti-platelet aggregation in addition to the endocrine functions in the human body. Several stimuli such as injury and cardiovascular risk factors could cause the impaired function of the vascular endothelium [25]. Various studies indicated that endothelial cell dysfunction is an early pathophysiological indicator of cardiovascular diseases (CVD). VEGF, among other substances, is secreted by the vascular endothelium and could be a substantial indicator of endothelial cell function [26].

Different signalling pathways are activated by the binding of VEGF to their corresponding receptors [27]. Various stimuli such as growth factors, inflammatory cytokines, and hypoxia could induce the VEGF secretion. Previous studies reported significantly higher VEGF levels in overweight and obese patients compared with slim subjects [14]. It has been proposed that high adiposity in obese patients induce secretion of various inflammatory cytokines, such as interleukin-6 (IL-6) that in turn causing increased VEGF secretion [25], [27].

However, other study suggested that the overexpression of VEGF-A in adipose tissue enhancing thermogenesis and energy expenditure resulting in decreasing obesity [28]. And in addition to the role of VEGF in vasculogenesis and angiogenesis, it also exerts metabolic effects and controls energy metabolism [29], [30]. In the present study, a significantly higher concentration of VEGF has been found in obese IR patients.

Previously, body mass index and blood pressure were found to be the independent determinants of VEGF [31]. It was also suggested that hyperglycemia without associated dyslipidemia is responsible for vascular endothelial lesions [32]. Additionally, higher levels of VEGF have been observed in patients with type -1 diabetes relative to healthy controls [33]. Significantly increased levels of VEGF have been found in metabolic syndrome cases compared to healthy control [34].

In conclusion, our results suggest an association between elevated serum levels of VEGF and IR in obese women. So elevation of VEGF might be responsible for the occurrence of metabolic abnormalities, highlighting the important role of obesity and visceral adiposity on insulin sensitivity.

Declarations

Ethics approval and consent to participate

After a complete description of the study, written informed consent has been obtained from all participants. The Ethical Committee of National Research Centre, Egypt (number = 16361) has authorised the research in conformity with the World Medical Association's Declaration of Helsinki (2013).

Availability of data and material

The data used and analysed during the current study are available from the corresponding author on reasonable request.

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Clinical Science

Comparison of the Effects of Albumin 5% versus Ringer's Lactate on Blood Loss and Coagulation after Vascular Surgery Using Thromboelastography

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Keywords: Albumin 5%; Ringer's lactate; Vascular surgery; Blood loss; Coagulation, Thromboelastography

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Abstract

AIM: Comparing the effects of Albumin 5% versus Ringer's lactate on blood loss and coagulation after vascular surgery using

METHODS: In this randomised study, 60 patients, aged (18-60 years) ASA physical status (I-III) undergoing vascular surgery were included in the study and randomly allocated into two groups using a random number generator, to receive either Human albumin or Ringer lactate after obtaining written informed consent. Group A received 1-2 ml per minute of human albumin 5% combined with normal saline (0.9%). Group B received Ringer's lactate only as of the main solution. Variables were measured after administration of fluids as postoperative measures. The amount of blood needed for testing was 4 ml drawn before the operation and at the end of surgery with a citrate tube (blue tube) from the venous line or using a regular needle. The standard time of 15 minutes was considered to begin processing.

RESULTS: There was no statistically significant difference observed between both groups regarding demographic data, surgical wound drainage, haemoglobin level, hematocrit level and coagulation profile. Regarding ROTEM thermoelectrometry variables showed that there was no statistically significant difference was found between the two groups In-TEM variables (Ex-TEM Clotting time, TEM Clot Formation Time) but In-TEM Alpha Angel measured in degrees showed a Statistically significant difference between the two groups. $P < 0.001$ and Ex-TEM Maximum Clotting Firmness MCF values measured in mm, there was a statistically significant difference between the two groups $P = 0.045$.

CONCLUSION: This study concluded that the use of human albumin (5%) in vascular surgeries before reaching the trigger point for blood transfusion didn't improve blood loss or coagulation profile compared to the use of ringer lactate only. Therefore, ringer lactate can be used as a good replacement for human albumin. Ringer lactate is readily available and inexpensive while human albumin may be costly.

Introduction

Anesthesiologists always seek for the optimum fluid to be used especially in bloody operations like vascular surgeries before reaching the trigger for blood transfusion, Crystalloids, in the form of Ringer's lactate (RL), or and colloids such as 5% human serum albumin (HA) are commonly used for intra-operative fluid management during surgery [1].

Monitoring perioperative coagulation competence has relied on clinical estimates besides on plasma coagulation tests [2]. Plasma coagulation tests were, however, introduced to evaluate the lack of coagulation factors and not to predict the risk of bleeding or for guiding hemostatic therapy [3].

In contrast, viscoelastic evaluation of whole blood enables rapid diagnosis of coagulation competence and may be displayed in real time within the operating theatre. Thus, the use of perioperative coagulation monitoring by, e.g. Thromboelastography (TEG) is recommended [4].

Rotation thermoelectrometry (ROTEM®, TEM® Innovations, Munich, Germany) offers a recent alternative approach to assess perioperative coagulation disorders using the visco-elastic analysis of clotting. Trials specifically examining bleeding management in vascular surgery are lacking, and much of the literature and guideline are derived from studies on patients with trauma [5].

ROTEM® provides the most complete and rapid information on hemostasis.

The ROTEM for the evaluation of fluid management during vascular surgery using either Albumin or Ringer lactate was adopted in this study.

Methods

After obtaining an approval from the departmental ethics and research committee of Cairo University hospitals (Kasr Alainy hospital) 60 patients aged (18-60 years) ASA physical status (I-III) undergoing vascular surgery were included in the study and randomly allocated into two groups using a random number generator, to receive either Human albumin or Ringer lactate after obtaining a written informed consent (Figure 1).

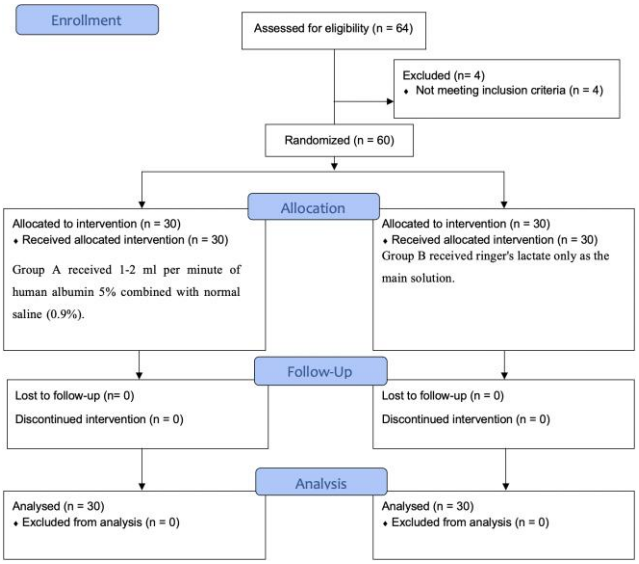

Figure 1: CONSORT Flow Diagram

After preoperative assessment and full medical history as well as laboratory investigations, patients were premedicated with 3 mg midazolam, and standard monitoring was applied.

General anaesthesia was induced with Propofol (2 mg/kg), Rocuronium (0.6 mg/kg) and Fentanyl (2 µg/kg), followed by endotracheal intubation, isoflurane 1.5%. Mechanical ventilation was initiated using 50% oxygen in air through a closed-circuit system to keep SaO_2 > 97% and end-expiratory CO_2 between 35 and 40 mm Hg.

Additional Fentanyl 50 mcg was given when heart rate or arterial blood pressure increased 20% above the baseline. Rocuronium top up was given as 1/5 of the initial dose every 20 minutes.

Fluid requirements were given based on fluid losses before the start of anaesthesia, maintenance requirements, normal fluid losses that occur during surgery, and response to unanticipated fluid (blood) loss.

At the end of the surgical procedure, muscle relaxant was reversed with neostigmine 0.08 mg/kg and atropine 0.01 mg/kg. All patients were extubated and transferred to the postoperative care unit (PACU).

ROTEM is a modern modification of the TEG technology originally described by Hartert in 1948 [6], and it was used in the present study.

These technologies provide a visual assessment of clot formation and subsequent lysis under low shear conditions (0.1/sec) similar to those present in the vena cava and well below those seen in venules, large veins, and the arterial system. Multiple test reagents are available for ROTEM; EXTEM (tissue factor activation), INTEM (ellagic acid/phospholipid activation). The commonly used ROTEM variables include clotting time (CT sec), clot formation time (CFT sec), α-angle (degree), maximum clot firmness (mm), and Lysis index (%). CT represents the onset of clotting, while CFT and α-angle both represent the initial rate of fibrin polymerisation. MCF is a measure of the maximal viscoelastic strength of clot. Lysis index issued for the diagnosis of premature lysis or hyper-fibrinolysis.

Enrolled patients were randomly allocated in two groups:

Group A received 1-2 ml per minute of human albumin 5% combined with normal saline (0.9%).

Group B received Ringer's lactate only as of the main solution.

ROTEM variables, coagulation profile, and complete blood count were recorded for all patients' pre-operative (baseline). ROTEM variables were measured after administration of fluids as postoperative measures.

The amount of blood needed for testing was 4 ml drawn before the operation and at the end of surgery with a citrate tube (blue tube) from the venous line or using a regular needle. The standard time of 15 minutes was considered to begin processing.

For ROTEM analysis, samples of blood were immediately mixed with 0.5 ml of a 3.2% citrate sodium solution (9 NC; Becton, Dickinson and Co., Franklin Lakes, NJ). After gentle mixing, the blood sample was analysed at 37°C.

The following ROTEM assays were performed, Ex-TEM which evaluates the extrinsic pathway, In-TEM evaluates the intrinsic pathway, and FIBTEM assess fibrinogen level after tissue. CT, CFT, ALP, MCF and ML parameters were measured in Ex-TEM and In-TEM assays while only the MCF was reported in the FIBTEM. All ROTEM analyses were observed for 60 minutes and then stopped.

Pain, vital signs, oxygen saturation, ventilation, and wound drainage were monitored during post-operative care.

Statistical Analysis

Data are presented as mean and standard deviation (SD). Non-normally distributed variables are expressed as median (25% and 75% percentiles). Non-parametric statistical tests were used for analysis if no normal distribution could be achieved by log transformation.

Repeated Measures Analysis of covariance (ANCOVA) is used to test the statistical differences between ROTEM thromboelastometry

All P-values are reported as results of two-sided tests and values of $P < 0.05$ were considered statistically significant.

Results

Demographic data

Patient characteristics including age and gender of both groups were comparable (Table 1).

Table 1: Demographic variable

Group	Range	Mean	μ ± σ (95%)[a]	Between-Groups Effects, P-Value
Age				
5% Human Serum Albumin	21 – 53	40.87	36.53 - 45.20	P-Value (F_c) =0.475
Ringers Lactate	21 - 60	43.07	38.73 - 47.40	
Gender	Male		Female	
5% Human Serum Albumin	17		13	*P* Value (χ^2) =0.2879
Ringers Lactate	21		9	

[a] μ = Mean; σ = Standard Deviation.

Regarding Surgical Wound Drainage: There was no statistically significant difference observed between both groups. Regarding haemoglobin level (Hb): No statistically significant difference was observed in Hb level between both groups. Regarding hematocrit level: No statistically significant difference was observed between both groups. Regarding coagulation profile: international normalised ratio (INR), prothrombin time (PT), and partial thromboplastin time (PTT). There was no statistically significant difference between both groups (Table 2).

Table 2: Coagulation profile

Parameter	Mean	μ ± σ (95%)[a]	Pairwise comparisons, P-Value
Coagulation Profile			
INR			
5% Human Serum Albumin	1.04	1.00 - 1.08	0.286
Ringers Lactate	1.07	1.03 - 1.11	
PT (seconds)			
5% Human Serum Albumin	12.80	12.39 - 13.21	0.302
Ringers Lactate	12.50	12.09 - 12.91	
aPPT(seconds)			
5% Human Serum Albumin	30.00	28.83 - 31.17	0.503
Ringers Lactate	30.10	28.93 - 31.27	

[a] μ = Mean; σ = Standard Deviation.

ROTEM thromboelastometry variables

In-TEM variables

In-TEM clotting time measured in seconds showed that there was no statistically significant difference was found between the two groups = 0.75. Within-group effects (the difference between baseline and postoperative regardless of the type of liquid) showed significant differences, $P < 0.001$. That revealed that both liquids have a significant impact on this parameter. The interaction between different effects and liquid effects showed significant differences, $P < 0.001$, (Figure 2).

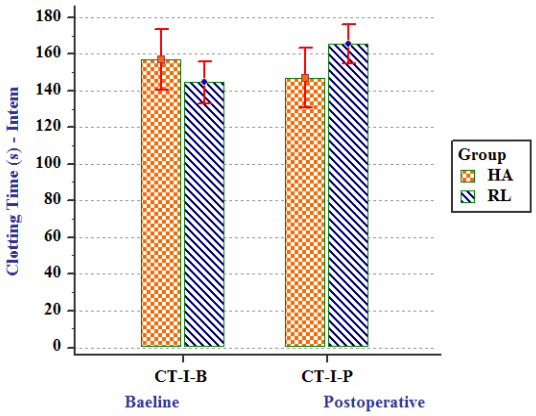

Figure 2: Clotting time, In-TEM

In-TEM Clot Formation Time measured in seconds; there was no statistically significant difference between the two groups p = 0.77. Within-group effects (the difference between baseline and postoperative regardless of the type of liquid) showed non-significant differences, $P < 0.357$. That revealed both liquids have a non-significant impact on this parameter. The interaction between different effects and liquid effects showed significant differences, $P < 0.001$, (Figure 3).

In-TEM Alpha Angel measured in degrees showed a statistically significant difference between the two groups, $P < 0.001$. Within-group effects (the difference between baseline and postoperative

regardless of the type of liquid) showed significant differences, P < 0.001.

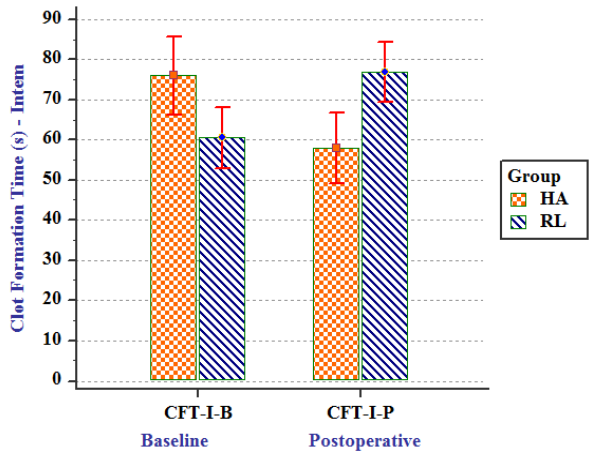

Figure 3: Clot Formation Time, In-TEM

That revealed both liquids have a non-significant impact on this parameter. The interaction between different effects and liquid effects showed significant differences, P < 0.001, (Figure 4).

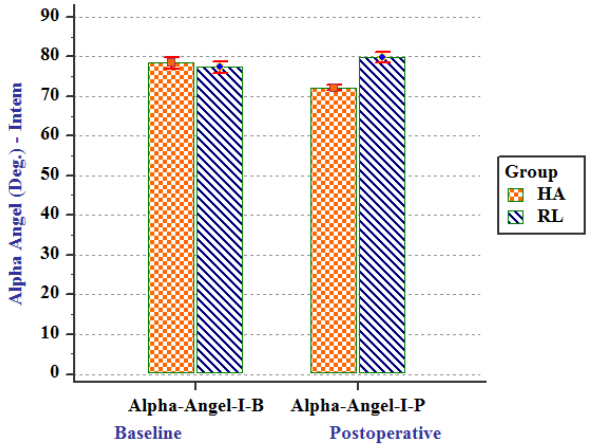

Figure 4: Alpha Angel, In-TEM

In-TEM Maximum Clot Firmness measured in mm there was no statistically significant difference between the two groups P = 0.085. Within-group effects (the difference between baseline and postoperative regardless of the type of liquid) showed significant differences, P < 0.001. That revealed that both liquids have a significant impact on this parameter. The interaction between different effects and liquid effects showed significant differences, P < 0.001, (Figure 5).

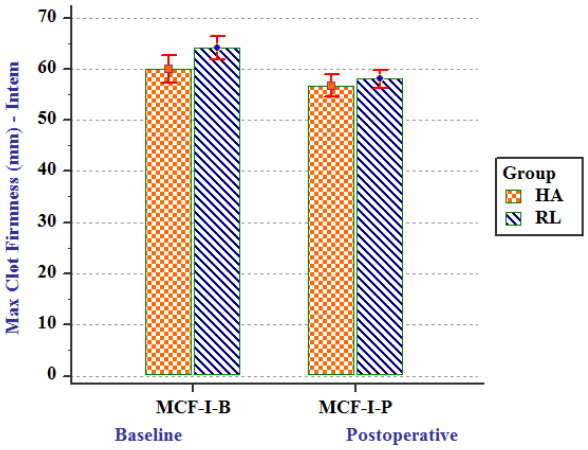

Figure 5: Max Clot Firmness, In-TEM

Ex-TEM variables

Ex-TEM Clotting time values measured in seconds. There was a statistically significant difference between the two groups P < 0.001. Within-group effects (the difference between baseline and postoperative regardless of the type of liquid) showed significant differences, P < 0.001. That revealed that both liquids have a significant impact on this parameter. The interaction between different effects and liquid effects showed significant differences, P < 0.001, (Figure 6).

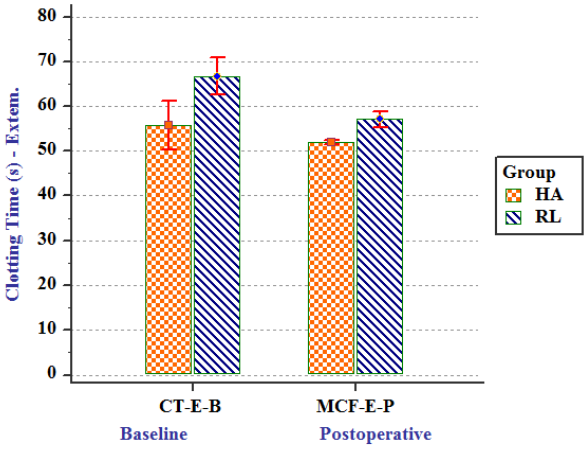

Figure 6: Clotting Time, Ex-TEM

Ex-TEM Clot Formation Time CFT values measured in seconds, Showed no statistically significant difference between the two groups P = 0.104. Within-group effects (the difference between baseline and postoperative regardless of the type of liquid) showed significant differences, P < 0.008. That revealed that both liquids have a significant impact on this parameter. The interaction between different effects and liquid effects showed significant differences, P < 0.001 (Figure 7).

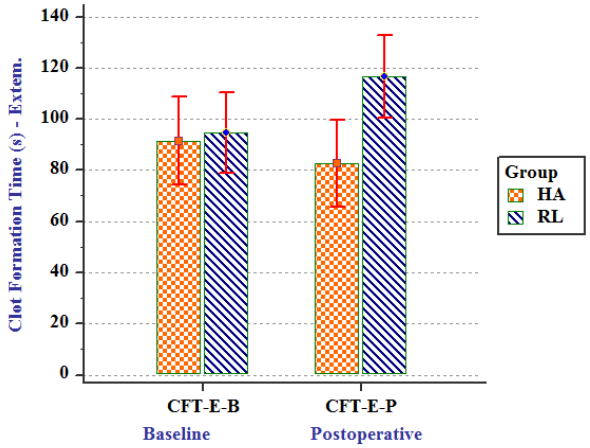

Figure 7: Clot Formation Time, Ex-TEM

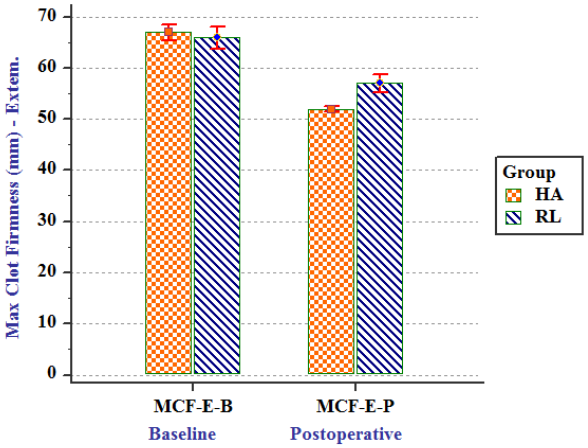

Figure 9: Max Clot Firmness, Ex-TEM

Ex-TEM Alpha Angel values measured in degree showed no statistically significant difference between the two groups P = 0.902. Within-group effects (the difference between baseline and postoperative regardless of the type of liquid) showed significant differences, P < 0.008. That revealed that both liquids have a significant impact on this parameter. The interaction between different effects and liquid effects showed non-significant differences, P = 0.208, (Figure 8).

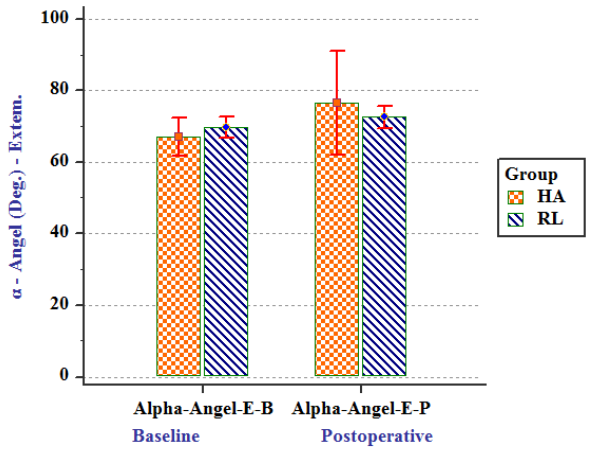

Figure 8: Alpha Angel, Ex-TEM

Ex-TEM Maximum Clotting Firmness MCF values measured in mm, There was a statistically significant difference between the two groups P = 0.045. Within-group effects (the difference between baseline and postoperative regardless of the type of liquid) showed significant differences, P < 0.001. That revealed that both liquids have a significant impact on this parameter. The interaction between different effects and liquid effects showed significant differences, P < 0.001 (Figure 9).

Discussion

Maintenance of intravascular volume is important to achieve optimal perioperative outcomes. There is continuing debate regarding the quantity and the type of fluid resuscitation during elective major surgery [7].

In terms of the effect of albumin versus ringer lactate on blood loss, the results of this study were in line with published studies and meta-analyses comparing crystalloids and colloids for cardiac surgery [8] postoperative bleeding often did not differ between crystalloids and colloids. Studies comparing albumin with non-protein colloids during cardiac surgery were in the majority in favour of albumin regarding transfusion requirements and mortality [9], [10], [11].

In contrast to this study, Johan AB Groeneveld found that Colloids are more efficient than crystalloids in attaining resuscitation endpoints since much less fluid volume is required [12].

Thromboelastography (TEG) and rotational thromboelastometry (ROTEM); are devices that provide the continuous measurement and display of the viscoelastic properties of a whole blood sample from the initial phase of fibrin formation to clot retraction and ultimately fibrinolysis [13].

Numerous studies have reported the utilisation of TEG and ROTEM as a monitoring device for hemostasis and transfusion management in various clinical settings, for example, cardiac surgery, liver transplantation, identification of patients with overt disseminated intravascular coagulation [14], hypercoagulability, and prediction thromboembolic events in surgical patients [15].

Studies claim that TEG is a point of care device for rapid diagnosis and differentiation of hypercoagulable and hyperfibrinolytic conditions [16].

TEG's ability to assess hemostasis in whole blood renders it to be ideal for rapidly identifying patients with trauma-induced coagulation and transfusion guidance [17]. Rotation thromboelastometry (ROTEM®, TEM® Innovations, Munich, Germany) offers an alternative approach to assess perioperative coagulation disorders using the visco-elastic analysis of clotting in vitro. First results are available within 10 min of test initiation, and clot formation can be observed online by a bedside monitor. ROTEM® measurements of EXTEM and INTEM are reproducible and stable over time, regardless of a delay from blood withdrawal to analysis (range 0–120 min after blood withdrawal).

Along with this present study, some studies report TEG and ROTEM to be a useful research tool in comparison to six common tests, hematocrit, platelet count, fibrinogen, PT, aPTT, and fibrin split degradation [18], suggesting a strong relationship.

Tripodi and colleagues [19] stated that standard coagulation tests failed to reflect the balance between the actions of pro- and anticoagulant factors.

By this study, Wang and colleagues [20] showed a significant decrease in transfused allogeneic blood products following a transfusion algorithm using ROTEM® compared with standard laboratory tests in liver transplant surgery.

Data from Schöchl and colleagues revealed the effective use of ROTEM®-guided coagulation management in trauma patients by reducing the amount of allogeneic blood product transfusion [21].

Koray et al. have demonstrated that a ROTEM®-based coagulation algorithm decreased total transfusion costs in cardiac surgery which is consistent with results of the current study [22].

In line with this study, a previous thrombelastographic study in patients undergoing knee replacement surgery and exhibiting even minor blood loss and intravascular volume replacement already showed that colloid administration reduces final clot strength more than Ringer lactate solution does [23].

Limitations: 1. Although ROTEM® can guide the clinician as to which type of treatment may be most helpful to treat coagulopathy during surgery or in trauma ROTEM® results cannot give exact recommendations on the number of blood products or coagulation factors to be administered; 2. Thromboelastometry/Thromboelastography is a valuable addition to the diagnostics of perioperative coagulation management, but it should not be overlooked that this method by no means provides a complete picture of clotting SO as to avoid this drawback correlation between thrombo-elastographic coagulation time and conventional PT and PTT should be considered; 3. Thromboelastography should be performed by trained personnel, and its technique requires standardisation. Standard parameters of ROTEM do not directly examine platelet function; recently specific "platelet mapping assay" for ROTEM had been introduced to practice; 4. ROTEM® analyses were performed not as bedside tests but in a central laboratory with a certain time delay for sample transport.

This study concluded that the use of human albumin (5%) in vascular surgeries before reaching the trigger point for blood transfusion didn't improve blood loss or coagulation profile compared to the use of ringer lactate only. Therefore, ringer lactate can be used as a good replacement for human albumin. Ringer lactate is readily available and inexpensive while human albumin may be costly.

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Case Report

Goldenhar Syndrome: A Case Report

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Keywords: Goldenhar syndrome; Congenital; Deformity

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Abstract

BACKGROUND: Goldenhar syndrome is a multiple congenital disorder with classic characteristics regarding the face, eyes, ears. The incidence of this case is between 1:3.500 to 1:5.600. Early detection and good management can have good outcomes. A newborn with this condition can have a normal life and intelligence.

CASE PRESENTATION: A baby girl was born spontaneously at Wangaya General Hospital with APGAR minutes 1, 5, and 10 scores respectively 3, 5, and 8. Infant birth weight was 2.600 grams, body length was 47 cm, and head circumference was 31 cm with estimated age 32-34 weeks. Clinically showing an asymmetrical face, mouth toward the right side without hypoplasia, the eyelids appear asymmetrical with the right eyelid not open when the left eyelid is open, and the two ears not fully formed and diagnosed as Goldenhar Syndrome. From the physical examination, the ear canal is not formed, intact palate, normal eyeball no abnormalities in the spine and found murmur during the systolic-diastolic phase of the heart. Evaluation of the function of vision and hearing has not been concluded. Abdominal ultrasound showed first-degree picture of bilateral hydronephrosis, and from echocardiography, a PDA was found. TORCH profiles in infant were positive for IgG anti-CMV, IgG anti-rubella, and IgG anti-HSV 1. The prognosis, in this case, is good and periodic evaluation needs to be done in 6 months.

CONCLUSION: Multidisciplinary examination and management, in this case, are needed so that appropriate therapeutic planning can be carried out as well as periodic evaluations in monitoring the child's growth and development.

Introduction

Goldenhar syndrome, known as oculo-auriculo-vertebral (OAV) dysplasia is a multiple congenital disorder with classic characteristics involve the face, eyes, ears [1]. This case was first reported in 1952 [2].

The incidence of this case is between 1: 3.500 to 1:5.600 where the ratio of men: women is 3:2 [3]. The underlying cause is still poorly understood [4]. This syndrome can also affect the heart, lungs, kidneys and nervous system [5]. A newborn with this condition can have a normal life and intelligence.

Case Report

A baby girl was born spontaneously at Wangaya General Hospital and did not immediately cry with APGAR scores at 1, 5 and 10 minutes respectively 3, 5, and 8. Infant birth weight was 2.600 grams, body length 47 cm and head circumference 31 cm. After the examination of the new Ballard score, a score of 22 was obtained with estimates of age 32-34 weeks. Clinically showing an asymmetrical face, mouth toward the right side without hypoplasia, the eyelids appear asymmetrical with the right eyelid not open when the left eyelid is open, and the two ears not fully formed (Figure 1) and diagnosed as Goldenhar Syndrome. Maternal diagnosis is 40 years old, gravida 2, para 1, 32 weeks 4 days gestation age,

premature rupture of membranes (ROM) 12 hours and HBsAg (+). Baby's mother never continued immunisation before pregnancy, no history of illness or perceived fever during pregnancy, no history of miscarriage and only taking vitamin supplementation from the primary health centre. There is no similar history in the family.

Figure 1: The face is not symmetrical

From the physical examination, the ear canal is not formed (Figure 2), intact palate, normal eyeball, no abnormalities in the spine and found murmur during the systolic-diastolic phase of the heart. Evaluation of the function of vision and hearing has not been concluded. Based on routine blood results, leukocytes 10.610/uL, haemoglobin 19.8 g/dL, platelets 189.000/uL, neutrophils 48%, lymphocytes 33.6%, monocytes 16.6%, so it was concluded that there were no signs of acute infection.

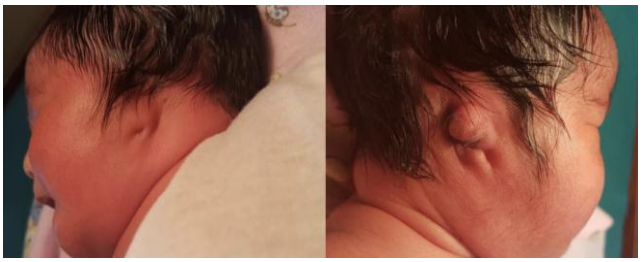

Figure 2: The earlobe and ear canal are not formed

The babygram examination shows the heart is not enlarged, the lungs do not appear infiltrates and normal bronchovascular patterns, the left-right diaphragm is normal, no abnormalities in the projected spine (Figure 3), the contour of the right-left kidney is not clear, psoas line is symmetrical right, the shadow of the liver and spleen do not appear enlarged and the corpus, pedicle, intervertebral spatium are good. From the abdominal ultrasound, first-degree bilateral hydronephrosis was obtained. From echocardiography, a patent ductus arteriosus (PDA) was found. TORCH profiles in infant were positive for IgG anti-cytomegalovirus (CMV), anti-rubella IgG, and IgG anti-HSV (herpes simplex virus) 1. From the physical examination and supporting data, it was concluded that Goldenhar syndrome.

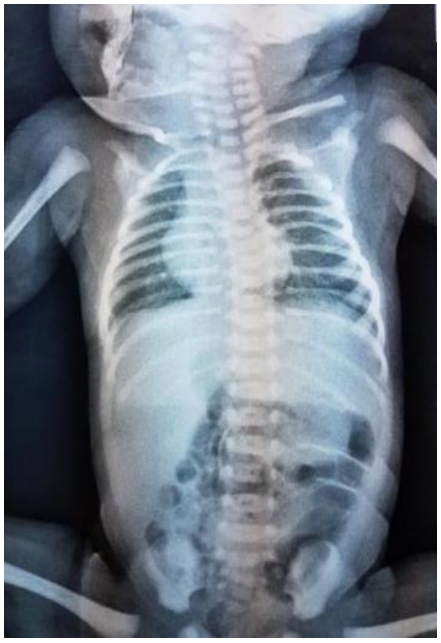

Figure 3: Radiological examination of the anteroposterior spine. No abnormalities were found

Discussion

Goldenhar syndrome is a congenital disorder known as dysplasia/oculo-auricular-vertebral syndrome (OAV) with classic triad syndrome, namely mandibular hypoplasia (facial asymmetry), ear malformation (microtia/anotia and preauricular fistula) and/or eyes (bulbar dermoid cyst, microphthalmia), as well as vertebral anomalies [7]. This case was first reported in 1952 by Maurice Goldenhar [1], [2].

The incidence of this case is between 1:3.500 to 1:5.600 where the ratio of men: women is 3:2 [3]. Approximately 1-2% of people affected have other family members with this condition, which indicates that genes can play a role in some cases [5]. This case cannot be concluded because genetic testing cannot be carried out in Bali because it can only be done at the Eijkman Institute Jakarta and can be a consideration for further examinations. In family history, no or negative abnormalities were found.

The underlying cause of this disorder is still poorly understood, but it is possible that the embryonic vascular supply to the first and second branch arches at 4-8 weeks of conception is abnormal, disturbances in mesoderm migration or several other factors cause the formation of branch arches and a damaged spinal system [6], [8], [9]. The history of pregnancy such as heavy alcohol

consumption, use of drugs such as thalidomide, retinoic acid, tamoxifen, and cocaine may be related as a risk factor to the development of this syndrome. Pregnancy with diabetes, rubella infection and influenza are possible etiological factors [10]. The causative factor in this case probably due to rubella infection during before or early pregnancy is seen from the presence of positive anti-rubella IgG from baby and mother without immunization. It maybe the baby's mother had been infected before. From birth defects are found, we considered to rubella infection.

Facial asymmetry and mandibular hypoplasia are typical features of OAV syndrome [11]. In addition to these abnormalities, Goldenhar syndrome may be associated with other disorders such as mental retardation, hearing loss, cleft palate, abnormal hands or fingers, pulmonary hypoplasia, lymphoma, kidney agenesis, ectopic kidney, ureter duplication, hydronephrosis, hydroureter, and genitourinary system anomalies [7]. Cardiovascular malformations occur in patients with Goldenhar syndrome between 5-58 per cent, the most common being ventricular septal defect and tetralogy of Fallot, PDA [12]. In this case, a small PDA was found and no symptoms during hospitalised. It must be observed because a small PDA may close spontaneously. If a small PDA does not close and no symptoms, it requires medical treatment and possible to surgical repair after 6 months of age.

The diagnosis of Goldenhar syndrome through the identification of abnormalities on physical examination, no genetic examination. The multidisciplinary examination of ophthalmology, otolaryngology, odontology, radiology and neurology contributes to the diagnosis and treatment of this case [13].

Management of this case is by abnormalities and age. In patients with mandibular hypoplasia, reconstruction is performed. Structural abnormalities in the eyes and ears are corrected by plastic surgery. Surgery to repair cleft lip and cleft palate [14].

Early management is very important in this case. By improving hearing function with hearing aids, bone-anchored hearing aids for conductive hearing loss, cochlear implants in cases of severe sensorineural hearing loss, and reconstruction at 4-10 years of age, operating time depends on the severity of the disability and the age of the child [15], [16]. After the Goldenhar syndrome is diagnosed, flexion-extension must be measured every 6 months and must be considered for scoliosis [12]. Evaluations carried out in this case, among others, the examination of vision, hearing, spine, heart in 6 months of monitoring and growth and development. Scan computerized tomography (CT) inspection plans to look for problems that are not seen with babygram.

In conclusion, Goldenhar syndrome patients can have multiple congenital abnormalities and must be thoroughly examined with other multidisciplinary. In making a diagnosis, must be with the other multidisciplinary so that appropriate therapeutic planning can be carried out and periodic evaluations in monitoring the child's growth and development. The prognosis is determined by the abnormality and severity obtained, and periodic evaluation needs to be done in 6 months to determine its progress. In this case, the prognosis of this baby is good; there are no serious problems in facial malformations and inclusion abnormalities such as abnormalities of the heart and kidneys. Patients reported here have several problems in the eyes, ears and heart. At present, patients are in medical care and observation for other possible problems.

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Case Report

Giant Pelvic Neurofibroma in Patient with Plexiform Sciatic Neurofibroma and Neurofibromatosis Type 1

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Keywords: Neurofibromas; Plexiform neurofibroma; Surgical treatment; Sirolimus; MPNST; Neurofibrosarcoma

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Abstract

BACKGROUND: Neurofibromatosis is a genetic disease with an autosomal dominant type of inheritance. It is a multisystem disease in which, besides skin manifestations, there is a possibility for the involvement of other organs and systems, and an atypical variant of neurofibromatosis type 1 can also be observed- the so-called plexiform neurofibroma. In patients with this inherited disease, mortality is higher due to the existing risk for malignant transformation and development of malignant peripheral nerve sheath tumours (MPNSTs) or neurofibrosarcoma.

CASE REPORT: We present a 25-year-old woman with neurofibromatosis type 1 and a family history of the disease-father and grandmother with NF-1, with fatal outcome in the grandmother as a result of malignant transformation to neurofibrosarcoma. The patient has clinical data for multiple cafés- au- lait spots on the skin of the trunk, upper and lower limbs, and plexiform tumour formation in the seating area. From the performed imaging diagnostic there are available MRT data for 1) giant pelvic neurofibroma, 2) plexiform giant neurofibroma in the subcutaneous fat on the right thigh and gluteal fat tissue to the right, passing through the midline in the area of the external genitalia, leading to deformation of the front wall of the sacrum with bilateral meningoceles and 3) diffuse involvement of the bladder wall from the process in the area of the trigonum vesicae felleae/the two urethral ostium, as well as 4) the presence of neurofibromas in the course of the iliac vessels on the right. Surgical removal of the oval pelvic formation, identified as neurofibroma was planned, as well as the initiation of systemic therapy with Sirolimus for the plexiform sciatic formation, infiltrating the bladder.

CONCLUSION: Neurofibromatosis type-1 is a problematic disease due to the parallel systemic involvement of different organs and systems, which can be both limited and diffuse. Limited tumour lesions in the form of neurofibromas with diverse localisation (as in the patient we describe) could be surgically removed without difficulty. On the other hand, the diffuse involvement of internal organs within a giant, network-3spreading plexiform neurofibromas (as in the described patient) makes interdisciplinary interventions impossible, and therefore therapeutic alternatives should be considered.

Introduction

Neurofibromatosis was first described in 1882 by Von Recklinghausen as a genetic neuroectodermal abnormality with systemic and progressive involvement, which mainly affected the skin, nervous system, bones, eyes and possibly other organs [1]. It is estimated that about one million people worldwide are living with NF [1]. Neurofibromatosis type 1 is a multisystem autosomal dominant disease, with cutaneous manifestations such as café-au-lait spots, multiple neurofibromas, ephelides in the skin fold areas, and hamartomatous lesions in the eyes, bones, glands and the central nervous system [1], [2]. Uncommon variants of NF-1 are the so-called plexiform neurofibromas in which neurofibromas arise from multiple nerves that may also engage connective tissue and skin folds and are clinically described as "bags of worms" [3]. Patients with neurofibromatosis are at risk for malignant transformation, as on the one hand having an increased risk of developing malignant peripheral nerve sheath tumours (MPNSTs), and on the other are threatened by a transformation to neurofibrosarcoma, which should be suspected if the initial lesion grows rapidly and

significantly [4], [5], [6]. We describe a patient with neurofibromatosis type 1, miction disorders and giant pelvic neurofibroma.

Case Report

We present a 25-year-old woman with a family history for neurofibromatosis type 1. According to anamnestic data, father and grandmother suffer from neurofibromatosis type 1, as in the grandmother being observed lethal outcome as a result of the transition from plexiform neurofibroma to neurofibrosarcoma in the neck area. The first symptoms date back to the age of 2-3 when multiple café-au- lait spots were observed all over the body and appearance of tumour formation in the neck and genital area (Figures 1a-1d). At the age of 3 labiaplasties were performed on the pubic lips. By entering puberty, tumour formations in the neck area and genitals begin to increase in size. In 2015, the patient was hospitalised in a plastic-restorative and aesthetic surgery department, where step by step the skin and some parts of the plexiform neurofibromas in the lumbosacral, the sciatic, genital, and upper-medial area of the right thigh have been removed.

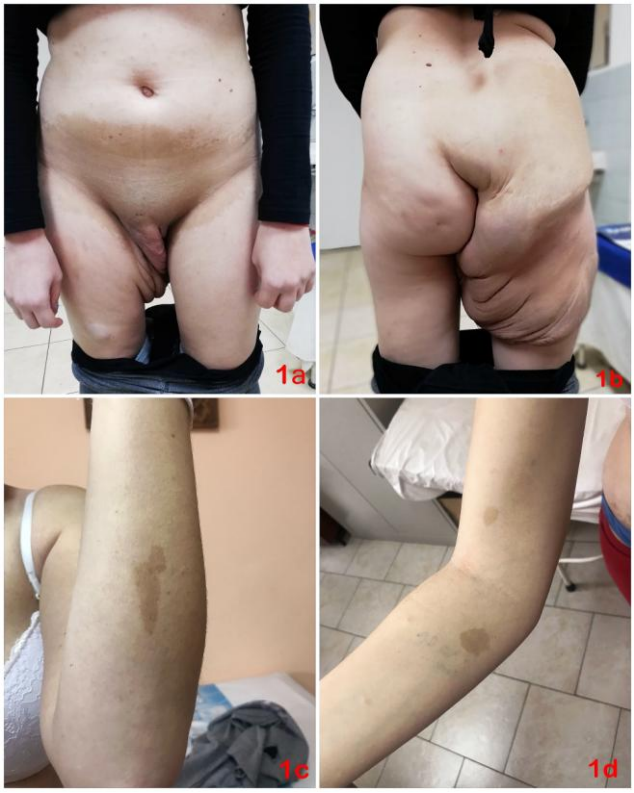

Figure 1: A), B) Clinical examination: in the area of the genitals, the gluteal region and the upper medial part of the right hip, a plexiform tumour-like formation with a darker-skin-colour appearance, which resembles clinically as "worm bag" (Figures 1a and 1b) was observed; C), D): Clinical view of numerous café-au-lait spots on the skin of the upper limbs

In 2016, due to recurrent bladder infections, the patient was directed to a urology department where small pelvis MRI was performed. The results showed an enlarged, hypotonic bladder with multiple diverticula (Figures 2b, 2d). In addition to the right of the bladder and ovary, an 82/50/50 mm formation with sharp and smooth outlines was visualised (Figures 2a-2c). The formation has a characteristic of giant pelvic neurofibroma (Figures 2a-2c).

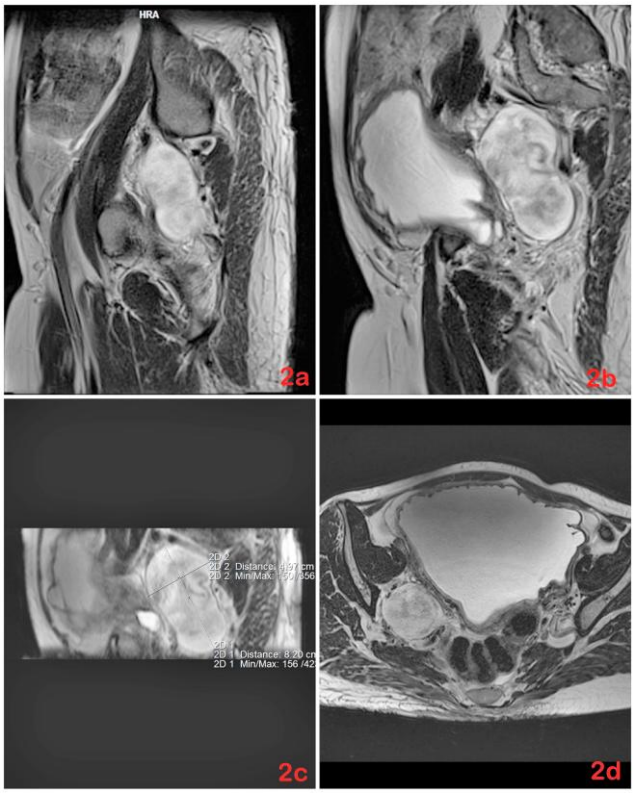

Figure 2: A), B), and C) An MRI images of abdomen, small pelvis, gluteal area and thighs with data for a 9cm mass with irregular shape, lying on the right side of the pelvis and a conclusion about giant pelvic neurofibroma; D) MRI of a small pelvis showing an enlarged, hypotonic bladder with multiple diverticulitis

Within the dermatological examination, a tumour formation was observed in the genital, buttock area and upper-medial part of the right thigh with darker skin colour, resembling clinically as a "worm bag"-plexiform neurofibroma in the sciatic area (Figures 1a-1b). Also, the presence of multiple cafés-au- lait on the skin of the trunk, upper and lower extremities were found (Figures 1c-1d).

An additional MRI of a small pelvis with contrast revealed data for deformation of the sacrum on the front wall with bilateral meningoceles at Ec1 and Ec2 levels (Figures 3a,3c), as well as the presence of plexiform giant neurofibroma in the subcutaneous fat of the right thigh and gluteal fat on the right, with the changes crossing the midline in the area of the external genitalia (Figure 3a).

The presence of neurofibromas in the course of the iliac vessels on the right, with the larger one measuring 75/49 mm (Figure 3a). Bladder with

irregularly thickened walls and uneven internal outline contours (Figures 3a-3b, 3d). According to the image data, the finding engages the back wall diffusely in the area of the trigonum and the two urethral ostium, and in the trigonum area, the bladder wall is engaged in its entire volume (Figures 3a-3d).

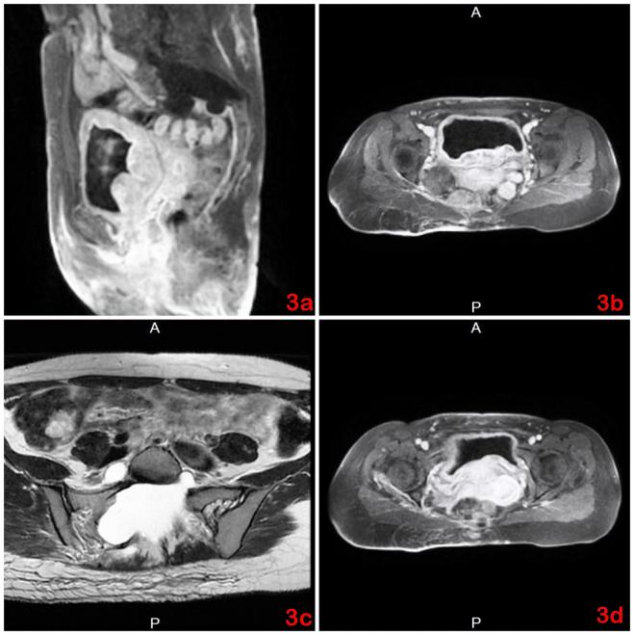

Figure 3: A), B) MRI images showing irregularly thickened walls and uneven internal contours of the bladder wall in the area of the trigonum and the two urethral ostia; C), D) Deformation of the anterior sacral wall with bilateral meningoceles at the Ec1 and Ec2 levels and diffuse engagement of the posterior wall of the bladder in its entire volume

The hospitalisation was planned in a neurosurgical unit for eventual surgical treatment.

Discussion

Data on patients with neurofibromatosis have existed since XIII and XVI centuries, and as early as 1785- Mark Akensidi describes a case about a patient who was nicknamed "wart man" [1]. Subsequently, various descriptions and definitions of the disease arise, and it is currently thought that Neurofibromatosis type 1 (NF-1) or von Recklinghausen's disease is a disease of autosomal dominant transmission that is thought to have mutations in the NF1 gene on the long arm of chromosome 17 (17q11.2), resulting in a decrease in the production of neurofibromin protein, resulting in a lack of control of cell growth and division [2].

Classically, the skin manifestations of NF1 are characterised by the presence of neurofibromas and café au lait spots variably distributed throughout the skin [7]. In some cases, however, neurofibromas are located along the nerves, in the subcutaneous tissue and follow the nerves affecting large areas and are defined as plexiform neurofibromas [8]. Patients with neurofibromatosis have a higher mortality rate which is mostly related to the possibility of malignant transformation of tumours [1].

Patients are at risk for the transformation of NFs to malignant peripheral nerve sheath tumours (MPNSTs) or neurofibrosarcoma [4], [5], [6]. According to the literature data, particular attention should be paid to four markers (epidermal growth factor receptor, interferon-γ, interleukin-6, and tumour necrosis factor-α) to distinguish between patients with NF-1 and healthy subjects [9].

Even more significant (from a prognostic point of view) should be the determination of two additional markers as potential early risk predictors of developing MPNST (insulin-like growth factor binding protein 1 (IGFBP1)) and regulated upon activation, normal T-cell expressed and secreted (RANTES) in patients with type 1 neurofibromatosis and plexiform neurofibromas [9].

According to the majority of authors' collectives, patients with neurofibromatosis should be closely monitored and, where possible, excision of skin tumours or surgical treatment should be performed [1], [2], [3]. In many cases, however, surgical excision is complicated due to the involvement of the main nervous branches within the plexiform neurofibromas, and then the possibility of relapse after resection depends on the possibility of total or partial resection [4].

We describe an interesting case of a patient with a giant, well-defined neurofibroma behind the bladder, as well as giant network-like sciatic plexiform neurofibroma engaging the genital area of the right, the gluteal region and the upper-medial part of the right thigh. Surgical excision of well-defined neurofibroma in the pelvic region should not be problematic and was planned in a neurosurgical ward at a later stage. Due to evidence of meningoceles in the sacral region, as well as an unfortunate localization of plerixiform neurofibroma in the pelvic/genital area, neurosurgeons currently restrain from surgical intervention.

Alternative treatment for patients with type 1 neurofibromatosis could be systemic therapy with Sirolimus (0.8 mg/m² body surface area by mouth for a 28-day course) [10]. Based on the available data, sirolimus treatment is appropriate for patients with progressive NF-1 type, inoperable, and it is not recommended for non-progressive forms of NF-1 [11]. Therapeutic alternatives should be considered.

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Case Report

Surgical Management (Microsurgery) of Traumatic Penile Amputation: A Case Report

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Keywords: Anastomosis; Microsurgery; Traumatic Penile Amputation

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Abstract

BACKGROUND: Traumatic penile amputation is an uncommon surgical emergency with various etiologies, carrying major functional and psychological consequences concerning the patient's overall quality of life. Regardless of the aetiology, penile amputation represents a surgical emergency that must be addressed quickly and efficiently to maximise functional outcomes.

CASE PRESENTATION: We herein describe a case of psychiatric disorder that resulted in a complete self-amputation of the patient's penis. The author presents a case of a 20-year-old single Indonesian male with no significant past medical or psychiatric history, who was presented to our Regional Referral Hospital with traumatic penile self-amputation. Immediately, the patients were taken to the operating room, and careful examination under anaesthesia revealed fully and transversally transected urethra as well as corporal bodies at the level of penis base. Viable artery and vein were then searched using a microscope after suturing through the tunica albuginea of the corporal bodies on the ventral aspect and snapped them for future tying. After microvascular re-anastomosis of the left dorsal artery and only one dorsal vein done. We attached the urethra and placed a 16 Fr silicon catheter. The result was an excellent tension-free, widely spatulated urethra anastomosis, which was then reattached to the corporal bodies. The penis had significant oedema and swelling in the distal penile shaft; however, pain sensation was gradually returning.

CONCLUSION: The authors noted that microsurgical reimplantation is the treatment of choice for penile amputation, with a minimum one of the penile vascular was successfully anastomosis.

Introduction

Traumatic penile amputation is an uncommon surgical emergency with various etiologies. It carries major functional and psychological consequences regarding the patient's overall quality of life. There is a paucity of case reports of traumatic penile amputation during circumcision; however, most of the cases reported with self-mutilation are a result or severe substance-induced psychosis or underlying psychiatric disorder [1]. Nonetheless, the incidence of traumatic penile amputation remains low, limiting our understanding mainly to case reports and reviews. Regardless of the aetiology, penile amputation represents a surgical emergency that must be addressed quickly and efficiently to maximise functional outcomes [2]. We herein describe a case of psychiatric disorder that resulted in a complete self-amputation of the patient's penis.

Case Report

A 20-year-old single Indonesian male with no significant past medical or psychiatric history was presented to our Regional Referral Hospital with traumatic penile self-amputation. The patient brought in his distal penile stump placed in dry gauze (3-hour warm ischemic time), the stump was placed on the ice after arrived in the emergency department (4 hours cold ischemic time). The patient had some family problems and developed severe depression; it begun almost 3 weeks and never talk any word. Earlier to the

patient's presentation, the patient had done wooden carving tools, and suddenly he cut the penis with a sharp pocket blade due to auditory hallucinations.

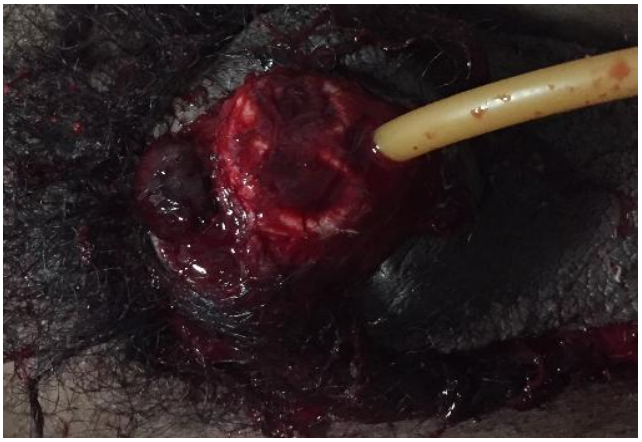

Figure 1: Complete penile amputation at the base of the penis

A detailed discussion regarding surgical reimplantation of the amputated penile stump was undertaken. All risks, benefits, alternative treatments, and potential complications were discussed, and formal consent to the family was obtained. Immediately, the patients were taken to the operating room, and careful examination under anaesthesia revealed fully and transversally transected urethra as well as corporal bodies at the level of penis base. The skin along with the penile stump and amputated penis were intact with no evidence of ischemia or necrotic changes. Prophylactic intravenous antibiotics (cefazolin 1 gr) were given.

Figure 2: Amputated penis before reimplantation

Meanwhile, the urology team began to look for the dorsal artery and vein using a microscope. We did sutures through the tunica albuginea of the corporal bodies on the ventral aspect and snapped them for future tying. Next, we success to do microvascular re-anastomosis of the left dorsal artery and only one dorsal vein. We attached the urethra in a 360-degree fashion using interrupted 6-0 vicryl sutures. Halfway through the anastomosis, we placed a 16 Fr silicon catheter. We had an excellent tension-free, widely spatulated urethra anastomosis. We reattached in interrupted fashion using 3-0 vicryl sutures to the corporal bodies.

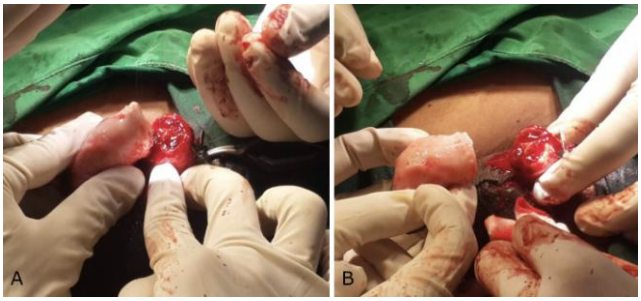

Figure 3: (A and B) Process of reimplantation and re-anastomosis under the microscope

The penis was then covered in sufratule and gauze. Postoperatively, the patient had adequate flow to the distal end. During his postoperative course, he was under strict bed rest until postoperative day 14, with given an antidepressant from psychiatry. The penis had significant oedema and swelling in the distal penile shaft; however, pain sensation was gradually returning.

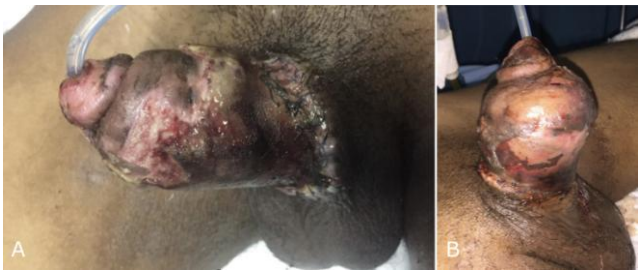

Figure 4: (A and B) Postoperative image day 1 after re-anastomosis and 16 French Silicon Foley catheter in place

Discussion

Penile amputation is a rare urologic emergency, with only a few microsurgeons have or will experience managing this patient. Therefore, the course of management has to be carefully [1].

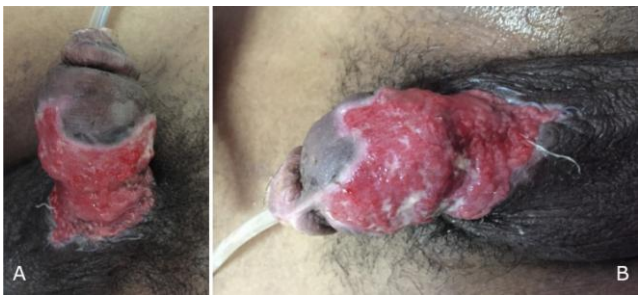

Figure 5: (A and B) Penile skin was necrotic and was done wound care until the granulated raw surface

Tamai and Cohen in 1977, has successfully

microsurgical reimplantation of the penis, repair involved suturing of the major part – corpora cavernosa, urethra, and skin without repair of the nerves and vessels. This was often complicated with skin and glans necrosis, urethral fistula, stricture, incomplete erection, and failure of sensory sensation.[1] Skin loss has been a problem in microsurgical repair; data showed from 28 patients reviewed by Landstrom et al. showed 15 patients with skin loss of which 2 were a complete loss [3].

Figure 6: (A and B) Penile after FTSG (Full-thickness Skin Graft) (5 weeks after the first reimplantation)

In our case, penile skin was partial loss after 14 days reimplantation. In a systematic meta-analysis detailed by Li et al., a total of 109 patients with penile amputation were successfully reimplanted in China for over 48 years. Among all cases, 53/109 (49%) cases were performed microsurgery. Postoperative complications identified were skin necrosis in 58 patients, penile sensation alteration in 31 patients, urethral strictures in 16 patients, erectile dysfunction in 14 patients, and urethral fistula in 8 patients. Penile skin necrosis was negatively correlated with the total number of anastomosed blood vessels ($P < 0.05$) [4].

Correlation of the number of arteries or veins repaired with skin loss did not give a clear conclusion. Skin loss was observed in patients in whom both dorsal arteries and deep arteries were repaired. The anastomosis of the deep arteries only was insufficient to prevent skin complication [3]. Wei et al. suggested that at least one dorsal artery was repaired. Even after both superficial and deep venous anastomosis, skin necrosis was occurred, suggesting that this may not be the only reason. Oedemas of the penile, prolonged ischemia, use of heparin postoperatively have been implicated in contributing skin necrosis [3].

With the involvement of complex neuro-circulatory reflex, involving various factors, which influenced by medication, psychiatric background, and general shyness, erectile function is difficult to assess. Erectile function tests with the nocturnal penile test and prostaglandin test as early as 3 weeks following repair [5]. A consensus in the contemporary literature acknowledges that microsurgery revascularization and reimplantation of the penile structure provide early and adequate restoration of penile blood flow with the best outcome of penile reimplant survival, erectile, and voiding functions [4]. Penile replantation is not a contraindication in psychiatric patients, is superior to any presently available method of reconstruction [3].

In conclusion, traumatic penile amputation is an emergency surgical case that needs immediate treatment. Microsurgical reimplantation is the treatment of choice for penile amputation, with a minimum one of the penile vascular was successfully anastomosis.

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Case Report

Cerebellar Cryptococcal Abscess in HIV-Negative Patient: A Case Report and Literature Review

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Keywords: Cryptococcosis; Abscess; Immune-competent; Cerebellum; Fungal

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Abstract

BACKGROUND: Cryptococcus is a common cause of opportunistic infection in HIV-positive patients. While the incidence of this disease has decreased in AIDS-associated cases, cryptococcal infection in immune-competent person has been increased.

CASE PRESENTATION: We report a case of cryptococcosis and literature review of pathogenesis and clinical aspects of cryptococcal central nervous system infection. A 64-year-old man, from Flores, complaining of severe headache since a few days before admitted to hospital. Head MRI showed multiple hypointense lesions in the left cerebellar hemisphere, suspected abscess or metastatic process. HIV testing was non-reactive. Surgery was performed, and microscopic evaluation revealed multiple abscesses containing PAS-positive budding yeasts consistent with cryptococcal abscesses.

CONCLUSION: Cryptococcosis rarely occur in immunocompetent patients. The clinical manifestation depends on pathogenic factors of pathogen and host factor. Treatment is with the administration of antifungal drugs, and the prognosis mostly depends on the underlying disease.

Introduction

Cryptococcus is the fourth leading cause of opportunistic infections after Pneumocystis jiroveci, Cytomegalovirus, and Mycobacteria with central nervous system (CNS) manifestations. Manifestations of the CNS are much more frequent than those of other organs. In patients infected with human immunodeficiency virus (HIV) in the United States, the incidence of cryptococcosis is about 2-7 cases per 1000 per year, with 89% of manifestations in the CNS [1]. The incidence in HIV patients has declined recently due to widespread antifungal and antiretroviral use. Meanwhile, the incidence in immune-competent person is increasing. Cryptococcosis is an infection caused by encapsulated fungi, *Cryptococcus neoformans*, and *Cryptococcus gattii.* In CNS cryptococcosis, the organisms spread through the respiratory tract and infect the CNS hematogenously [2]. Pathogenesis of disease in immune-competent person is not clear; the pathogenic factor of the pathogen and host immune response may involve. Here we report one case of cerebellar cryptococcal abscesses in an immune-competent patient, with a literature review of its pathogenic and clinical aspects.

Case Presentation

A 62-year-old man from Flores, exposed to poultry, complaining of severe headache since a few

days before hospital admission. He was suffered from intermittent headache previously. Neurologic examination revealed left cranial nerve VII, XII supranuclear paresis, left spastic hemiparesis, negative meningeal sign, and positive left Hoffmann-Tromner reflex. Laboratory examination showed normal complete blood count and non-reactive for HIV testing.

Head MRI examination revealed multiple hypointense lesions in the left cerebellum hemisphere at T1W1, the lesion became hyperintense on T2W1 and restricted to DW1, which was with contrast enhancement. The lesions suppress the IV ventricle. The ventricular system of lateral and III ventricular widen with periventricular hyperintense (Figure 1). The conclusion was multiple hypointense lesions in the left hemisphere of the cerebellum with differential diagnoses of abscesses and metastasis tumour, non-communicating hydrocephalus, and ventriculitis.

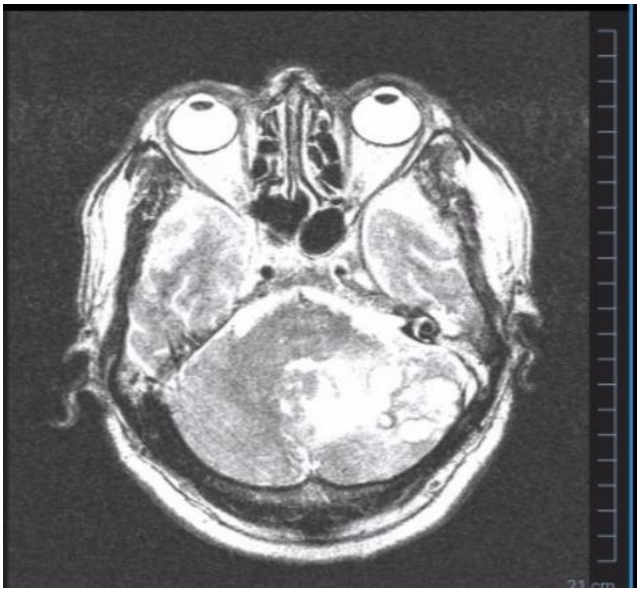

Figure 1: Head MRI. Multiple hypointense lesions in the left cerebellar hemisphere of T1W1, hypertense on T2W1 and restricted to DW1, with contrast enhancement rim. The lesion suppresses the IV ventricle

Based on the results from anamnesis, physical examination and investigation, a working diagnosis on this patient were space-occupying lesions of the cerebellum with differential diagnoses of tumour and abscess. Brain surgery was being performed and based on microscopic examination; there were areas of brain tissue necrosis. The edges of necrosis were surrounded by granulation tissue containing lymphoplasmacytic inflammatory cells. In the necrotic area, there were many unstained ovoid structures. PAS staining examination demonstrated oval-shaped organisms, with bright red double contour wall consistent with cerebellar cryptococcal abscesses (Figure 2). The patient was treated with fluconazole, and the symptoms were improved.

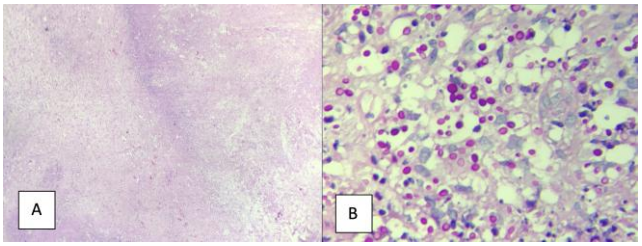

Figure 2: A) Diffuse necrosis with lymphoplasmacytic inflammatory cells. In the necrotic area, there are many unstained ovoid structures; B) PAS staining showed bright red yeast consistent with Cryptococcus

Discussion

Invasive fungal infections are estimated to cause over 1.5 million death per year worldwide — the majority of cases occurring in immunocompromised patients [3]. Cryptococcus can be isolated from the environment in many regions of the world. However symptomatic disease after exposure is relatively rare. The defect in cellular immunity is the most commonly found risk factor. Meanwhile, other predisposing factors are tissue transplant, malignancy, sarcoidosis, chronic liver disease, and renal failure [3], [4].

Cryptococcus neoformans and *Cryptococcus gattii* are two etiologic agents of cryptococcosis. Brain cryptococcosis more frequently associated with *C neoformans*. Meanwhile, lung infection more frequently caused *by C gattii*. They can be distinguished from other pathogenic yeasts by the presence of polysaccharide capsule, melanin formation, and urease activity, which all function as virulence factors of this pathogen. *C neoformans* primarily reaches the lung after inhalation and disseminates to the brain [5]. Several pieces of evidence showed that *C neoformans* invades the central nervous system by two mechanisms, direct invasion of endothelial cells lining of brain vasculature and by Trojan horse mechanism whereby the pathogen enters the central nervous system after macrophage ingestion [4], [6].

Neurological cryptococcosis can encompass several different presentations, including meningitis, encephalitis, ventriculitis, increased intracranial pressure, and space-occupying lesion (cerebral abscesses, cyst, and granuloma) [5]. Meningeal infection is one of the most common presentations. Meanwhile, cerebellar infection is rare. Several cases of cerebellar cryptococcosis have been reported [6], [7], [8], [9], [10], [11], [12]. The clinical manifestations of cerebellar cryptococcosis is commonly present with signs of increased intracranial pressure and abnormality of cerebellar functions (ataxia and dysdiadochokinesis). Extension of infection to the surrounding structures can be observed by the presence of cranial nerve functions disorder,

meningeal signs, ventriculitis, and hydrocephalus. The typical imaging appearances of cryptococcal meningoencephalitis include dilated Virchow Robin Space, pseudocysts, abscess, cryptococcoma, leptomeningeal or parenchymal enhancing lesions, and hazy brain base [13]. Cultures of CSF and analysis of soluble cryptococcal polysaccharides are the standard laboratory diagnosis for neurological cryptococcosis [5]. Tissue examination from the space-occupying lesion of brain cryptococcosis may reveal the specific morphologic appearance of this fungi as pathogen proliferation within space surrounded by chronic inflammation or with the granulomatous formation. The fungi which appeared as unstained ovoid structures in H-E staining will be looked like bright red encapsulated ovoid yeast when it is highlighted with PAS staining [14]. Most of the CNS cryptococcosis are successfully treated with fluconazole. A severe case can be treated with amphotericin B and flucytosine combination. A study by Pappas *et al*. showed significant factors that contributed to mortality included late age, hematologic malignancy, and the presence of organ failure [15].

In conclusion, cryptococcosis is an infection caused by fungi and rarely occurs in immunocompetent patients. The clinical manifestation depends on pathogenic factors of pathogen and host factor. Treatment is with the administration of antifungal drugs. The prognosis of this disease mostly depends on the underlying disease.

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Case Report

Case Report: A Simple Thoraco - Abdominal Flap to Reconstruct Wide Defect of Radical Mastectomy in Squamous Cell Carcinoma of Breast

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Keywords: Thoraco-abdominal flap; Squamous cell carcinoma; Mastectomy

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Abstract

BACKGROUND: Squamous cell carcinoma (SCC) of the breast is very unusual. Thoraco-abdominal (TA) flap is a simple flap, and it is a rotation advancement fasciocutaneous flap. Here, we present a case of using TA flap for chest wall reconstruction in quick in-quick outpatient.

CASE PRESENTATION: A Russian woman 48 years old presented enlarging lump on her left breast for the last 5 years. She was diagnosed as stage IV low-differentiated breast cancer luminal B and had a history of 4 cycles chemotherapy. Patient getting worsening and no response to chemotherapy. We decided to perform palliative radical mastectomy to improve quality of life. Primary skin closure was not possible due to the wide defect of skin and soft tissue. We decided to use TA flap to cover the defect. Histopathology result was compatible with SCC.

CONCLUSION: TA flap can be the choice in patients with a wide defect of skin and soft tissue after a radical mastectomy. Given its simplicity and shorter operative time, TA flap is an ideal option for quick in-quick outpatient.

Introduction

Squamous cell carcinoma (SCC) of the breast is very unusual. The incidence of primary SCC of the breast was 0.04-0.1% of all breast cancer. In 1908, the SCC of the breast was first time reported, and until 2002, there were 85 cases [1]. SCC of the breast may originate from a neoplasm of the breast skin or spreading of SCC another side of the body. Breast metastasis of SCC may be from skin, cervix, pharynx, stomach, and lung. Pure SCC of the breast can be from the epidermis, the nipple or epithelium of a deep-seated dermoid cyst or metaplasia on chronic inflammation [2].

The wide defect produced during surgery, thoraco-abdominal (TA) flap is a simple flap and suitable for quick in-quick outpatient. This flap is a rotation advancement fasciocutaneous flap. This flap uses the skin and subcutaneous tissue of the anterior abdominal. Here, we present a case of using TA flap for chest wall reconstruction in a patient with SCC of the breast. This patient was not in her optimum state, so we choose the best action for quick in-quick outpatient.

Case Presentation

A-Russian woman 48 years old presented enlarging lump on her left breast for the last 5 years. Patient neither visited the doctor nor been examined

or treated. On 2014, lump getting bigger and patient lost 7 kg during a month. She was hospitalised in Moscow for follow up examination. CT scan showed a mass in the left breast, lymph node enlargement on the left axilla, and pleural effusion on the left lung. The core biopsy revealed as low-differentiated breast cancer, luminal type-B. MRI brain showed no metastasis.

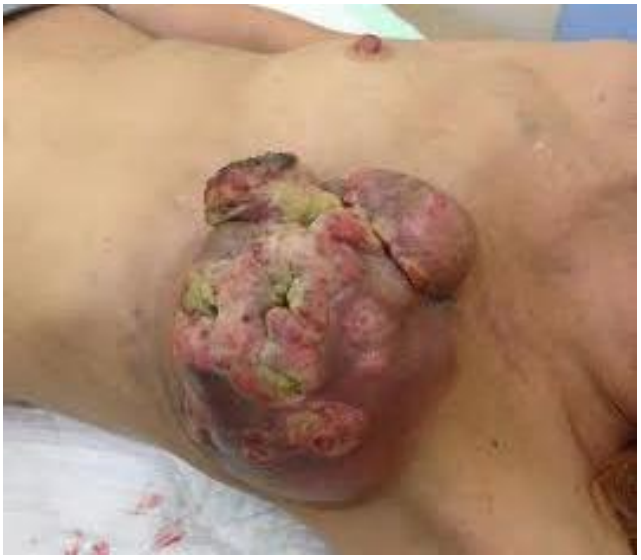

Figure 1: Lump with an ulcer on the left breast

Patients had history 4 cycles of chemotherapy (Adriamycin + Cyclophosphamide 2 cycles and switched to Docetaxel 40 mg/m^2 + Capecitabine 2000 mg/m^2 per day, 1-14 days about 2 cycles). There was no response to chemotherapy but follow up on the chest and abdominal CT scan, no fluid in the lung, and no metastatic on the liver. Laboratory finding was within normal limit. The patient then continued her treatment at the place residence in Bali.

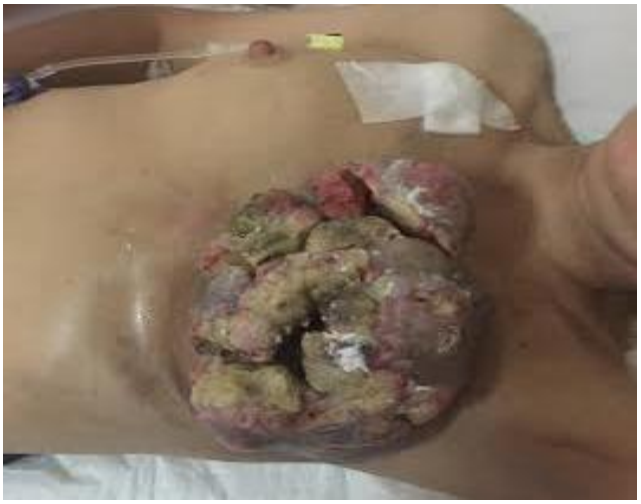

Figure 2: Lump with no response after 6 cycles of chemotherapy

Reevaluation, lump located centrally in the left breast with an ulcer, poorly defined border, measuring about 14 centimetres across, nipple areola complex was involved without fixation to the chest wall (Figure 1).

Enlargement of the left lymph node was apparent and multiple. So, based on TNM staging, she diagnosed stage IV breast cancer (T4bN2M1). She continued with Docetaxel 65 mg/m^2 + Capecitabine about 2 cycles.

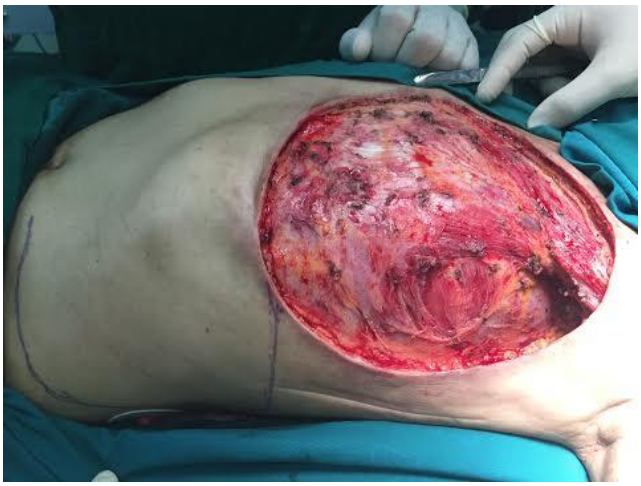

Figure 3: Wide defect of skin and soft tissue after radical mastectomy

Restaging post-chemotherapy course was complicated. She getting worse and no response to chemotherapy was noticed. The wound became smell odour (Figure 2).

Laboratory findings result in anaemia and hypoalbuminemia. We decided to perform palliative radical mastectomy to improve quality of life. Primary skin closure was not possible due to the wide defect of skin and soft tissue (Figure 3).

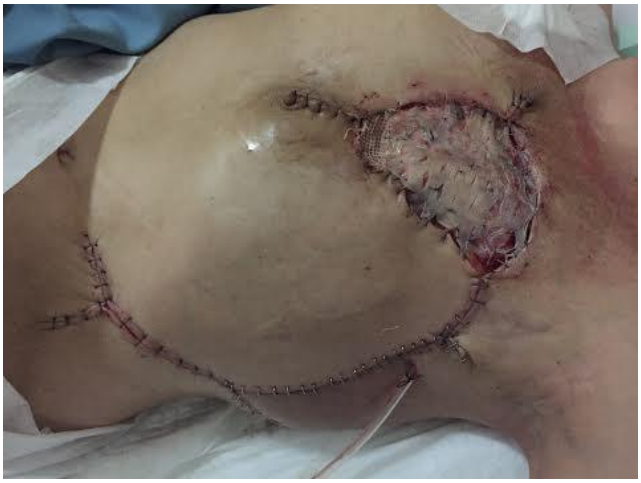

Figure 4: The defect was covered by TA flap and split-thickness skin graft on the medial side

Considering the patient's condition was not in an optimum state, we have to choose simple action for reconstruction. We decided to use thoraco-abdominal (TA) flap to cover the defect and defect on

the medial side we used a split-thickness skin graft from the left thigh (Figure 4).

Histopathology result was compatible with SCC (Figure 5). After the operation, she started three cycles of chemotherapy Gemcitabine 1800 mg, and Carboplatin 600 mg then continue with Tamoxifen 20 mg daily and Goserelin acetate 3.6 mg per month.

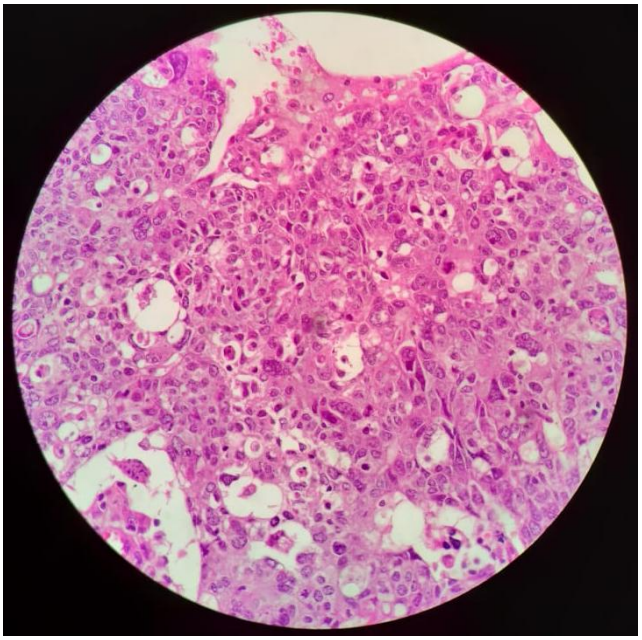

Figure 5: Histopathology result

The TA flap was viable, and there was no complication (Figure 6). The wound was well healed after two months of the operation (Figure 7).

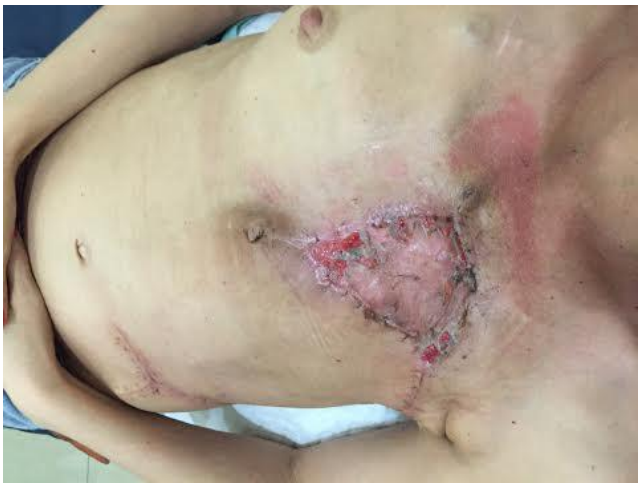

Figure 6: Wound healing after three weeks of operation

Discussion

This patient in the early had diagnosed as low-differentiated breast cancer, luminal type-B and has the change to be SCC after mastectomy. This is a primary cause of SCC of the breast.

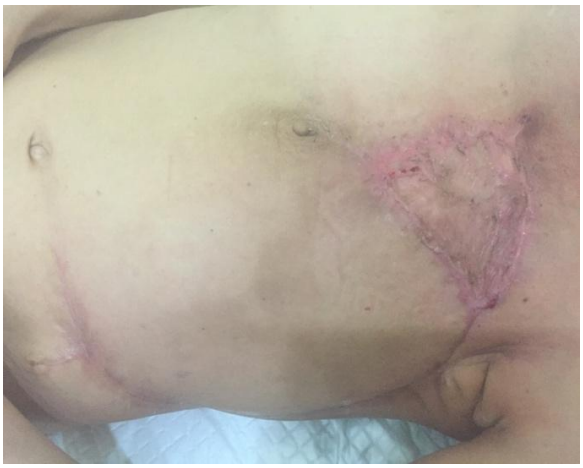

Figure 7: The scar slowly disappeared after two months of operation

SCC of the breast is the tumour of the elderly group [3]. Based on the literature, tumours frequently huge and can expand more than 5 cm in diameter. This case was 48 years old woman and the presented lesion measured 14 x 11 x 8.5 cm and would probably be the first case of this size. In Menes et al., study, SCC was associated with a lower risk of lymph node metastasis at the tumour site and a significant risk of distant metastasis without lymph node involvement [4]. In our case, she had distant metastatic to the lung without involvement malignant metastatic of the lymph node.

The result of histopathology examination on the removed tumour was SCC grade III. No metastatic deposit on 14 lymph nodes observed. Microscopically, the lump is consisting of malignant epithelium, invasive into connective tissue, partially covered by the epidermis. Mitotic index more than 20. No evident of intralymphatic and intravascular invasion. There also some intraductal carcinoma.

Approximately 30% of the patients with SCC of the breast will spread to other organs [5]. The treatment of pure SCC of the breast is unclear. Dejager et al. showed that cisplatin chemotherapy could be thought over in the chemotherapy regimen. In this type of cancer, Weigel et al. recommended the combination treatment using radiotherapy, because SCCs are radiosensitive. The small size of the primary SCCs of the breast could be managed with lumpectomy and axillary dissection followed by radiotherapy [6].

A lump of this patient was 14 cm, and she had a metastatic process on her left lung. The patient had chemotherapy like an adenocarcinoma in the same stage. After chemotherapy 6 cycles, pleural effusion on the lung was disappeared, but the lump had no response to chemotherapy and getting worsen with unstable laboratory result. The patient got anaemia

and hypoalbuminemia at the end of 6 cycles of chemotherapy. Radical mastectomy was performed to excision the lump. Surprisingly, histology of removal tumour after mastectomy showed differences in the result. The results were SCC which might be the reason why the lump did not respond to chemotherapy. Because the wide defect produced during surgery, and considering this patient was not in her optimum state, we have already chosen thoraco-abdominal (TA) flap as a simple flap for this patient.

The TA flap is a simple flap and suitable for quick in-quick outpatient. TA flap is a rotation advancement fasciocutaneous flap. This flap uses the skin and subcutaneous tissue of the anterior abdominal [7]. This flap used a mark of two sets of direct perforating segmental arteries. The medial border was arising from the deep epigastric arcade at the lateral border of the rectus abdominis. The lateral border was arising from the lumber and subcostal arteries at the level of the anterior border of the latissimus dorsi [8]. Sub fascia anastomoses are present between the medial and lateral perforators. The border of the flap is anterior axillary line laterally for medially based flap and midline medially for the laterally based flap with the horizontal plane at the level of anterior superior iliac spine inferiorly [9]. To cover the defects on the medial side of the TA flap, a split-thickness skin graft from the left thigh was used. We used two vacuum drains in the axilla and under the TA flap. The skin closure used skin stapler and 2-0 nylon suture. This operation was taken 4 hours long.

The prognosis of SCC of breast cancer was still controversial [10]. Several cases showed a slowly clinical presentation and had a good prognosis. But some studies showed SCC of the breast had an aggressive clinical presentation, and the outcome was similar to poorly differentiated breast cancer [4]. In this case, she is still in our routine follow up and started on three cycles adjuvant chemotherapy then continue with hormonal therapy Tamoxifen 20 mg daily and Goserelin acetate 3.6 mg per month. The lung metastasis was relapsed in 13 months follow up after surgery.

In conclusion, pure SCC of the breast is an extremely unusual malignancy, so a primary SCC of the breast is an extraordinary phenomenon. Based on the literature, outcome and appropriate approach for treatment is still controversial. Every new case report would help to determine the right management to this disease.

In this case, we had got different histopathology finding after mastectomy. Learn from here; we should think probability another diagnosis when we accept a new case from the patient who had been treated by a doctor before, moreover without improvement of the disease. For surgery approach, TA flap can be the choice in patients with a wide defect of skin and soft tissue after a radical mastectomy. In view of its simplicity and shorter operative time, TA flap is an ideal option for quick in-quick outpatient.

Acknowledgement

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Dental Science

Clinical Behaviour and Marginal Sealing of Bulk-Fill Resin Composite Restorations Using Light Amplified High-Intensity LEDs Curing: A Randomized Controlled Clinical Trial

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Keywords: LAHI LED curing; Bulk-fill; Clinical performance; Marginal sealing

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Abstract

BACKGROUND: Delivering sufficient intensity output of curing lights is mandatory to ensure optimum cure and clinical success of bulk-fill resin composite restorations and to avoid undesirable clinical outcomes.

AIM: To evaluate the effectiveness of using light amplified high intensity LED curing on the clinical performance and marginal sealing of posterior bulk-fill resin composite restorations.

MATERIAL AND METHODS: This study was designed as a randomised, controlled, double-blind, Unicenter, parallel, two arms, superiority trial with 1:1 allocation ratio. Adult patients who required posterior tooth-coloured restorations were asked to participate in this trial. All participants signed written informed consent after being completely aware of the settings of the study. The participants who fulfilled the eligibility criteria were divided into two groups according to the type of light curing mode used. Adhesive compound proximal cavities were prepared. All restorative materials were applied according to the respective manufacturer's instructions. Assessments of the restorations were done at baseline (one week after placement of the restoration), after 6 months and after 12 months using the modified US Public Health Service (USPHS) criteria. For quantitative assessment of the marginal sealing, resin replicas were analysed using scanning electron microscopy. Statistical analysis was done using Chi-square, Mann Whitney, independent t-test and dependent t-tests.

RESULTS: There were no statistical differences between the two groups for the tested clinical parameters along the study periods. For marginal analysis, there were no statistical differences between the intervention and control group at baseline and six months (p-value = 0.347 and 0.516) respectively. At 12 months the control group showed statistically significant higher percentages (p-value = 0.031).

CONCLUSION: Light amplified high-intensity curing units have clinical performance comparable with the conventional LED.

Introduction

The improvements in resin composite formulations and newer generation bonding agents, as well as the increasing demand for esthetics, have made composite resin restoration the optimal choice for restoring posterior teeth. Several efforts have been continuously made to improve its physical and mechanical properties and to simplify its application techniques [1], [2]. Application of resin composite restorations requires a dry field to guarantee effective enamel and dentin etching, priming, and bonding. The application has usually been incremental, with the maximum incremental thickness of two millimetres, to ensure complete curing of resin composites, in addition to minimisation of the polymerisation shrinkage and associated shrinkage-induced stress [3].

A new resin composite material class has been introduced in the past years relying upon bulk-fill technology. These newly introduced bulk-fill resin composites have additional light penetration and deeper cure depth properties due to both increased translucency and developments in photoinitiator dynamics, so they can be used to fill cavities up to 4-5 mm at once allowing for more convenient procedure and reducing the operatory times required for

restoration of large cavities [4], [5], [6].

Delivering sufficient intensity output of curing lights is mandatory to ensure the longevity of restorations and to avoid undesirable clinical outcomes. Light-emitting diodes (LEDs), which were introduced in the late 1990s, are becoming increasingly popular for curing of resin-based restorations. Although first-generation LEDs had power density ranged from 160 to 400 mW/cm^2, newer generations LED curing units had higher power intensities, ranging from approximately 500 to 1,400 mW/cm^2 [7], [8].

Therefore, it is of prime importance to assess the effect of using light amplified high intensity LED curing (1400 mW/cm^2) on the clinical performance and marginal sealing of posterior bulk-fill resin composite restorations.

Material and Methods

Trial registration

The trial was designed following the SPIRIT 2013 Statement (Standard Protocol Items: Recommendations for Interventional Trials) and approved from Evidence-Based Dentistry Committee (EBD) – Faculty of Dentistry, Cairo University (Date of approval: 13-7-2015). The trial was registered in the Pan African Clinical Trial Registry (PACTR). The identification number for the registry was PACTR201609001768135 (Date of approval: 22-9-2016). The ethical issues of this trial were reviewed and approved by the Research Ethics Committee (REC) – Faculty of Dentistry, Cairo University (Approval no. 1579/Date of approval: 27-7-2015).

Sample size calculation

Based on a previous study [9], the secondary outcome variable is normally distributed (marginal sealing by µm), and an approximately 0.21 Cohen F effect size was expected which was the standard deviation of the population means divided by their common standard deviation. A total sample size of 38 was required. This number had been increased to a total sample size of 44 (22 per group), to adjust for using a nonparametric test. This sample would be sufficient to detect an effect size of 0.21, a power of 80%, and a significance level of 5%. The sample size was calculated using the G*Power program (University of Düsseldorf, Düsseldorf, Germany).

Participants' recruitment

During the period from 3rd December 2016 to 22nd April 2017, adult patients attending the conservative dentistry clinic, Faculty of Dentistry – Cairo University who required posterior tooth-coloured restorations were asked to participate in this trial. Medical and dental histories were carefully assessed. Thorough extra- and intra-oral examinations of the volunteers were performed and recorded in the diagnostic chart to identify volunteers fulfilling the eligibility criteria of the trial which were determined from previous studies [10], [11], [12], [13]. The inclusion and exclusion criteria are listed in Table 1.

Table 1: Eligibility criteria of the trial

Inclusion criteria	Exclusion criteria
Patient inclusion:	*Patient exclusion:*
1-Patients ageing ≥18 years.	1-Participants with general/systemic illness.
2-Patients with a high level of oral hygiene.	2-Pregnant or lactating females.
3-Patients have at least 12 posterior teeth in occlusion.	3-Concomitant participation in another research study.
4-Patients with a good likelihood of recall availability.	4-Inability to comply with study procedures.
	5-Heavy bruxism habits.
	6-Last experience with allergic reactions against any components of the used materials.
Tooth inclusion:	7-Patients are receiving orthodontic treatment.
1-Permanent premolars or molars.	*Tooth exclusion:*
2-Moderate to deep compound class II cavities.	1-Teeth with clinical symptoms of pulpitis such as spontaneous pain or sensitivity to pressure.
3-Primary carious lesions.	2-Non-vital teeth.
4-Vital with the positive reaction to a cold thermal stimulus.	3-Secondary carious lesions.
5-Well-formed and fully-erupted in normal functional occlusion with the natural antagonist and adjacent teeth.	

Participants' grouping

All volunteers who gave their written informed consent for participation and fulfilled the eligibility criteria were randomly assigned using computer-generated randomisation *www.random.org* to either intervention (Light amplified high intensity (LAHI) LED curing) or control (Low intensity LED curing) groups

Preoperative assessments

The pulp sensibility was assessed with a cold test using (Endo-Frost, Coltène/Whaledent GmbH+ Co. KG; Langenau, Germany). After cold application on the centre of the buccal surface of the examined tooth for 10 seconds, participants were expected to answer positively to this test providing a short and transient pain response.

A digital periapical radiograph was taken before restorative procedures to assess the degree of approximation of carious lesion to a pulp, intactness of lamina dura and/or presence of any periapical radiolucency. A digital bitewing radiograph with 1:1 ratio was further taken. The carious cavities had to be at least 3 mm deep All radiographs were taken using (FONA XDC, FONA SRL; Assago, Italy) and processed by (Sordex DIGORA Optime, KaVo; Charlotte, NC, USA)

Cavity preparation

The operative field was isolated by the

application of medium consistency latex rubber dam sheet (Sanctuary Dental Dam, Sanctuary Health SDN BHD; Malaysia). Local anaesthetic agent; articaine hydrochloride 4% with 1/100 000 epinephrine (Septanest SP, Septodont; Saint-Maur des Fossés, France) was administrated before cavity preparation to control patient discomfort during the restorative procedures. The adhesive cavity preparation design was employed according to the principles of minimally invasive dentistry. All cavities were prepared by the same operator (S.E.F). The cavities were prepared using #330 and #245 carbide burs (Komet®, Brasseler; Lemgo, Germany) rotating in high-speed handpiece (COMFORTdrive 200 XD, KaVo Dental; Fruehauf, Germany) with copious amounts of water coolant. Remaining soft caries -if present- were removed using sharp excavator (Maillefer, Dentsuply; Switzerland). Control of the depth of the prepared floor was done by visual inspection and confirmed by probing with a sharp explorer to assess the hardness of discoloured underlying dentin. The operator measured the depth of the prepared cavities with a periodontal probe.

Restorative procedures

All restorative materials were applied according to the respective manufacturer's instructions. The restorative materials used as well as their descriptions, compositions, manufacturers and lot numbers were listed in Table 2.

Table 2: Materials' specifications, compositions, manufacturers and lot numbers

Material	Specification	Composition	Manufacturer	Lot number
Vococid	Etching gel	Orthophosphoric acid (34.9%).	Voco, Cuxhaven, Germany *www.voco.com*	132704
Futurabond M+	Universal Adhesive	Liquid A: methacrylic phosphorus acid ester and carbonic acid modified methacrylic ester Liquid B: water, ethanol, silicon ph = 1.4	Voco, Cuxhaven, Germany *www.voco.com*	1612531
X-tra fil	Bulk-fill posterior resin composite	Resins: Bis-GMA(1) UDMA(2) TEGDMA(3) Bis-EMA(4) PEGDMA(5)-Fillers: (Combination of non-agglomerated/nonaggregated silica filler, zirconia filler, zirconia/silica cluster filler	Voco, Cuxhaven, Germany *www.voco.com*	1612219
Ionoseal	Light-curing glass ionomer cement	-Fluoro-aluminosilicate glass -An acrylic acid copolymer containing pendant methacryloxy groups, HEMA	Voco, Cuxhaven, Germany *www.voco.com*	1645424
Impregum™ Soft	Polyether impression material	*Base:* -Prepolymer-ethylene amine -Inert filler silica -Plasticiser glycol ether *Catalyst:* -Ester derivatives of aromatic sulphonic acid - Plasticiser phthalate -Thinner-octyl phthalate -Methyl cellulose	3M ESPE, Neuss, Germany *www.3mespe.com*	665796

(1) Bis-GMA: Bisphenol A diglycidylmthacrylate; (2) UDMA: Urethane dimethacrylate; (3) TEGDMA: Triethyleneglycoldimethacrylate; (4) Bis-EMA: Bisphenol A polyethylene glycol dietherdimethacrylate; (5) PEGDMA: Polyethyleneglycoldimethacrylate.

All cavities were restored using sectional matrix system (Palodent® Plus, DENTSPLY Caulk, Milford, DE, USA) to reestablish the interproximal contacts of the teeth. Light-curing glass ionomer cement was applied as a liner in very deep cavities (≥ 5 mm) over the deepest area in the prepared cavity and cured for 20 seconds using Dr's light AT CL-AT24 light curing system (Good Doctors Co., Ltd., Korea) with a light intensity of 650 mW/cm^2. The enamel surfaces of each prepared cavity were etched with 34.9% phosphoric acid for 15 seconds. The cavity was then thoroughly rinsed with air-water spray for 15 seconds; the excessive water was then removed, using oil-free air, to avoid dehydrating the dentin. The bonding system was applied once using a micro brush in rubbing motion for 20 seconds, followed by gentle blow of oil-free air for 10 seconds then light cured for 10 seconds with a low light intensity of 650 mW/cm^2.

The bulk-fill resin composite was available in one semi-translucent universal shade. The material was applied as one increment into the prepared cavity then light-cured in either high-intensity mode (1400 mW/cm^2 for 5 seconds) or low-intensity mode (650 mW/cm^2 for 20 seconds). The light was directed perpendicular to the occlusal surface. The light output of the curing unit was monitored with a light meter (Curing Radiometer Model 100; Demetron Corp, USA). The occlusal adjustment was performed with carbon articulating paper (HANEL Articulating Paper, Coltène/Whaledent GmbH + Co. KG; Langenau, Germany) to establish appropriate occlusal morphology and contacts. The quality of interproximal contacts and cervical adaptation was checked using dental floss. Finishing and polishing of all restorations were done under water cooling with fine-grit diamond burs, polishing discs and strips (Sof-Lex, 3 M, St. Paul, MN, US). Abrasive strips were used (3M ESPE, St Paul, MN, USA) on the proximal surfaces.

Clinical evaluation of the restorations

Assessments of the restorations were done at baseline (one week after placement of the restoration), after 6 and 12 months using the modified US Public Health Service (USPHS) criteria [14], [15] for the following parameters; marginal discolouration, marginal adaptation, secondary caries and postoperative sensitivity. The criteria are listed in Table 3.

Table 3: Modified USPHS criteria

Criteria	Test procedure	Score		Criteria
		Accepted	Unaccepted	
Marginal discolouration	Visual inspection with a mirror at 18 inches	0		No discolouration evident
		1		Slight staining can be polished away
			2	Gross staining cannot be polished away
Marginal adaptation	Visual inspection with explorer and mirror, if needed	0		Continuity between the restoration and the tooth surface. The explorer tip does not engage at the interface
		1		The explorer tip does engage at the interface, but no gap is visible
		2		Gap at tooth/restoration interface, exposed enamel
			3	Gap at tooth/restoration interface exposed dentin
Secondary caries	Visual inspection with explorer and mirror, if needed	0		No caries present adjacent to the restoration margins
			1	Caries present adjacent to the restoration margins
Postoperative sensitivity	Ask patients	0		Not present
		1		Sensitive but diminishing in intensity
			2	Constant sensitivity, not diminishing in intensity

Two independent assessors (M.A.M & M.H.A) who had no preliminary information about the type of interventions evaluated the restorations.

Disagreements between examiners over assessments were solved by reexamination of the restorations, and a consensus was obtained through discussion between examiners.

Assessment of marginal sealing

For quantitative assessment of the marginal sealing, impressions were taken at the predetermined periods (baseline, after 6 and 12 months) using custom-constructed sectional trays with polyether impression material (impregnate Soft, 3M ESPE) and then poured with epoxy resin (Kemapoxy 150, CMB International; Khofo Gate Hdaek El-Ahram, Giza, Egypt) to obtain epoxy replicas. Scanning electron microscopy (SEM) Model Quanta™ 250 FEG (Field Emission Gun) attached with EDX Unit (Energy Dispersive X-ray Analyses), with accelerating voltage 30 K.V. at a magnification of 200×. The values are expressed as a percentage of the continuous margin over the total margin length for the occlusal margins [16], [17]. The marginal analyses were performed by an evaluator (H.M.T) who was blinded to the interventions.

Statistical methods

For the results of clinical evaluation of the restorations; data showed nonparametric distribution, the Chi-square test was used to compare between groups and follow-up periods. Mann Whitney test was used to compare between tested light curing modes. For the results of the assessment of marginal sealing; data showed parametric distribution, so independent t-test was used to compare between tested groups. A dependent t-test was used to compare between follow-up periods in each group ($\alpha = 0.05$). Statistical analysis was performed with IBM® SPSS® (SPSS Inc., IBM Corporation, NY, USA) Statistics Version 25 for Windows.

Results

In this study, a total of 44 restorations were placed in the cavities of 36 patients and re-evaluated for 6 and 12 months. Thirty patients had one restoration while four patients had two restorations and two patients had three restorations. In the first recall after 6 months, 42 restorations in 34 patients were evaluated (one restoration from each group was lost). After 12 months, another evaluation was performed, and two more restorations from the intervention group and one from the control group were lost. The study model employed was illustrated in Figure 1.

Of the 44 restorations that were placed, 45.5% were in the maxilla, 54.5% were in the mandible, 40.9% were in the premolar teeth, and 59.1% were in the molar teeth. Table 4 presents an overview of the distribution of the restorations according to the type of tooth and arch.

Table 4: Distribution and tooth locations of the restorations

	Premolars	Molars	Total
Maxillary	8	12	20
Mandibular	10	14	24
Total	18	26	44

The volunteers who participated in the study comprised 21 females (61.4%, restorations = 27) and 15 males (38.6%, restorations = 17) ranging in age from 19 to 32 years (median age was 21.6 years).

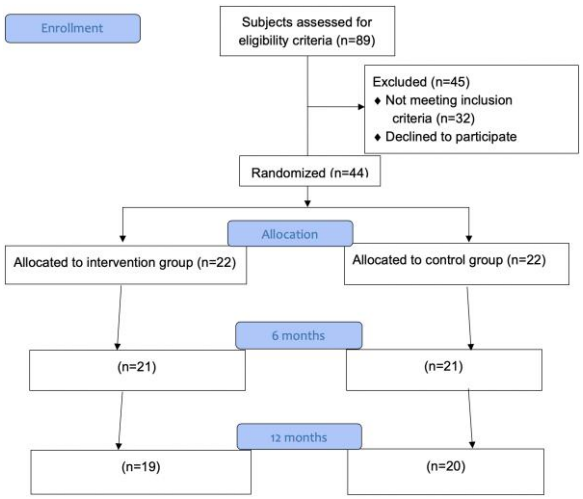

Figure 1: Flowchart of the model under study

Results of clinical evaluation of the restorations (Marginal discolouration)

At baseline, all restorations showed *score 0* (100%). After 6 months, 19 restoration of the intervention group (LAHI) representing 90.5% showed *score 0* while 2 restorations representing 9.5% showed *score 1*. However, 20 restorations of the control group (low intensity) representing 95.2% showed *score 0* while 1 restoration representing 4.8% showed *score 1*. No statistical differences did exist between the two groups (p-value = 0.5539).

Table 5: Frequency (N) and percentage (%) for marginal discolouration

			LAHI		Low		p-value
			N	%	N	%	
Marginal Discoloration	Baseline	0	22	100.0%	22	100.0%	1.00 NS
		1	0	0.0%	0	0.0%	
		2	0	0.0%	0	0.0%	
	6 Months	0	19	90.5%	20	95.2%	0.5539 NS
		1	2	9.5%	1	4.8%	
		2	0	0.0%	0	0.0%	
	12 Months	0	17	89.5%	19	95.0%	0.5228 NS
		1	2	10.5%	1	5.0%	
		2	0	0.0%	0	0.0%	
p-value			0.3060 NS		0.5740 NS		

NS = Non-Significant.

After 12 months, 17 restorations of the intervention group (LAHI) representing 89.5% showed *score 0* while 2 restorations representing 10.5% showed *score 1*. However, 19 restorations of the control group (low intensity) representing 95.0% showed *score 0* while 1 restoration representing 5.0% showed *score 1*. No statistical differences did exist between the two groups (p-value = 0. 5228). The frequency (N) and percentage (%) of scores of marginal discolourations were presented in Table 5 and Figure 2.

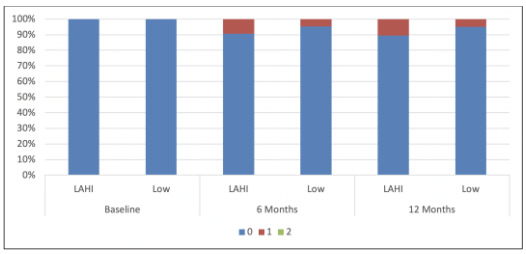

Figure 2: Stacked bar chart showing the marginal discolouration scores

Results of clinical evaluation of the restorations (Marginal adaptation)

For both intervention (LAHI) and control (low intensity) groups, all restorations (100.0%) showed *score 0* at different evaluation periods.

Table 6: Frequency (N) and percentage (%) for marginal adaptation

			LAHI		Low		p-value
			N	%	N	%	
Marginal Adaptation	Baseline	0	22	100.0%	22	100.0%	1.00 NS
		1	0	0.0%	0	0.0%	
		2	0	0.0%	0	0.0%	
		3	0	0.0%	0	0.0%	
	6 Months	0	21	100.0%	21	100.0%	1.00 NS
		1	0	0.0%	0	0.0%	
		2	0	0.0%	0	0.0%	
		3	0	0.0%	0	0.0%	
	12 Months	0	19	100.0%	20	100.0%	0.8728 NS
		1	0	0.0%	0	0.0%	
		2	0	0.0%	0	0.0%	
		3	0	0.0%	0	0.0%	
p-value			0.8932 NS		0.9535 NS		

NS = Non-Significant.

No statistical differences did exist between the two groups at baseline (p-value = 1.00), 6 months (p-value =1.00) and 12 months (p-value = 0.8728). The frequency (N) and percentage (%) of scores of marginal adaptations were presented in Table 6 and Figure 3.

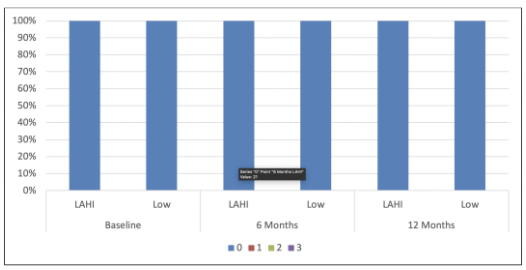

Figure 3: Stacked bar chart showing the marginal adaptation scores

Results of clinical evaluation of the restorations (Secondary caries)

For both intervention (LAHI) and control (low intensity) groups, all restorations (100.0%) showed *score 0* at different evaluation periods.

Table 7: Frequency (N) and percentage (%) for secondary caries

			LAHI		Low		p-value
			N	%	N	%	
Secondary Caries	Baseline	0	22	100.0%	22	100.0%	1.00 NS
		1	0	0.0%	0	0.0%	
	6 Months	0	21	100.0%	21	100.0%	1.00 NS
		1	0	0.0%	0	0.0%	
	12 Months	0	19	100.0%	20	100.0%	0.8728 NS
		1	0	0.0%	0	0.0%	
p-value			0.8932 NS		0.9535 NS		

NS = Non-Significant.

No statistical differences did exist between the two groups at baseline (p-value = 1.00), 6 months (p-value = 1.00) and 12 months (p-value = 0.8728). The frequency (N) and percentage (%) of scores of secondary caries were presented in Table 7 and Figure 4.

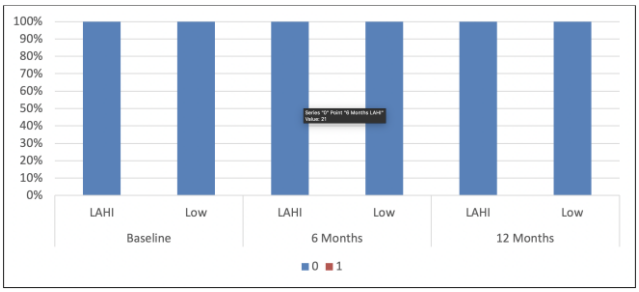

Figure 4: Stacked bar chart showing the secondary caries scores

Results of clinical evaluation of the restorations (Postoperative sensitivity)

At baseline, 19 restorations of the intervention group (LAHI) representing 86.4% showed *score 0* while 3 restorations representing 13.6% showed *score 1*. However, 18 restorations of the control group (low intensity) representing 81.8% showed *score 0* while 4 restoration showed *score 1* representing 18.2%.

Table 8: Frequency (N) and percentage (%) for postoperative sensitivity

			LAHI		Low		p-value
			N	%	N	%	
Postoperative sensitivity	Baseline	0	19	86.4%	18	81.8%	0.6837 NS
		1	3	13.6%	4	18.2%	
		2	0	0.0%	0	0.0%	
	6 Months	0	20	100.0%	20	100.0%	1.00 NS
		1	0	0.0%	0	0.0%	
		2	0	0.0%	0	0.0%	
	12 Months	0	19	100.0%	20	100.0%	0.8728 NS
		1	0	0.0%	0	0.0%	
		2	0	0.0%	0	0.0%	
p-value			0.0569 NS		0.0187*		

*Different letters within each column indicate a significant difference; NS = Non-Significant, * significant.*

No statistical differences did exist between the two groups (p-value = 0.6837). After 6 and 12 months, all restorations in both groups showed *score*

0. The frequency (N) and percentage (%) of scores of postoperative sensitivities were presented in Table 8 and Figure 5.

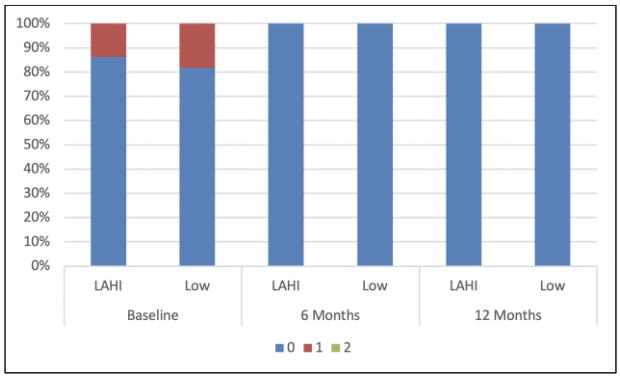

Figure 5: Stacked bar chart showing the postoperative sensitivity scores

Results of the assessment of marginal sealing

The values are expressed as a percentage of the continuous margin over the total margin length for the occlusal margins Figure 6. At baseline, the intervention group showed statistically non-significant lower mean marginal sealing values (93.98 ± 3.58 %) than the control group (94.89 ± 2.77 %) (p-value = 0. 347).

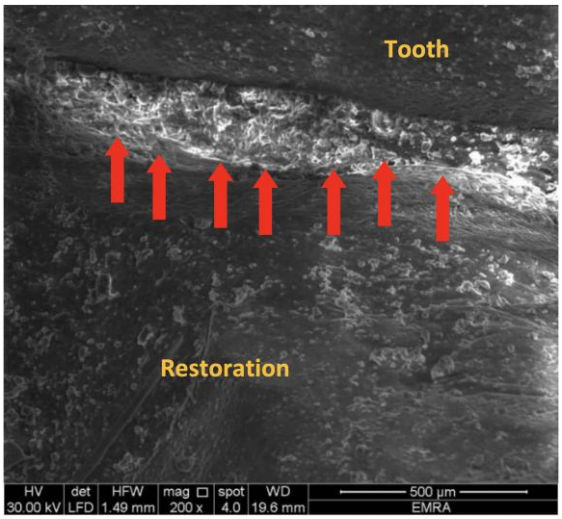

Figure 6: Representative SEM 200× image of non-continuous margins (arrows)

After 6 months, the intervention group showed statistically non-significant lower mean marginal sealing values (91.44 ± 3.30 %) than the control group (92.89 ± 2.94 %) (p-value = 0. 516).

Table 9: Mean and standard deviation (SD) for a percentage of marginal sealing

		LAHI		Low		p-value
		Mean	SD	Mean	SD	
% of Continuous margins	Baseline	93.98	3.58	94.89	2.77	0.347 NS
	6 Months	91.44	3.30	92.08	2.94	0.516 NS
	12 Months	87.22	3.19	89.39	2.72	0.031*

NS = Non-Significant; = significant.*

After 12 months, the intervention group showed statistically significant lower mean marginal sealing values (87.22 ± 3.19 %) than the control group (89.39 ± 2.72 %) (p-value = 0. 031). The mean and standard deviation (SD) for a percentage of marginal sealing were presented in Table 9 and Figure 7.

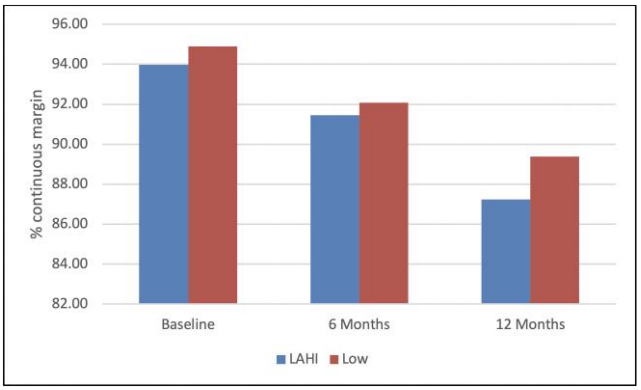

Figure 7: Bar chart showing the percentage of continuous margins for tested groups

Discussion

The use of resin composite as a restorative material for class I and II cavities has become a popular option in daily practice. However, the placement of posterior resin composite restorations has many limitations. Among these limitations are the increased time required for incremental packing in deep cavities and increased incidence of postoperative sensitivity [18]. Although several major developments in resin composite formulations and light curing units have been done to overcome these limitations, there is limited good quality in vitro research regarding the effect of light curing intensity while clinical trials are scarce apart from a few studies [19]. Long term results with some of these newly developed lights curing units are lacking and remain controversial as studies report inconsistent clinical results. There is a paucity of well-conducted clinical trials assessing the clinical effectiveness of different light curing intensities. The present prospective clinical trial was conducted to evaluate the clinical performance and marginal sealing of bulk-fill resin composite restorations using different light curing intensities.

The USPHS criteria had served well for clinical evaluation of different types of dental restorations. However, the sensitivity of these criteria in the short term and medium-term clinical evaluations (< 3-5 years) was questioned. The USPHS system lacks the sensitivity to record small early changes, therefore, in 2007 the FDI published new recommendations, which were updated in 2010, for conducting clinical studies of dental restorative materials with detailed assessment criteria [20], [21].

Despite the increasingly wide use of FDI criteria, USPHS criteria were used in this study because they are still being used in the clinical researches more than FDI criteria [22]. Only outcomes that may be related to polymerisation stress effects on restorations were chosen for clinical evaluation in the current study. These outcomes include marginal discolouration, marginal adaptation, secondary caries, and postoperative sensitivity [23], [24].

Among the 36 volunteers participating in this study, 5 participants did not complete the study. The recall rate was 95.5% at 6 months and 88.6% at 12 months, which are comparable to the rates reported in similar clinical trials [13], [25], [26]. The dropout rate (11.4%) did not increase the risk of attrition bias because the sample size was calculated allowing for losses of around 25%.

The first 6 up to 24 months are considered the critical period for evaluation of deteriorations of resin composite restorations [10]. Although the present study can be criticised that the duration of follow up (12 months) is insufficient to confirm long-term suitability of the tested light curing intensities. However, the obtained clinical findings may provide an initial indication of their clinical performance.

The potentially deleterious effects of polymerisation stress, despite the absence of clear evidence, remain clinically meaningful. Several modifications have been made in the monomer and composite organic matrix of bulk-fill resin composites to allow optimal curing efficiency. These include increased translucency [27]; increased flowability [28]; incorporation of “booster” photoinitiators and polymerisation modulators [8]. These modifications have been reported to reduce polymerisation shrinkage stresses up to 70% [29], [30], [31].

In this study, none of the evaluated restorations over 12 months showed manifestations of clinical failure. All restorations recorded clinically ideal or accepted scores (score 0 and 1). The marginal discolouration is one of the early clinical signs of failure of resin composite restorations. In the current study, the majority of the scores allocated for the marginal discolouration criteria was 0. These results were by the findings of Van Dijken and Pallesen, 2015 [13], Çolak et al., 2017 [26], Yazici et al., 2017 [32]. The slight colour changes observed after 6 and 12 months at the restoration margins were not associated with secondary caries or loss of marginal adaptation. None of the evaluated restorations showed secondary caries at different evaluation periods. This is closely related to the good marginal adaptation of the restorations.

Postoperative hypersensitivity (POH) is one of the common patients’ complaints following resin composite restorations [33]. This occurs as a consequence of polymerisation shrinkage stress [34]. In the present study, only spontaneous postoperative sensitivity was measured. This is by a recent systematic review and meta-analysis [35] that investigated the correlation between the risk and intensity of POH in posterior resin composite restorations and the adhesive strategy. In that review, POH was assessed in approximately 50% of the included studies by asking patients whether they experienced spontaneous POH during a specific time frame. In our study, 3 restorations of the intervention group and 4 of the control group showed score 1 representing 13.6% and 18.2% respectively of the restorations evaluated at baseline. These results were in line with the results obtained by (Costa et al., 2017) who found that overall risk of postoperative sensitivity was 20.3% and typically occurred within 48 hours after the restorative procedure. None of the patients involved in the study reported postoperative sensitivity at 6- and 12-months evaluation point. The lack of long-term sensitivity may be related to the application of resin-modified glass ionomer liner in deep and very deep cavities.

The use of intraoral impressions to fabricate accurate replicas is a challenge. Unless an adjacent tooth is not present, the proximal margins of the restoration can rarely be replicated. Therefore, most studies can only assess the marginal integrity of the occlusal margins of the restoration. However, this is of limited value because caries adjacent to restoration occur more frequently at proximal margins rather than occlusal margins [23]. In the current study, the restorations had marginal deficiencies already at baseline. The percentages increased over time. However, there was no correlation between increasing marginal deficiencies with the clinical performance of this restoration.

Marginal deficiencies may result from excessive polymerisation contraction stresses at tooth-restoration interface [36], [37]. The statistically significant increase in marginal deficiencies over time could also be attributed to slow hydrolysis which causes degradation of the resin/bond interface [38], [39], [40].

In conclusion, the results of the current study revealed no significant differences between the intervention and the control group over different evaluation periods regarding the clinical performance. A significant increase in the percentage of discontinuities at the tooth-restoration interface was observed over the 12-month evaluation period. Based on these findings it could be concluded that light amplified high-intensity curing units have clinical performance comparable with the conventional LED. However, the in vitro assessment of marginal sealing at the tooth-restoration interface has limited clinical relevance. Finally, further well-conducted randomised clinical trials with extended evaluation periods are highly recommended to confirm the findings obtained from the current study.

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Alveolar Cleft Reconstruction Using Different Grafting Techniques

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Abstract

BACKGROUND: Cleft lip and palate CLP is a frequent congenital malformation that manifests in several varieties including unilateral or bilateral anomalies due to either genetic or acquired causes. Alveolar cleft graft ACG remains controversial as regard timing, grafting materials and surgical techniques. The primary goal of alveolar cleft grafting in ACG patients is to provide an intact bony ridge at the cleft site to allow maxillary continuity for teeth eruption, proper orthodontic treatment for dental arch alignment, oronasal fistula closure and providing alar support for nasal symmetry.

AIM: This study aims to compare different grafting techniques to treat the alveolar cleft defect.

METHODS: This study included 24 cases divided into three groups of patients: Group A was treated with autogenous iliac crest bone; Group B was treated with nano calcium hydroxyapatite with collagen membrane and Group C was treated with tissue engineering method using bone marrow stem cells extract and PRF membrane.

RESULTS: According to clinical and radiographic examination measuring bone density in the CT preoperatively compared to six months postoperatively. Group C with bone marrow stem cells extract showed superior results among all followed by group B, while group A with autogenous iliac crest showed resorption in some cases and gave the least values, in addition to its drawbacks as regard donor site affection with pain & scar formation.

CONCLUSION: Bone substitutes as Nano calcium hydroxyapatite and bone marrow stem cells extract showed to be reliable methods for bone grafting than autogenous iliac crest.

Introduction

The alveolar cleft is a bony defect present in 75 % of CLP patients. Repair of the alveolar cleft is mandatory for both function and esthetics especially in syndromic patients with genetic malformations. Although secondary ACG is commonly accepted for these patients, controversy remains regarding the surgical technique and type of grafting material used [1], [2], [3]. A primary alveolar cleft repair usually takes place at an early age of life. Secondary alveolar bone grafting for patients with a cleft involving maxillary alveolus was first advocated by Boyne and Sands in 1972 [4]. The grafting procedures are usually taken around the age of 9-12 years by dental development, most notably at the cleft side of permanent canine as stated by Bergland et al., 1986 [5], [6]. Bone grafting can be performed using either autogenous bone or allogenic bone substitutes. Autogenous bone graft harvested from the iliac crest or rib graft with bone morphogenic proteins BMP has shown success rates. It supports the tooth in the alveolar arch and establish nasal bone morphology and ensures the stability of orthodontic treatment [7]. There are several benefits of bone grafting in patients with alveolar clefts, 1: to obtain arch continuity, which is a universal goal in cleft management. 2: to maximise bone support for dentition. 3: to stabilise the maxillary segments after orthodontic treatment, 4: to eliminate the oronasal

fistulae. 5: to provide alar cartilage support 6: to establish ideal alveolar morphology and 7: to provide available bone with attached soft tissue for future implant placement in cases where there is residual dental space. In order to achieve these objectives, sufficient height and volume of bone must be provided [8], [9]. The concerns associated with an iliac crest or rib harvesting have focused primarily on the possible effects on growth, gait disturbances, hematoma, and donor site morbidity. Most of these complications can be overcome with a careful surgical technique and using allograft materials [10]. With the advent of new biomaterials, which may include or consist of allogenic bone source such as collagen membranes, hydroxyapatite crystals, tricalcium phosphate powder that has been increased consideration for their placement in the repair of alveolar clefts as well as other dental applications [11], [12]. Recently the technique for tissue engineering using bone marrow stem cells BMSC, mesenchymal stem cells MSC extract seeded on a scaffold as polylactic acid PLA, collagen, fibrin, tri calcium phosphate, calcium carbonate used for ACG [13]. BMSC & MSC are self renew cells isolated from the non hematopoietic compartment of bone marrow that can be induced to differentiate into other cells as osteoblasts, chondroblasts and fibroblasts [14]. Growth factors obtained from platelet rich plasma PRP by Chokroun in 2006 aids in tissue healing and accelerates recovery. PRP seems to enhance bone formation in alveolar clefts mixed with graft materials with less rate of postoperative complications. Its autologous, easy to prepare with a low cost that can be used as a source of growth factors [15], [16]. Orthodontic treatment has a major role in dental preparations preoperatively including maxillary expansion and teeth alignment allowing relief of crowding resulting from arch collapse and hypodontia it also creates space needed for the eruption of missing teeth as well as exposure of impacted teeth resulting from the cleft deformity. Cone beam CT is a low dose and effective method of radiological evaluation of the amount of bone defect at cleft side preoperatively measuring height and faciolingual depth. It measures bone density formed postoperatively to evaluate bone quality & quantity compared to the normal side [17], [18].

This study aims to compare different grafting techniques to treat the alveolar cleft defect.

Methods

This study included twenty-four patients with unilateral alveolar clefts who were randomly selected from Orodental Genetics clinic at National Research Center and Oral Surgery clinic at Faculty of Oral & Dental Medicine Al Azhar University. The sample was divided into 3 groups with different grafting techniques each containing 8 patients.

Group A: Included 8 patients who were treated with autogenous bone graft harvested from the iliac crest.

Group B: included 8 patients who were treated with GBR graft of nano calcium hydroxylapatite and collagen membrane.

Group C: included 8 patients who were treated with tissue engineering of bone marrow stem cells extract with the addition of PRP growth factors.

All patients were informed about all the details of the surgery & signed consent. Ethical approval of the scientific committee at the National Research Center was obtained. Preoperative patients preparation included clinical photographs intraorally as well as extra orally. Also digital radiographs including panoramic xrays and multi slice CT measuring the size of the defect. Medical history was recorded excluding any systemic diseases. Blood investigations were made prior to each surgery including hemoglobin level, bleeding profile (bleeding time, clotting time & prothrombin time). Kidney functions (Urea & Creatinine), liver functions (SGOT & SGPT), blood sugar, ECG electrocardiogram and chest examination for each patient.

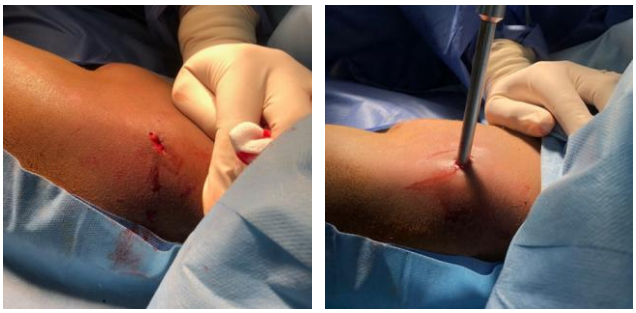

Figure 1: Iliac crest technique (left); Bone marrow stem cells trocar (right)

Surgical procedures: Under GA with full aseptic conditions. A full mucoperiosteal flap was reflected from first premolar region to the central incisor. Separation of oral and nasal layers and closure of fistula was done.

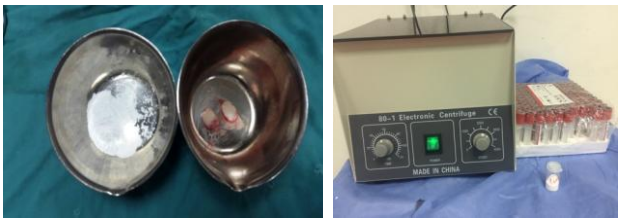

Figure 2: Nano calcium & collagen (left); PRP centrifuge (right)

Group A: Superior anterior iliac spine approach incision at the pelvis with trocar bone particles harvesting minimal invasive rather than traditional chisels and osteotome method. Bone

crushed and placed into the cleft site Figure 1.

Group B: 2 gm of hydroxyapatite powder was placed on the collagen membrane and placed into the defect site Figure 2.

Group C: Stem cells extracted from bone marrow aspirated from iliac crest using a biopsy needle. PRP obtained by citrated 10 cc syringe after being centrifuged for 15 minutes with 2500 rpm speed to separate the plasma portion rich with growth factors and mixed with bone marrow cells aspirate and packed into cleft site Figure 3.

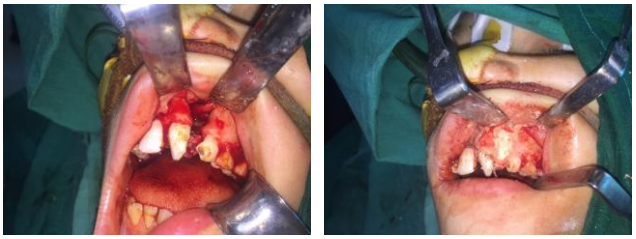

Figure 3: Alveolar cleft defect (left); Alveolar cleft grafting (right)

Following the surgery, all patients were prescribed proper antibiotics, analgesics and anti-inflammatory with oral hygiene instructions and soft food diet. Postoperative clinical evaluation (Figure 4) and radiographic evaluation after 6 months with panoramic x rays (Figure 5) and measuring bone density at graft site from CT and comparing it to the normal side (Figure 6). All data were subjected to statistical analysis.

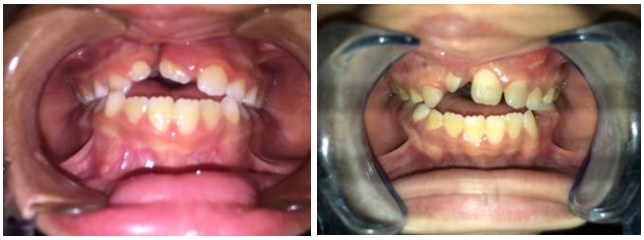

Figure 4: Preoperative cleft site (left); Postoperative cleft site (right)

Statistical methodology

All test data was converted and manipulated by using the SPSS software program version 20.0. Data were analysed, mean and standard deviation, range and median were calculated as regarding the three groups (Autogenous graft of iliac crest, Graft with Nano calcium hydroxyapatite and collagen membrane and Graft with bone marrow stem cells extract) in normal and grafted sides and the mean difference between normal and grafted side. Comparisons between normal and grafted results using paired t-test were made as well as a comparison between subjects undergone different methods of grafting using the t-test. P value was established to determine the statistically significant difference between the two groups. The difference between the two groups was considered statistically significant when $p < 0.05$, and considered highly statistically significant when $p < 0.01$.

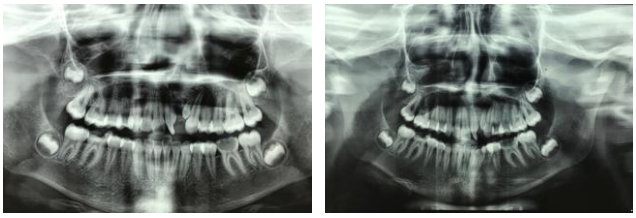

Figure 5: Preoperative panorama (left); Postoperative panorama (right)

Results

This study included twenty-four patients suffering from unilateral cleft and needed alveolar cleft grafting. Group A patients were grafted by autogenous bone harvested from the iliac crest. Group B patients were grafted by nano calcium hydroxyapatite particles and collagen membrane. Group C patients were grafted by bone marrow mesenchymal stem cells extract with platelet-rich plasma PRP membrane as a scaffold. Group C showed superior results with Mean ± SD 242.4 ± 47.8 with statistically highly significant P value < 0.001** followed by group B with Mean ± SD 144.6 ± 51.6 with statistically highly significant P value < 0.00 ** then group A with Mean ± SD 92.5 ± 35.8 and statistically significant P value 0.033* Table 1. Methods of the evaluation were clinically Figure (7 and 8) and radiographically preoperatively and six months postoperatively Figure (9 and 10) using panoramic x-ray and CT Figure (11 and 12).

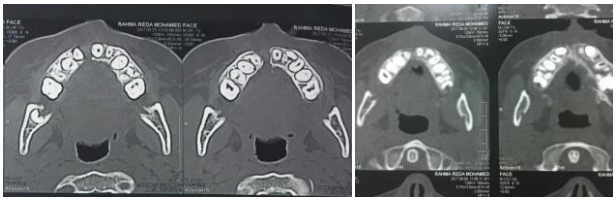

Figure 6: Preoperative CT (left); Postoperative CT (right)

Bone density was measured in the normal noncleft side and compared to the grafted cleft side in Housefield unit HU. Group C grafted side mean ± SD 618 ± 60.2 compared to normal side mean ± SD 375.6 ± 67.9 with P-value statistically highly significant < 0.001 **. Group B grafted side Mean ± SD 539.9 ± 84.5 compared to normal side with mean ± SD 395.3 ± 65.9 with P-value statistically significant < 0.001* And Group A grafted side mean ± SD 461.0 ± 66.3 compared to normal side mean ± SD 368.5 ± 68.3 with P-value highly significant < 0.001* Table 2. Accordingly, the use of bone substitute materials proved to be a reliable method rather than doner site

affecting iliac with scar and pain as well as patient's gait problems in case of autogenous bone. All grafted patients procedures went uneventful. However, group A showed some bone resorption later on while groups B & C showed bone regeneration due to osteoinductive properties of the graft material used.

Table 1: Comparison among 24 cases undergone different methods of grafting according to the mean difference of bone densities of grafted side and normal side

	Group A	Group B	Group C	P
Mean ± SD	92.5 ± 35.8	144.6 ± 51.6	242.4 ± 47.8	< 0.001**
Range (min-max)	46-158	79-245	148-299	
Median	91.5	132	251.5	
P value	0.033*	< 0.001**	< 0.001**	

p value between group A&B #between B&C between A&C; *statistically significant difference p < 0.05; **statistically highly significant difference p< 0.01.

Group A: Autogenous graft of iliac crest bone.

Group B: Graft with Nano calcium hydroxylapatite and collagen membrane.

Group C: Graft with bone marrow stem cells extract and PRF.

Table 2: Comparison between bone density in the normal side and grafted side in 24 cases

	Grafted area Mean ± SD	*Normal side Mean ± SD*	*P*
Autogenous graft of iliac crets bone	461.0 ± 66.3	368.5 ± 68.3	< 0.001*
Nano Ca hydroxy apatite& collagen membrane	539.9 ± 84.5	395.3 ± 65.9	< 0.001*
Bone marrow stem cells extract with PRF	618.0 ± 60.2	375.6 ± 67.9	< 0.001**

** Statistically highly significant difference p < 0.01.

Discussion

Cleft lip and palate is a congenital problem that happens for 1:700 child at birth according to UK studies. The alveolar cleft is a bony defect present in 75 % of CLP patients [1]. Repair of the alveolar cleft is mandatory for both function and esthetics. A primary alveolar cleft repair usually takes place at an early age of life. Secondary alveolar bone grafting for patients with a cleft involving maxillary alveolus was first advocated by Boyne and Sands in 1972 [4]. Secondary Alveolar bone grafting between the ages of 9-11 years is a routine procedure for children with cleft involving the alveolus [19]. The main advantages can be summarised as follows: stabilisation of the maxillary arch, allowing eruption of the canine and sometimes the lateral incisor, providing bony support for adjacent teeth, oroantral fistula closure and raising the alar base [20]. Von Eisenberg in 1901 & Lexer in 1908 was the first to use autogenous bone graft in the maxillary alveolar cleft. Iliac crest donor site seems to be the most preferred by surgeons however there are possible complications from the iliac crest as excessive blood loss, haematoma, delayed wound healing, pain lasting for two weeks to two months, and painful scars under belts or clothing and hypoesthesia or anaesthesia as observed by patients in the first group A. Radiographic evaluation showed that after 6 months of follow up bone volume loss in some cases. According to Masashi et al., [21] who compared the use of autogenous bone grafting versus using hydroxyl appetite bone combined with collagen membrane in 15 patients and observed that the there was no difference in radiographic results as regard the bone volume formed postoperatively while after 6 months postoperatively he concluded that the group grafted with iliac crest resulted in bone resorption while the group grafted with hydroxylapatite particles resulted in bone formation with no doner site complications with matching results to our current research in the second group B patients. The present study also concluded that using nano hydroxyl apatite on collagen sponge in alveolar cleft grafting was more successful and the same finding occurred with Al Ahmady HH et al., [22] who studied the merits of nano calcium hydroxyapatite with 90% success rate over the autologous iliac crest bone grafting with 70 % success rate in 20 patients divided into two groups during a period of 12 months follow up radiographically. The third group C in this study that was treated with bone marrow mesenchymal stem cells along with PRF membrane showed promising outcome according to its osteoinductive and osteoconductive properties in addition to overcoming the draw backs of standard autogenous method of bone grafting. Bajestan MN et al., [23] also proved that stem cell therapy with bone marrow derived cells can promote regeneration of bone in 18 cleft and trauma patients. He showed that the ability of stem cells to treat large alveolar cleft defects is safe. Our findings in the present study showed the best outcome in group C patients who were treated with tissue engineering technology combined with PRF method [1] as Choukran [15] who stated all the merits of the platelet-derived growth factors.

In conclusion, bone substitutes as Nano calcium hydroxyapatite and bone marrow stem cells extract showed to be reliable methods for bone grafting than autogenous iliac crest.

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Dental Science

Influence of Motivation on Academic Performance among Dental College Students

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Keywords: Motivation; Academic Performance; Learning; GPA; Academic Success; Dental Students; Motivated Strategies for Learning Questionnaire; MSLQ

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Abstract

AIM: This study aimed to assess the influence of motivation on academic performance among dental undergraduate students.

METHODS: A cross-sectional study was carried out among a sample of 187 undergraduate dental students from the main dental colleges in the Riyadh region of Saudi Arabia using an electronic questionnaire. Students' academic performance was measured by their current grade point average (GPA). Motivation was assessed using the Motivated Strategies for Learning Questionnaire (MSLQ), which is a self-report instrument designed to assess students' motivational orientations and learning strategies in college, including goals and value beliefs for the studied program (intrinsic, extrinsic goals orientation and task value), beliefs about their skills to succeed in their studies (control of learning beliefs, self-efficacy for learning and performance), and their anxiety about program tests.

RESULTS: The results showed positive correlations between GPA and the motivation scale ($r = 0.2296$, $p = 0.0019$) and most of its subscales, including self-efficacy for learning performance ($r = 0.2997$, $p = 0.0001$), control of learning beliefs ($r = 0.2305$, $p = 0.0021$) and task value ($r = 0.2243$, $p = 0.0021$). Test anxiety showed negative correlation with GPA ($r = -0.1943$, $p = 0.0100$). Compared to their counterparts, male students, students perceived to be from middle class families and students living with their families were consistently showing significant correlations between GPA and most of the motivation subscales.

CONCLUSION: It can be concluded that motivation for learning can influence the academic performance of dental students. This influence can be affected by factors such as sex, socioeconomic factors and family support of the students.

Introduction

Dental students are usually chosen based on proofing superior cognitive abilities in standardised admissions tests as this showed to be a predictor of college grades [1]. Although this indicator might be helpful in the early years of college studies as it mainly mandates knowledge and cognitive abilities, dental college programs differ from other Academic programs by having extensive pre-clinical and clinical courses that demand other skills such as psychomotor, interpersonal, responsibility and communication skills. Therefore, non-cognitive differences might be useful in accurately predicting academic performance among dental students.

In this regards, many well established theories, such as Vroom's Expectancy theory, Locke's Goal-Setting Theory and Eccles's Expectancy-Value Theory, as well as an extensive body of literature have emphasized the importance of non-cognitive factors in enhancing academic performance [2], [3], [4], [5]. Motivation is one of the key non-cognitive factors in this context, which is linked to progress and achievement behaviours [6], [7]. It also seems to be potential key players in accurately predicting academic performance [8].

The potential importance of motivation in predicting academic performance among dental

students is based on previous research findings that link motivation to the improvement of several academic conducts [9], [10], [11]. This influence seems to be related to the relationship found between high motivation and self-regulation, in which highly motivated students showed to be more capable of planning and mastering their learning processes independently [12], [13], [14]. Also, researchers from multiple disciplines found that students with high levels of motivation have a superior learning outcome compared to their colleagues with lower levels of motivation [11]. Initial research on student learning and performance made a distinction between motivational and cognitive aspects and researched each of these topics in isolation. However, later work in this field recognised the importance of having both motivation and cognitive skills for students to have better academic performance [11].

The significance of this combination in improving academic performance is derived from social–cognitive theories and the notion that it could provide insights into the mechanism of this process as a determinant of how students can effectively and efficiently regulate their learning process and acquire new knowledge [15], [16]. This is because students who have appropriate cognitive skills and motivated enough to engage themselves in self-regulated learning are arguably more capable of viewing the learning duties as intrinsically stimulating and valuable and have both high levels of self-efficacy and capabilities of monitoring their own set goals which lead them to be more persistent with appropriate learning behaviors that eventually boost the learning outcomes [17], [18].

Also, researchers advocate that motivation and self-regulation can be adaptive to specific situations [12], [16]. This could explain why some students perform better than others (between-person variation) or better on some tasks but not on others (within-person variation). The suggested variation in motivations and the resulting behaviours based on the targeted tasks differentiate this prospect from meta-cognitive and self-regulatory abilities in which their nature is to be more stable between tasks. An example of this variation among motivation and learning behaviour is the different levels of motivation usually showed by students when they chose to study an optional subject compared to being forced to study a mandatory one. Another example can be seen when students are asked to do group presentations on interesting topics compared to more formal tasks, such as written reports.

There is some limited evidence related to the medical and nursing disciplines showing that high motivation was correlated with high academic outcome [19], [20], [21]. However, there is no evidence that could be found to show how these theories are applicable in the dental field. Therefore, there is a clear knowledge gap in the dental education field regarding the relationship between motivation and academic performance which need to be addressed. Advancement in this topic can help to teach staff and policymakers in the dental field to determine and focus on the factors that influence students' academic learning and performance. This can also help in facilitating early identification of at-risk students and in improving admission procedures to help select the best candidate who can fit the requirement of the dental programs.

This research aimed to assess the influence of motivational orientations on academic performance. The objectives were to assess, among a sample of dental undergraduate students in Riyadh region of Saudi Arabia, the relationship between student academic performance and several constructs of motivation orientations, including students' goals and value beliefs for the dentistry program (intrinsic, extrinsic goals orientation and task value), their beliefs about their skills to succeed in this program (control of learning beliefs, self-efficacy for learning and performance), and their anxiety about tests in the program.

Methods

Ethical approval for the study was granted from the Ethical Committee at Aston University, United Kingdom. This was a cross-sectional study using a self-report questionnaire assessing the students' Academic performance, demographics and motivational orientations among a sample of dental undergraduate students randomly selected from the main dental colleges in Riyadh region of Saudi Arabia. The estimated numbers of students in these schools were 1030 students. Using a margin of error of 5% and a confidence interval of 85%, the recommended sample size was 173. An extra 20% was added to count for potential non-response. This yielded a required sample of 208 students who were randomly invited to participate in this study. An online questionnaire was designed for this study in which an access link to it was sent to the invited students. A pilot study showed that the questionnaire takes less than 10 minutes to complete, which was explained to the participants in the information sheet attached to the invitation link and at the beginning of the questionnaire. Out of the 208 who were invited to participate in the study, 187 students participated (90%).

Students' academic performance was measured by their current grade point average (GPA), which is a commonly used measure to study undergraduate academic performance by calculating the mean of grades over weighted courses contributing to assessment of the final degree. GPA is also the most common measure for employment and postgraduate admission and is linked to both success

and working status [22]. It also has shown good validity and reliability as a measure of academic performance [23]. In this study, students were asked to provide their GPA, which was in a range of 0 to 5.

The motivation was assessed using the motivation section of the Motivated Strategies for Learning Questionnaire (MSLQ) [24]. The MSLQ is a self-report instrument designed to assess college students' motivational orientations and learning strategies in college. The MSLQ theoretical structure, validity and reliability is well established in the literature [25], [26], [27]. Another key advantage of the MSLQ is that it has been developed using a social-cognitive concept of motivation and learning strategies that are based on the theoretical framework assuming that motivation is a dynamic process that reacts to the surrounding contexts and can vary between different situations [26]. The motivation part of the questionnaire consists of Thirty-one items in Six subscales that assess students' motivational orientations towards intrinsic and extrinsic goal orientation, value beliefs, their beliefs about their skills to succeed, and their anxiety about tests. Students responded to each item using a seven-point Likert scale ranging from 'not at all true of me' to 'very true of me'. Student's motivation level was attained by averaging the item responses in the MSLQ's motivation section. Covariates variables collected for assessment included age, sex, type of home and perceived family socioeconomic status (SES).

The study used the MSLQ which have been tested and used extensively showing acceptability among college students. Also, all the invited students were sent information sheets explaining the study content and the expected time needed for completing the survey as well as explaining clearly that the participation is voluntary and anonymous with the data only accessible by the main researcher. The design of the survey allowed the students to exit the survey at any time, if they wish, without being noticed or identified. After collecting this data, it was kept in a password-protected laptop during the data analysis and then stored safely in a password-protected storage desk.

Stata version 13.1 (STATA Corp, College Station, TX, USA) was used for the analyses. Data was first cleaned and checked for consistency. Then, the MSLQ scale and subscales were constructed by taking the mean of the items that made up that scale. Next, variables were inspected for normality using both graphical and numerical methods to determine appropriate statistical tests, which included using an unpaired t-test and one-way analysis of variance test (ANOVA) to compare the mean scores for motivation strategy for the variables that have two and more independent groups, respectively. Subsequently, correlations between the different MSQL subscales of motivation and GPA were tested. This was carried out for the MSQL subscales first. Then, MSQL subscales were further assessed while stratifying by covariate variables to assess for any confounding effect on the relationship between GPA and motivation subscales.

Results

Differences in the Mean Composite Scores for Motivation Strategy by the Characteristics of Students

Regarding the mean composite scores for motivation strategy among the study participants (Table 1), the results of the independent sample t-tests showed no statistically significant differences of the mean composite scores for motivation strategy between male and female students (p = 0.14). However, the results of one-way analyses of variance (ANOVA) indicate that there were statistically significant differences between the mean composite scores for motivation strategy within the different groups of ages (p = 0.03) and study years (p = 0.004).

Table 1: Comparison of mean composite scores for motivation strategy among the study participants (n=175)

Student characteristics	n	Mean	SD	p-value
Sex				
Male	127	4.81	0.61	0.14 [a]
Female	47	4.96	0.53	
Age				
< 21	48	4.97	0.55	0.03 [b]
21-23	95	4.86	0.58	
> 23	31	4.63	0.60	
Year of study				
Year 1	56	5.91	0.54	0.004 [b]
Year 2	16	5.05	0.67	
Year 3	29	5.10	0.53	
Year 4	40	4.73	0.58	
Year 5	34	4.61	0.59	
Living				
Alone	22	4.90	0.47	0.92 [b]
With family	127	4.84	0.58	
With friends	25	4.85	0.71	
Perceived family SES				
Working class	13	4.62	0.63	0.49 [b]
Lower-middle class	14	4.84	0.49	
Middle class	98	4.85	0.61	
Upper-middleclass	9	4.81	0.62	
Upper class	34	4.97	0.54	

[a] Independent sample t-test; [b] One-way analysis of variance (ANOVA).

Correlations between Academic Performance and Motivation Subscales

As presented in Table 2, the Pearson product-moment correlation coefficient results showed statistically significant correlations between academic performance and most of the motivation subscales. The strongest correlation with academic performance was Self-efficacy for learning performance subscale (r = 0.2997, p = 0.0001) (Figures 1), followed by control of learning beliefs subscale (r = 0.2305, p = 0.0021) and Task value (r = 0.2243, p = 0.0028). The general MSQL scale was also found positively correlated with academic performance (r = 0.2296, p = 0.0019). On the other hand, test anxiety subscale showed negative correlation with academic performance (r = -0.1943, p = 0.0100).

Table 2: Correlations between subscales of motivation and academic performance (n = 175)

Subscales	r [a]	p-value
Intrinsic goal orientation (average subscore)	0.0740	0.3303
Extrinsic goal orientation (average subscore)	0.0233	0.7594
Task value (average subscore)	0.2243	0.0028
Control of learning beliefs (average subscore)	0.2305	0.0021
Self-efficacy for learning performance (average subscore)	0.2997	0.0001
Test anxiety (average subscore)	-0.1943	0.0100
The composite score for motivation (combining the above average subscores)	0.2296	0.0019

[a] Pearson's product moment correlation coefficient.

The only subscales that showed no significant correlation were those related to goal orientation (Intrinsic goal orientation and extrinsic goal orientation).

Figure 1: Correlations between self-efficacy for learning performance subscale of motivation and academic performance

Correlations between Task Value Subscale of Motivation and Academic Performance by the Characteristics of Students

Further analysis showed an effect of some factors on the relationship between academic performance and task value subscale of motivation. As shown in Table 3, the correlations between academic performance and task value subscale of motivation was statistically significant only among male students (r = 0.1969, p = 0.0265) compared to female, students aged above 23 years (r = 0.4763, p = 0.0068) compared to other age groups (Figure 2), students of the first and last academic year (r = 0.3324, p = 0.0123 and r = 0.5066, p = 0.0022, respectively) compared to students of middle years, and students living with their families (r = 0.2300, p = 0.7575) compared to those living in other types of accommodation. In regard to SES, this correlation was statistically significant only among students who perceived their family SES as middle and upper classes (r = 0.2839, p = 0.0042 and r = 0.4159, p = 0.0116, respectively) compared to lower class.

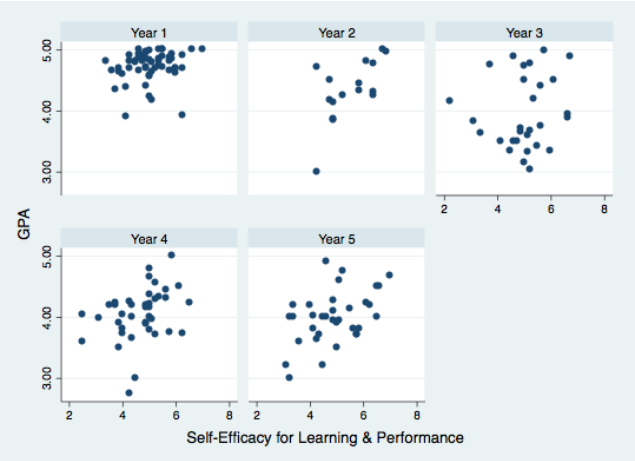

Figure 2: Correlations between Self-efficacy for learning performance subscale of motivation and academic performance, stratified by Academic year

Correlations between Control of Learning Beliefs Subscale of Motivation and Academic Performance by the Characteristics of Students

Looking at the confounding effect of the correlations between academic performance and control of learning beliefs subscale of motivation, the results indicated a statistically significant correlation only among male students (r = 0.2265, p = 0.0104) compared to female students, students aged below 21 years (r = 0.3605, p = 0.0118) compared to other age group, students of the fourth year (r = 0.3592, p = 0.0228) compared to other academic years, and students living with their families (r = 0.2517, p = 0.0043) compared to other accommodation types. In regards to SES, the correlation was statistically significant only among students who perceived their family SES as middle class (r = 0.2434, p = 0.0157) compared to other SES (Table 3).

Table 3: Correlations between motivation subscale and academic performance, stratified by students' characteristics

		Motivation Subscales							
		Task Value		Control of Learning Beliefs		Self-Efficacy for Learning Performance		Test Anxiety	
	n	r	p-value	r	p-value	r	p-value	r	p-value
Sex									
Male	127	0.1969	0.0265	0.2265	0.0104	0.2921	0.0009	-0.1024	0.2518
Female	47	0.0704	0.6382	0.2672	0.0695	0.1811	0.2233	-0.2399	0.1043
Age									
<21	48	0.1230	0.4048	0.3605	0.0118	0.2300	0.1158	-0.2636	0.0703
21-23	95	0.0849	0.4135	0.1118	0.2809	0.2971	0.0035	-0.1779	0.0846
>23	31	0.4763	0.0068	0.2185	0.2376	0.2761	0.1327	-0.0394	0.8334
Year of study									
Year 1	56	0.3324	0.0123	0.1052	0.4401	0.1886	0.1639	-0.2517	0.0613
Year 2	16	0.4948	0.0514	0.1350	0.6181	0.6333	0.0084	-0.3764	0.1507
Year 3	29	0.0398	0.8374	0.2662	0.1628	0.1172	0.5448	-0.1172	0.5450
Year 4	40	-0.2059	0.2024	0.3592	0.0228	0.3364	0.0338	-0.1347	0.4072
Year 5	34	0.5066	0.0022	0.1720	0.3306	0.4544	0.0069	-0.2256	0.1996
Living									
Alone	22	0.0698	0.7575	0.3349	0.1276	0.1487	0.5088	0.0035	0.9876
With family	127	0.2300	0.0093	0.2517	0.0043	0.5710	0.0014	-0.2584	0.0034
With friends	25	0.3457	0.0905	0.0424	0.8407	0.5066	0.0029	-0.0426	0.8399
Perceived family SES									
Working class	13	0.3069	0.3078	0.4259	0.1467	0.5111	0.0743	-0.3113	0.3006
Lower-middle class	14	0.2473	0.3940	0.3343	0.2428	0.0161	0.9564	0.2764	0.3388
Middle class	98	0.2839	0.0042	0.2434	0.0157	0.3079	0.0020	-0.3247	0.0011
Upper-middleclass	9	0.2208	0.5680	-0.2302	0.5513	-0.0614	0.8754	0.1639	0.6734
Upper class	34	0.4159	0.0116	0.2502	0.1536	0.0390	0.8266	0.0414	0.8161

n: observations; r: Pearson correlation coefficient.

Correlations between Self-Efficacy for Learning Performance Subscale of Motivation and Academic Performance by the Characteristics of Students

The results also showed that the correlations between academic performance and Self-efficacy for learning performance subscale of motivation were statistically significant only among male students (r = 0.2921, p = 0.0009) compared to females, students aged 21 to 23 years (r = 0.2971, p = 0.0035) compared to other age groups, students of second, fourth and fifth years (r = 0.6333, p = 0.0084; r = 0.3364, p = 0.0338; r = 0.4544, p = 0.0069, respectively) compared to other Academic years (Figure 2), and students living with their families (r = 0. 5710, p = 0.0014) compared to living in other types of accommodation (Table 3). The analyses of SES indicators showed a statistically significant correlation only among students who perceived their family SES as middle class (r = 0.3079, p = 0.0020) compared to other perceived family SES.

Correlations between Test Anxiety Subscale of Motivation and Academic Performance by the Characteristics of Students

As shown in Table 3, a significant correlation between academic performance and test anxiety subscale of motivation was only evident among students aged below 21 (r = 0.2636, p = 0.0703) compared to other age groups, and students living with their families (r = 0. 2584, p = 0.0034) compared to living in other types of accommodation.

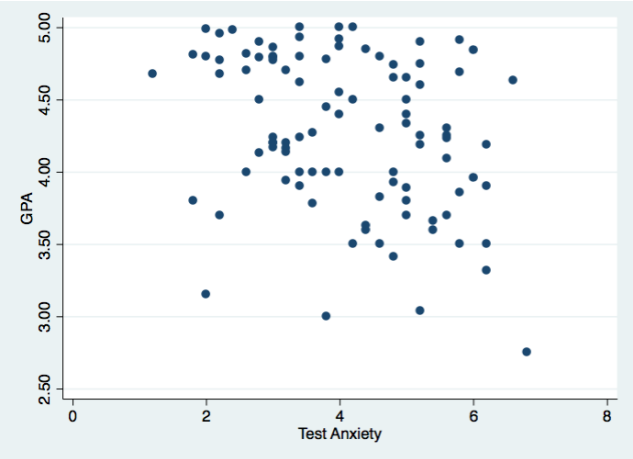

Figure 3: Correlation between test anxiety subscale of motivation and academic performance among students with middle-class families

The analyses of SES indicators showed that the correlation was statistically significant only among students who perceived their family SES as middle class (r = 0.3247, p = 0.0011) compared to other perceived family SES (Figure 3).

Discussion

This study examined the motivated strategies for learning and their association with the academic performances of a wide group of dental college undergraduate students from several dental schools in Riyadh region, Saudi Arabia. The study found statistically significant correlations between academic performance and the motivation scale as well as most of its subscales, including self-efficacy for learning performance, control of learning beliefs, task value and test anxiety. These results support the results of previous studies from other fields that showed such a relationship between academic performance and motivation strategy [8], [10], [28], [29]. The subscales that showed no significant correlation were those related to goal orientation (Intrinsic goal orientation and Extrinsic goal orientation) which was in line with previous research on the same topic [28].

The more in-depth analyses revealed that these correlations differ from one subscale to another based on how they react to other factors that affect this relationship. Interestingly, students living with their families were the only students who consistently showed significant correlations between academic performance and the motivation subscales, including task value, control of learning beliefs, self-efficacy for learning performance and test anxiety. To the author knowledge, this is the first study that shows such a unique effect. This effect might be because students living with their families have more exposure to positive feedback from parents, which found to be increasing self-efficacy of students [30]. Also, it might be because living with families in some cultures increase the students feeling of obligations for not causing shame or disappointment to their families after all the support they received. This kind of influence is seen commonly in middle- and far-east cultures [31], [32], [33].

Another factor that consistently confounds the relationship between academic performance and the motivation subscales is SES. Only students perceived their families to be a middle-class family showed significant correlations between academic performance and motivation strategies. The effect of SES is not unusual as it showed to be a well-known predictor of motivational orientation and academic performance [34], [35]. However, this study showed that it was the middle-class students who had this effect, not the higher SES as some previous research had found. This might be related to the differences in methods used to assess SES. This study used a proxy measure of SES to help in overcoming the issues related to asking about income as respondents often view this subject as sensitive and personal [36]. Further research is needed to examine this unique finding in more depth as the literature indicate that such an effect could be contingent upon several factors, such as student's minority status and school

location [35].

Additionally, even though no significant differences were found initially in the composite score for motivation between male and female students, the more in-depth analysis for each subscale showed that only males were having significant correlations between academic performance and motivation subscales, compared to a female student. These differences might be a result of the strong sex difference found in task preferences between boys and girls [37]. However, some previous results showed females having higher motivation scores than males [28], [29], [38], [39]. This variation could be related to the differences between both sexes in several factors including patterns of course-taking, achievement motivation and even educational experiences [40]. Analysing these differences was out of the scope of this study. However, further research is needed to analyse further sex differences for a better understanding of its role in this context as it has potential implications in education strategies.

This study used GPA as the outcome measure for Academic performance as it is the main outcome measure for students' Academic performance in universities and later on for employment and even showed to be predictive for employment [22], [41]. Although GPA is an objective measure with good internal reliability and stability [23], some factors could affect its validity, such as grade inflation and grading differences between universities [42], [43]. Further research could add to these findings by assessing different aspects of academic performance indicators, such as job offers, employment status and job performance.

Also, the use of students' self-reports to acquire this data might have introduced potential bias as students might be more inclined to self-enhancement, self-presentation and social desirability bias [41]. Even when students are trying their best to be honest and truthful, their self-reports might still be subjected to other limitations such as self-deception and memory biases [44], [45]. Nevertheless, this was judged to be the best available option considering other alternatives. For example, using University administration data as the source of the GPA could have jeopardised the anonymity of the questionnaire since it would be needed to make the questionnaires traceable. That action could have affected the study privacy protocol and participant's responses because of the increased likelihood of a potential source of biases as a result, such as social desirability distortion [46].

Another limitation of this study is related to the stratified analysis that was carried out to investigate about confounders and effect modifiers that could have impacted the relationship between academic performance and motivation strategy. The stratified analysis is a good tool to start with in such situations as it gives a fairly good picture of the role of confounders and effect modifiers. However, a key limitation to stratification is its inability to control simultaneously for multiple confounding variables. This study was the first to tap into such a relationship in dental education and therefore was meant to explore the potential correlations and initially discovered the potential confounders and effect modifiers. Doing in-depth and simultaneous testing for confounders would demand different design and much bigger sample to have enough power for such a level of analysis which was out of the scope of this research. Therefore, further research is needed to comprehensively assess the role of confounders and effect modifiers simultaneously which could bring up more details about the mechanisms of many of the correlations found by this study.

Considering the findings of this research, it seems helpful to give adequate attention to the enhancement of motivation in dental education and incorporate elements that stimulate intrinsic motivation among students. This theory is supported by previous research on medical education [47]. However, this has to be done while carefully considering factors that could affect this relationship, such as the type of family support, sex and SES, as some groups might act differently to these initiatives.

In conclusion, motivated strategies for learning, specifically self-efficacy for learning performance, control of learning beliefs and task value, are key factors for better academic performance among dental students. On the other hand, test anxiety can negatively affect the academic performance of dental students. This relationship might be affected by multiple factors, including the type of family support, sex and SES of the students. This emphasises the importance of carefully considering motivation strategies throughout the planning, implementation and improvement stages of academic programs.

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Public Health

Analysis of Nitrosamines in Processed Meat Products in Medan City by Liquid Chromatography-Mass Spectrometry

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Abstract

BACKGROUND: Nitrosamine is a carcinogen and the maximum level in processed meat products set by WHO.

AIM: The purpose of this study was to determine nitrosamine levels in meat products in Medan City and compared nitrosamine levels with standards set by WHO.

METHODS: The samples analysed were obtained from Berastagi Supermarket, sausages, burgers, corned beef and smoked beef. Nitrosamine levels were determined by reverse phase liquid chromatography-mass spectrometry.

RESULTS: The results showed that only 5 out of 20 samples of N-nitroso-thiazolidine-4-carboxylic acid (NTCA) nitrosamine ranged from 501.290 to 4227.492 µg/kg. The highest level of NTCA was found in smoked beef (Chiefs), which is 4227.492 µg/kg. N-nitroso-2-methyl-thiazolidine-4-carboxylic acid (NMTCA) was contained in all the samples analysed which ranged from 20.50 to 989.175 µg/kg. The highest NMTCA nitrosamine content was found in smoked beef (Chiefs), which is 989.175 µg/kg.

CONCLUSION: From this study reveal that nitrosamines in meat products exceed the maximum standards set by WHO (10 µg/kg).

Introduction

Nitrite and nitrate are generally found widely in soil, water, and food (especially in vegetables) [1]. Also, nitrite and nitrate are also intentionally added to some foods such as processed meats as preservatives and colouring agents [2]. The use of nitrite in processed meat products may react with alkyl amines to form carcinogen nitrosamines. The maximum level in processed meat set by WHO WHO is 10 µg/kg [3], [4], [5], [6]. There are several factors affecting the formation of nitrosamines, namely the concentration of nitrite, acidity, temperature, storage condition, the alkalinity of amines, and the presence of catalysts or inhibitors. So it is very necessary to analyse the content of nitrosamines in food, especially in meat products [7], [8], [9], [10], [11]. Nitrosamine analysis in food can be done by high-performance liquid chromatography and gas chromatography with a detector system. The method of high-performance liquid chromatography using mass spectroscopy is very selective and sensitive and can be used as an alternative method compared to other chromatographic methods [12], [13], [14], [15], [16].

The purpose of this study was to determine nitrosamine levels in meat products in Medan City and compared nitrosamine levels in meat products with standards set by WHO.

Material and Method

Materials

The materials used in this study were acetonitrile, formic acid (98%), methanol. Nitrosamines standard are *N-Nitrosodietylamine* (NDEA), *N-nitroso-2-methyl-thiazolidine 4-carboxylic acid* (NMTCA) and *N-nitroso-thiazolidine-4-carboxylic acid* (NTCA).

Samples Preparation

The samples used in this study were meat products (sausages, corned beef, burgers, and smoked beef) were obtained from Berastagi Supermarket in Medan city. Samples that have been taken and then placed in a *cooling bag* containing ice, then stored in the Laboratory in the freezer (temperature -20°C) before testing.

Format Acid Solution 0.1% in Purified Double Distillation Water

Transferred 0.5 mL formic acid (E.Merck) in a 1000 mL beaker glass, then added distilled water up to 500 mL. The mixture was stirred and filtered with 0.2 µm of cellulose nitrate filter membrane. The results obtained was 0.1% formic acid solution in distilled water.

Format Acid Solution 0.1% in Acetonitrile

Transferred 0.5 ml of formic acid (E. Merck) in a 1000 ml beaker glass, then added acetonitrile to 500 ml. The mixture was stirred and filtered with a 0.5 µm polymeretraflouroethylene (PTFE) filter membrane. The results obtained was 0.1% formic acid solution in acetonitrile.

Preparation of NDEA Standard Solution

Weighed 100 mg NDEA standard, transferred into a 10 ml volumetric flask, dissolved with acetonitrile to the marked line and shaken. Then, it was stirred for 5 minutes until it dissolved to obtain NDEA 10000 µg/ml solutions.

Preparation NMTCA Standard Solution

Weighed 10 mg of NMTCA standard, transferred into a 10 ml volumetric flask, dissolved with acetonitrile to the mark and shaken. Then, it was stirred for 5 minutes until it dissolved to obtain NMTCA 1000 µg/ml solutions.

Preparation of NTCA Standard Solution

Weighed 10 mg of NTCA standard, transferred into a 10 ml volumetric flask, dissolved with acetonitrile to the mark and shaken. Then, it was stirred for 5 minutes until it dissolved to obtain NDEA 1000 µg/ml solutions.

Chromatographic Condition

The nitrogen generator was turned on until it is ready to produce nitrogen gas used for mass spectrometry detectors. Then high performance liquid chromatography (*Agilent* 1290 *HPLC*) mass spectrometry (ABSciex API 4000 Q TRAP type) was turned on, by: turning on the mass spectrometry detector, setting positive detection, leaving it for a while until the mass spectrometry conditions become vacuum, run the pump on the type mixture of mobile phase, comparison of ratio mobile phase and predetermined mobile phase flow rate, as well as column oven temperature set at predetermined conditions, then the mobile phase was flowed until a stable pressure was obtained, which indicates that the high-performance liquid chromatography system of mass spectrometry has been stable and ready used for analysis [14].

Mobile Phase

The mobile phase used was a mixture of 0.1% formic acid solution in water and 0.1% formic acid solution in acetonitrile. The ratio of the mobile phase in the mixture were 10:90, 30:70, 50:50, 70:30 and 90: 10. Each of this mobile phase was used to separate the standard solution to determine optimum mobile phase composition [3], [12].

Nitrosamine Extraction

Five (5) g of mashed meat samples were weighed and transferred into 25 ml volumetric flask. Acetonitrile was added until 25 ml and then homogenised. Then, it was transferred into a tube and then centrifuged for 10 minutes at 8000 rpm. Five (5) ml of supernatant was filtered through a 0.2 µm filter membrane; then the solution was transferred into a vial. Ten (10) µL of solution was injected into a high-performance liquid chromatography system through an automatic injector [3], [12].

Calibration Curves

From the standard solution (NDEA, NMTCA, and NTCA) with a concentration of 1.25 mg/ml, pipetted 0.4 ml; 1 ml; 2 ml; 4 ml, and 10 ml, respectively. Each solution was transferred into volumetric flask 25 ml, then added acetonitrile to the marked line. The concentration of nitrosamine solutions (NDEA, NMTCA, and NTCA)

is 20; 50; 100; 200; and 500 ng/ml). Each solution was filtered through a membrane filter politetraflouroetilen (PTFE) of 0.2 µm. Then, the filtered solutions were transferred into a vial. Ten (10) µL of solution was injected into a high-performance liquid chromatography system through an automatic injector. The peak area of chromatogram was obtained. Then a calibration curve is made by plotting peak area versus concentration. Then, the regression line equation (Y = aX + b) determined.

Analysis of Nitrosamines in Processed Meat Products

Five (5) g of mashed meat samples were weighed and transferred into 25 ml volumetric flask. Acetonitrile was added until 25 ml and then homogenised. Then, it was transferred into a tube and then centrifuged for 10 minutes at 8000 rpm. Five (5) ml of supernatant was filtered through a 0.2 µm filter membrane; then the solution was transferred into a vial. Ten (10) µL of solution was injected into a high-performance liquid chromatography system through an automatic injector. MS/MS detection was done by Multiple Reaction Monitoring (MRM). The mass spectrometer is operationalised using electrospray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI) technique. The ion monitored in mass spectrometry is a positive ion that has a mass of 103 for NDEA, and negative ions that have a mass of 175 for NMTCA, and 161 for NTCA. Chromatogram and peak area were obtained, and then the concentration was calculated by substituting the peak area into the regression equation (y = ax + b) obtained from the calibration curve on linearity testing [3], [12]. Nitrosamine levels in solution can be calculated by the regression equation Y = a X + b.

Nitrosamine concentration was calculated using this equation:

$$C = \frac{X \; x \; V \; x \; Fp}{sample \; weigt \; (5 \; g)}$$

Notes: Y = Peak Area

C = Nitrosamine concentration in the sample (µg/kg); X = Nitrosamine levels in sample solution (ng/ml); V = Volume of sample solution before dilution (25 ml); Fp = Dilution Factors (1).

Results

Determination of Mobile Phase Composition

Determination of the mobile phase composition was done to get the best separation method. NDEA, NMTCA, and NTCA standard solutions in water and acetonitrile (ratio 10:90, 30:70, 50:50, 70:30) were injected into LCMS- MS/MS system through an automatic injector. The ideal chromatogram peak was shown by a Gaussian form with asymmetric value (tailing factor) which of 1. Tailing factor data with a different ratio of mobile phase for the analysis of NDEA, NMTCA, and NTCA can be seen in Table 1.

Table 1: Tailing Factor with different ratio of mobile phase for analysis of NDEA, NMTCA, and NTCA

Motion Phase	Tailing Factor		
	NDEA	NMTCA	NTCA
0.1% Formic acid in water: 0.1% Formic acid acetonitrile (10:90)	NA	NA	1,2
0.1% Formic acid in water: 0.1% Formic acid acetonitrile (30:70)	0.95	NA	0.93
0.1% Formic acid in water: 0.1% Formic acid acetonitrile (50:50)	NA	NA	1.5
0.1% Formic acid in water: 0.1% Formic acid acetonitrile (70:30)	1.08	1	1
Water: Acetonitrile (Format acid 0.1%) 0.1% format) 90:10	NA	NA	1.07

(Description: NA = Not applied)

In Table 1 it is shown that the best chromatogram mobile phase ratio is 70:30 (water: acetonitrile) because a symmetrical and sharp peak was produced and meets the requirements for tailing factor of 1.08 for the NDEA, 1 for NMTCA and NTCA. The best mobile phase with a ratio of 70:30 was then used for analysis of mixed solutions of NDEA, NMTCA, and NTCA. Chromatogram of NTCA, NMTCA, and NDEA using optimum mobile phase detected by Multiple Reaction Monitoring (MRM) Mass Spectrometry can be seen in Figure 1.

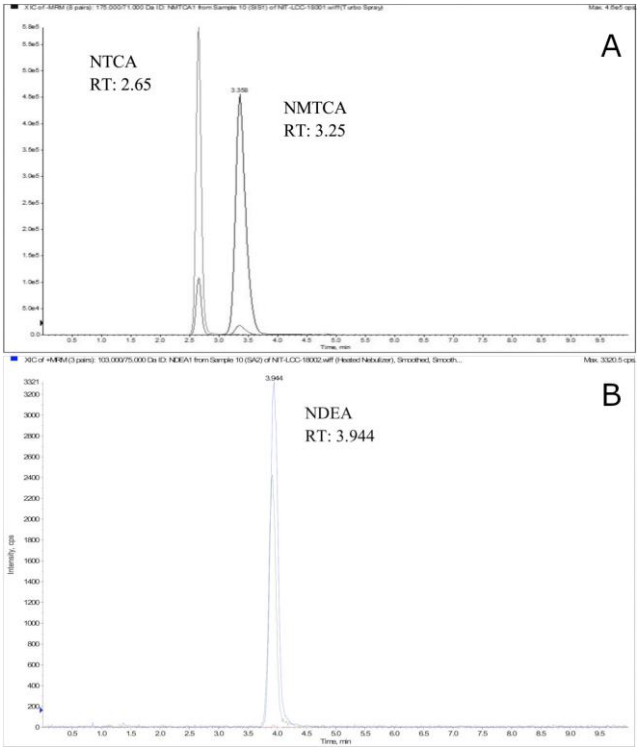

Figure 1: Chromatogram of NTCA, NMTCA, and NDEA using optimum mobile phase detected by Multiple Reaction Monitoring (MRM) Mass Spectrometry (A); N-nitroso-thiazolidine-4-carboxylic acid (NTCA) and N-nitroso-2-methyl-thiazolidine 4-carboxylic acid (NMTCA) detected with ESI ionisation and (B); N-Nitrosodietylamine (NDEA) with APCI ionisation

As seen Figure 1, it is shown that the optimum mobile phase for the analysis of both volatile and non-volatile nitrosamines is 0.1 % formic acid in water: 0.1% formic acid in acetonitrile (70:30) with flow rate 1 ml/minute. The standard mixture of NDEA, NMTCA, and NTCA eluted the optimum mobile phase shows a symmetrical and sharp peak. NDEA is analysed better using APCI compared to ESI. This is indicated by the high signal response obtained when NDEA standard solutions were analysed using APCI compared to ESI. APCI is usually used for the analysis of volatile nitrosamine compounds. On the other hand, NMTCA and NTCA are analysed better using ESI because higher signal responses were obtained [3], [12].

Calibration Curve

The concentration used for calibration curve preparation are: 20 ng/ml; 50 ng/ml; 100 ng/ml; 200 ng/ml; and 500 ng/ml for NDEA, NMTCA, and NTCA. Calibration curve of NDEA can be seen in Figure 2.

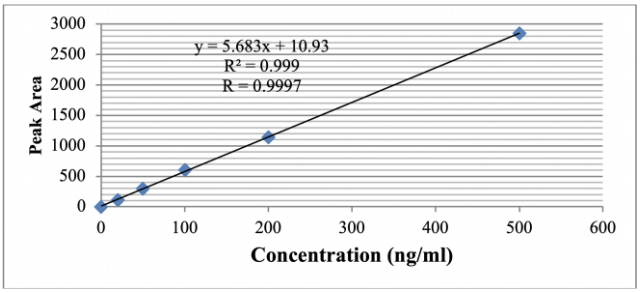

Figure 2: Calibration Curve of NDEA

In Figure 2 it can be seen that the calibration curve obtained has a linear relationship between peak area and concentration. The regression line equation obtained is y = 5.683 x + 10.93 with a correlation coefficient (r) of 0.9997. This shows a linear correlation between the area of the chromatogram and the concentration of NDEA [5], [6]. The calibration curve of NMTCA can be seen in Figure 3.

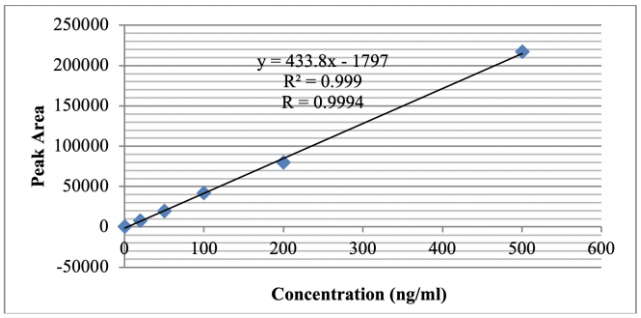

Figure 3: Calibration Curve of NMTCA

In Figure 3 it can be seen that the calibration curve obtained has a linear relationship between peak area and concentration. The regression line equation obtained is y = 433.8 x - 1797 with a correlation coefficient (r) of 0.9994. This shows a linear correlation between the area of the chromatogram and the concentration of N MTCA [5], [6]. The calibration curve of NTCA can be seen in Figure 4.

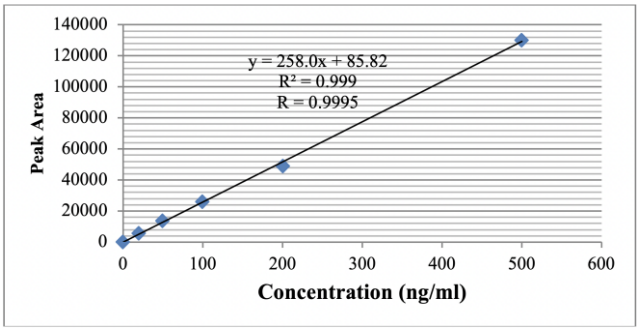

Figure 4: Calibration Curve of NTCA

In Figure 4 it can be seen that the calibration curve obtained has a linear relationship between peak and concentration. The regression line equation obtained is y = 258 x + 85.82 with a correlation coefficient (r) of 0.9995. This shows a linear correlation between the area of the chromatogram and the concentration of NTCA [5], [6].

Nitrosamine Levels in Various Types of Processed Meat Products

Determination of nitrosamines levels was carried out using reverse-phase high-performance liquid chromatography method which is then characterised by a mass spectrometer. Nitrosamine levels in various types of processed meat products can be seen in Table 2.

Discussion

Based on Table 3, it can be seen that there are differences in nitrosamine levels (NDEA, NMTCA, NTCA) in various types of processed meat products. There are 20 samples consisting of 5 types of sausage brands, 5 types of corned beef brands, 5 types of burger brands, and 5 types of smoked beef brands.

Of all samples analysed, NDEA (a volatile nitrosamine) was not detected. There are 2 possibilities, either NDEA is not present in the samples, or it is present in the sample, but below the limit of detection. On the other hand, non-volatile nitrosamine compounds such as NMTCA and NTCA are found in the samples and vary greatly. There are also some samples where these compounds are not detected. The highest nitrosamine level is NTCA (4227.492 µg/kg) which is found in smoked beef (Chiefs).

Table 2: Nitrosamine Levels (NDEA, NMTCA, and NTCA) in Various Types of Processed Meat Products

A. SAUSAGE			
No.	Nitrosamine	Brand	Nitrosamine ± SD (µg/kg)
1	NDEA	Fiesta	-
		Delicious	-
		Kenfood	-
		Kimbo	-
		So good	-
2	NMTCA	Fiesta	36.91 ± 6.2210
		Delicious	39.41 ± 3.0015
		Kenfood	35.64 ± 6.3940
		Kimbo	41.99 ± 6.6020
		So good	108.14 ± 6.5348
3	NTCA	Fiesta	585.45 ± 23.8477
		Delicious	-
		Kenfood	-
		Kimbo	-
		So good	2565.44 ± 60.8385
B. CORNED			
No.	Nitrosamine	Brand	Nitrosamine ± SD (µg/kg)
1	NDEA	Kornetku	-
		Pronas	-
		Milli	-
		Libbys	-
		Bernardi	-
2	NMTCA	Kornetku	20.54 ± 0.2951
		Pronas	20.50 ± 0.2604
		Milli	48.05 ± 2.8778
		Libbys	46.84 ± 37.3362
		Bernardi	90.52 ± 84.3906
3	NTCA	Kornetku	-
		Pronas	-
		Milli	-
		Libbys	-
		Bernardi	1508 ± 2975.7
C. BURGER			
No.	Nitrosamine	Brand	Nitrosamine ± SD (µg/kg)
1	NDEA	Kimbo	-
		Bernardi	-
		Vitalia	-
		Hemato	-
		Abbys	-
2	NMTCA	Kimbo	20.705 ± 0.0077
		Bernardi	20.703 ± 0.0043
		Vitalia	35.79 ± 2.7469
		Hemato	20.705 ± 0.0062
		Abbys	20.700 ± 0.0118
3	NTCA	Kimbo	-
		Bernardi	-
		Vitalia	-
		Hemato	-
		Abbys	-
D. SMOKED BEEF			
No.	Nitrosamine	Brand	Nitrosamine ± SD (µg/kg)
1	NDEA	Fiesta	-
		Yona	-
		Chiefs	-
		Bernardi	-
		Farmhouse	-
2	NMTCA	Fiesta	20.703 ± 0.0113
		Yona	20.702 ± 0.0107
		Chiefs	989.175 ± 116,403
		Bernardi	20.708 ± 0.0078
		Farmhouse	20.697 ± 0.0224
3	NTCA	Fiesta	-
		Yona	-
		Chiefs	4227.492 ± 605.5445
		Bernardi	501.290 ± 108.1681
		Farmhouse	-

Note: ((-): Not Detected, the data is an average of three Repeat times)

The level of NMTCA in all samples analysed varies greatly, ranging from 20.50 to 989.175 µg/kg. Whereas NTCA was only found in 5 of the 20 samples tested, ranging from 501.290-4227.492 µg/kg.

In this study, it is found that the levels of NMTCA in all samples exceed the limit set by the WHO, which is 10 µg/kg body weight. In sausage samples, NMTCA levels detected are 36.91 µg/kg (Fiesta), 39.41 µg/kg (Delicious), 35.64 µg/kg (Kenfood), 41.99 µg/kg (Kimbo) and 108.31 µg/kg (So Good). In corned beef samples, NMTCA levels detected are 20.54 µg/kg (Korenetku), 20.50 µg/kg (Pronas), 48.05 µg/kg (Mili), 46.84 µg/kg (libbys), and 90.52 µg/kg (Bernardi). In the burger samples, NMTCA levels detected are 20.705 µg/kg (Kimbo), 20.703 µg/kg (Bernanrdi), 35.79 µg/kg (Vitalia), 20.705 (Hemato), and 20.70 µg/kg (Abbys). In smoked beef samples, NMTCA levels detected are 20.703 µg/kg (Fiesta), 20.702 µg/kg (Yona), 989.175 µg/kg (Chiefs), 20.708 µg/kg (Bernardi), and 20.697 µg/kg (Farmhouse).

Besides that, it is also found that NTCA in some samples exceeds the limit allowed by the WHO. Those samples are Fiesta sausage (585.45 µg/kg) and So Good (2565.44 µg/kg), corned beef Bernardi (1508.00 µg/kg), smoked beef Chiefs (4229.492 µg/kg) and smoked beef Bernardi (501.29 µg/kg).

Based on previous research conducted by Herrmann (2014), nitrosamines levels obtained were also varied. The highest nitrosamine content found is NTCA which is 4030 µg/kg. The results of this study are the same as previous studies, where NDEA levels are not detected in all analysed samples. But in this study nitrosamine levels are higher than previous studies. The highest nitrosamine level is NTCA which is 4227.492 µg/kg (smoked beef Chiefs). The highest NMTCA and NTCA levels are found in smoked beef products. This is also corresponding to the previous study conducted by Herrmann (2014) where the levels of nitrosamines reach the maximum level of 2034-4030 µg/kg. The formation of these two nitrosamines is high in smoked beef samples because the high temperature applied during the smoking process triggers NMTCA and NTCA formation. It is also likely to occur due to several factors such as the concentration of nitrite, temperature, storage condition, the presence of catalysts or inhibitors. The higher the concentration of nitrite and amine compounds, the easier the formation of nitrosamines will be. However, the toxicological properties of both NMTCA and NTCA (nonvolatile nitrosamine) compounds have not been detected. Nonvolatile nitrosamines are weak carcinogens, but not enough data to determine the toxicological properties of these two compounds. However, NMTCA and NTCA nitrosamine levels in processed meat products need to be considered to prevent cancer prevalence [3], [9], [11], [12], [16], [17], [18], [19], [20].

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Public Health

The Content of Extracellular Nucleic Acids in the Blood and Ejaculate of Men of Reproductive Age Living in the Ecologically Unfavourable Regions of the Aral Sea

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Keywords: Extracellular DNA; Men of reproductive age; Ejaculate; Plasma acid soluble fractions (ASF); Plasma RNA; Plasma DNA

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Abstract

AIM: We aimed to study the influence of adverse environmental factors on molecular-cellular processes in the population living in the Aral Sea region.

METHODS: Extracellular RNA (ecRNA) and ecDNA were determined in blood. We obtained the ejaculate of the studied men after 4-5 days of abstinence. The ejaculate was placed in a warm tube with a ground stopper. The examination of the ejaculate was started in 20-30 minutes after receiving, as during this time it is subjected to liquefaction. Spectrophotometry of ASF, RNA and DNA hydrolysates was performed on an SF 26 at a wavelength of 290 nm against H_2O.

RESULTS: In the ejaculate of the studied groups of men, significant deviations in the content of extracellular nucleic acid fractions from the indicators of the comparison group were also detected. Statistically significant differences in the content of extracellular RNA were observed in men of the younger age group living in the territory of all study regions. A significant increase in the content of extracellular DNA was detected in two regions, but not in all age groups.

CONCLUSION: The study revealed a significant increase in the content of extracellular nucleic acids in the biological fluids of men of reproductive age living in the Aral Sea region. The most significant are the changes in the level of extracellular RNA in the blood plasma and ejaculate in men of the younger age group and the increase in ASF content in the ejaculate in men of all age groups.

Introduction

The Aral problem as the largest ecological catastrophe of the planet poses a direct threat to the sustainable development of the region, the health and future of the people living in it. Environmental pollution from exposure to various chemical and physical factors leads to the development of environmentally dependent pathologies, which manifest as clinical, pathophysiological, immunological and biochemical changes, which leads to several relevant diseases [1].

It has been revealed that effect of dust-salt aerosols and ecotoxicants (hydrazine, heavy metals and increased radiation background) in the Aral Sea regions can induce disturbances at the molecular-cellular and subcellular levels, leading to disruption of intercellular signaling, membrane transport and the genetic apparatus of the cell, which are starting mechanism of development of pathophysiological processes in the body [2].

Thus, it seems relevant to study the biochemical markers, indicating violations of molecular cell processes, to assess the impact of negative environmental factors on the body of the population in ecologically unfavourable regions [3]. We have investigated the content of various fractions of extracellular circulating nucleic acids in the blood and ejaculate in men of reproductive age living in the Aral Sea region.

We aimed to study the influence of adverse environmental factors on molecular-cellular processes in the population living in the Aral Sea region. For this purpose, the content of extracellular circulating nucleic acids was studied: the acid-soluble fraction (ASF) of nucleic acid precursors, extracellular RNA and DNA in the blood and ejaculate of men of reproductive age.

Material and Methods

The object of the study was the blood and ejaculate of men of reproductive age living in the Aral Sea regions. Depending on the age the examined persons were divided into three groups. As a comparison, we used the indicators of healthy men of reproductive age living in the territory of the Karaganda region, not exposed to dust-salt aerosols.

Blood sampling was carried out in the morning on an empty stomach; the ingestion of food and liquids was excluded 8-10 hours before the study. The volume of blood obtained from patients was 5.0 ml with the addition of 0.5 ml of heparin. The storage time of the biomaterial from the moment of collection to the research was no more than 1 hour. The biomaterial was stored in a special container at a temperature of +2 to +6°C.

Extracellular RNA (ecRNA) and ecDNA were determined in blood using the method of L. I. Markusheva and M. I. Savina [4]. The principle of ecRNA and ecDNA quantitative determination consists of the extraction of nucleic acids after hydrolysis at different temperatures in a water bath with perchloric acid of a certain concentration. The units of optical density/1.0 ml are used when calculating.

We obtained the ejaculate of the studied men after 4-5 days of abstinence. The ejaculate was placed in a warm tube with a ground stopper. The examination of the ejaculate was started in 20-30 minutes after receiving, as during this time it is subjected to liquefaction.

Spectrophotometry of ASF, RNA and DNA hydrolysates was performed on an SF 26 at a wavelength of 290 nm against H_2O.

Statistical Analysis

Statistical processing of research results was carried out by methods of variation statistics, non-parametric data processing methods. Statistical analysis was performed using SPSS 7.0, Windows Statistica 8.0 [5], [6].

Results

According to the results of a study of blood plasma, most often significant changes in the content of extracellular RNA were observed in men living in ecologically unfavourable regions of Kazakhstan. At the same time, the largest deviations from the corresponding indicators of the comparison group were observed in men of the younger and middle age groups in all regions of the study (Table 1).

Significant differences with the comparison group in the content of ASF and extracellular DNA in the blood plasma were not observed in all regions of the study. Thus, the greatest deviations in the content of extracellular DNA in the blood plasma are characteristic of residents of the South Kazakhstan region, and increased content of ASF was detected in residents of the Aktobe region.

Table 1: Indicators of extracellular nucleic acids in blood plasma in men (M ± m)

Region	Age	ASF, (standard units per ml)	RNA, (standard units per ml)	DNA, (standard units per ml)
South Kazakhstan region				
Arys-town, n = 300	18-29 years, n = 100	0.41 ± 0.03	1.25 ± 0.27***	1.18 ± 0.16***
	30-39 years, n = 100	0.58 ± 0.21	1.51 ± 0.82***	1.26 ± 0.21**
	40-49 years, n = 100	0.65 ± 0.09	1.81 ± 0.63**	1.35 ± 0.21***
Aktobe region				
v. Argyz, n = 150	18-29 years, n = 50	0.49 ± 0.06**	1.46 ± 0.27***	1.38 ± 0.51
	30-39 years, n = 50	0.60 ± 0.02***	1.47 ± 0.22***	1.39 ± 0.67
	40-49 years, n = 50	0.71 ± 0.03	1.51 ± 0.02***	1.67 ± 0.94
Shalkar-town, n = 225	18-29 years, n = 75	0.49 ± 0.15	1.57 ± 0.28***	1.28 ± 0.62
	30-39 years, n = 75	0.61 ± 0.29	1.63 ± 0.21***	1.30 ± 0.16***
	40-49 years, n = 75	0.69 ± 0.04*	1.18 ± 0.74	1.41 ± 0.26**

Note: the reliability of differences between the study groups and analogous indicators of the comparison group: * p < 0.05; ** p < 0.01; *** p < 0.001.

In the ejaculate of the studied groups of men, significant deviations in the content of extracellular nucleic acid fractions from the indicators of the comparison group were also detected (Table 2).

The increased content of ASF in the ejaculate is typical for men living in all regions of the study. Moreover, for two of the three study areas, a significant increase in the ASF content was observed in all age groups.

Table 2: Indicators of extracellular nucleic acids in the ejaculate in men, (M ± m)

Region	Age	ASF, (standard units per ml)	RNA, (standard units per ml)	DNA, (standard units per ml)
South Kazakhstan region				
Arys-town, n = 300	18-29 years, n = 100	0.41 ± 0.03**	1.25 ± 0.27**	1.18 ± 0.16***
	30-39 years, n = 100	0.58 ± 0.02***	1.51 ± 0.32	1.26 ± 0.21***
	40-49 years, n = 100	0.65 ± 0.1***	1.81 ± 0.63	1.35 ± 0.21***
Aktobe region				
v. Argyz, n = 150	18-29 years, n = 50	0.49 ± 0.06**	1.46 ± 0.27***	1.38 ± 0.51
	30-39 years, n = 50	0.60 ± 0.01***	1.47 ± 0.62	1.39 ± 0.67
	40-49 years, n = 50	0.71 ± 0.24***	1.51 ± 3.02	1.67 ± 0.94
Shalkar-town, n=225	18-29 years, n = 75	0.49 ± 0.15	1.57 ± 0.28***	1.28 ± 0.62
	30-39 years, n = 75	0.61 ± 0.29	1.63 ± 0.21*	1.30 ± 0.16***
	40-49 years, n = 75	0.69 ± 0.04***	1.18 ± 0.74	1.41 ± 0.26**

Note: the reliability of differences between the study groups and analogous indicators of the comparison group: * -p < 0,05; ** -p < 0,01; *** -p < 0,001.

Statistically significant differences in the content of extracellular RNA were observed in men of the younger age group living in the territory of all study

regions. A significant increase in the content of extracellular DNA was detected in two regions, but not in all age groups.

Discussion

Extracellular nucleic acids are actively studied under various pathological conditions. Increasing the concentration of extracellular nucleic acids in the blood leads to various metabolic consequences. Now it has been established that the content of circulating nucleic acids varies at diabetes, myocardial infarction, diseases of the kidneys and lungs, hepatitis, oncopathology [7], [8], [9].

The results of the study of extracellular nucleic acids have allowed developing new methods for diagnosing, determining the stage and monitoring the treatment of certain types of cancer, identifying certain congenital malformations [10], [11].

In conclusion, the study revealed a significant increase in the content of extracellular nucleic acids in the biological fluids of men of reproductive age living in the Aral Sea region. The most significant are the changes in the level of extracellular RNA in the blood plasma and ejaculate in men of the younger age group and the increase in ASF content in the ejaculate in men of all age groups.

It is possible that the change in the values of ASF, extracellular RNA and extracellular DNA is associated with the death of nucleated cell elements, as well as secretion of nucleic acids into the extracellular space, activated by exposure to dusty salt aerosols and other negative environmental factors in the Aral Sea region.

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Public Health

A Study of Noise Pollution Measurements and Possible Effects on Public Health in Ota Metropolis, Nigeria

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Abstract

BACKGROUND: Noise pollution has become a major environmental problem leading to nuisances and health issues.

AIM: This paper aims to study and analyse the noise pollution levels in major areas in Ota metropolis. A probability model which is capable of predicting the noise pollution level is also determined.

METHODS: Datasets on the noise pollution level in 41 locations across Ota metropolis were used in this research. The datasets were collected thrice per day; morning, afternoon and evening. Descriptive statistics were performed, and analysis of variance was also conducted using Minitab version 17.0 software. Easy fit software was however used to select the appropriate probability model that would best describe the dataset.

RESULTS: The noise levels are way far from the WHO recommendations. Also, there is no significant difference in the effects of the noise pollution level for all the times of the day considered. The log-logistic distribution provides the best fit to the dataset based on the Kolmogorov Smirnov goodness of fit test.

CONCLUSION: The fitted probability model can help in the prediction of noise pollution and act as a yardstick in the reduction of noise pollution, thereby improving the public health of the populace.

Introduction

Noise pollution is one of several environmental pollutions across the world. It can be described as the propagation of noise with a harmful impact on the physiological and psychological lives of humans or animals [1]. Noise or sound pollution is usually not studied compared with other forms of pollution such as air [2], [3], [4], water [5], soil [6], light and radioactive. The reason is that the adverse effects of other forms of pollution on humans are more pronounced. Notwithstanding, noise pollution remains a serious health concern in the study area (Ota, Nigeria) in particular and the entire planet [7], [8]. Some of the identified sources of noise pollution are loud music from concerts, religious buildings like churches and mosques, noise emitting generators [9], political rallies, road advertisement, traffic [10] and air transportation [11], sporting events, construction and industrial activities. In all the mentioned sources, areas that have high risk of noise pollution are residential places near to major roads [12] and airports and manufacturing industries [13]; for example, small scale industries [14], [15], steel rolling industries [16], oil and gas industry [17], [18] and so on.

The health effects of noise pollution cannot be over-emphasised. This has prompted the World Health Organization (WHO) and the Federal Environment Protection Agency (FEPA) (Nigeria) to set standards and limits of allowable noise levels. Noise pollution occurs when it is observed that those standards are exceeded as seen in [19], [20].

The most common manifestation of noise pollution is hearing loss or impairment [21]. Hearing impairment is mostly classified as occupational hazards especially when the individual is affiliated with industry that propagates loud sound or noise. Moreover, several physiological and psychological effects of noise pollution exist. The combination of

noise and air pollution is associated with respiratory ailments, dizziness and tiredness in school children [22], [23]. In adults, noise pollution has been found to be associated with high blood pressure [24] and cognitive difficulties [25].

A look at the literature showed the abundance of evidence of the adverse effects of noise pollution on the general public health. The worsening situation of noise pollution is that it has not been upgraded to the level of the other forms of pollution. Also, recommendations suggested by several authors on the different strategies on tackling noise pollution has not been considered and implemented. However, noise pollution continues to impact negatively on fetal development [26], annoyance and anxiety [27], mental health crisis [28], sleep disturbance and insomnia [29], [30], cardiovascular disorders in pregnant women [31], cardiocerebrovascular diseases [32], type 2 diabetes incidence [33] and medically unexplained physical symptoms [34]. Other auditory and non-auditory effects of noise on health are myocardial infarction incidence [35], peptic ulcers [36] and disruption of communication and retentive capabilities in children [37].

This paper aims to study and analyse the noise pollution levels in major areas in Ota metropolis. A probability model which is capable of predicting the noise pollution level is also determined.

Material and Methods

The dataset used in this research was gotten from [38]. It represents the noise level in 41 major locations in Ota metropolis, Nigeria. These major areas include industrial areas, commercial areas, passenger loading parks, busy roads and junctions. The readings were taken using the SLM (Sound Level Meter). Measurements were taken three different times of the day; morning (7 am to 9 am), afternoon (1 pm to 3 pm) and evening (6 pm to 8 pm). Particularly, the noise pollution level (NLP) was considered and analysed in this present research.

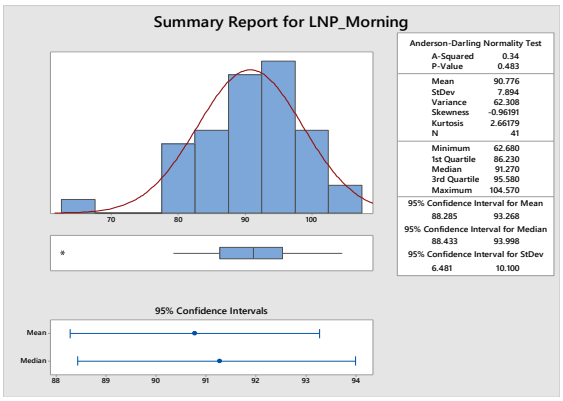

Figure 1: Summary report for morning measurements on LNP

Analysis of Variance

Analysis of variance is conducted in this research to know if there is a significant difference between the effect of noise pollution level in the morning, afternoon and evening in Ota metropolis. The hypothesis tested is:

H_0: The effects of the noise pollution level are the same for morning, afternoon and evening

Versus

H_1: The effects of the noise pollution level are not the same for at least one of either morning, afternoon or evening.

The level of significance used is 0.05, and the null hypothesis is considered rejected if the p-value is less or equal to the level of significance. The structure of the ANOVA table is such as presented in Table 1.

Table 1: A typical example of a one-way ANOVA Table

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	F-value
Factor	f-1	SSF	MSF = SSF/f-1	MSF/MSE
Error	n-f	SSE	MSE = SSE/n-f	
Total	n-1	SST		

where, 'f' is the number of factors which is 3 according to this research; morning, afternoon and evening. 'n' is the overall sample size.

The goodness of Fit Test

The goodness of fit test is performed in this research to select the probability model that best fits the dataset. The Kolmogorov Smirnov (KS) test, the Anderson Darling (AD) test and Chi-square test are examples of the goodness of fit tests.

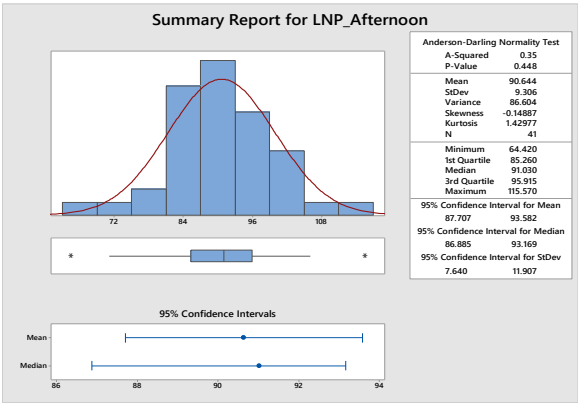

Figure 2: Summary report for afternoon measurements on LNP

The KS test was adopted in this research because it is the most popular and others might give similar results. The null hypothesis tests whether the data follow a specified distribution. If $X_1, X_2, ..., X_n$ represent ordered data points, the KS statistic is:

$$D \equiv \max_{1 \le i \le n} \left[F\left(X_i\right) - \frac{i-1}{N}, \frac{i}{N} - F\left(X_i\right) \right]$$

where X_i are the ordered data and $F\left(.\right)$ is

the cumulative distribution function (cdf) of the continuous distribution tested.

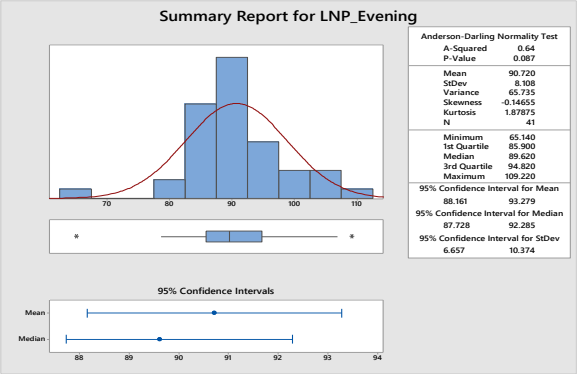

Figure 3: Summary report for evening measurements on LNP

Results

Descriptive Analysis of the Dataset

The summary for the LNP measurements is provided in Figures 1 to 3 while the summary for the mean measurement across the 41 locations is provided in Figure 4.

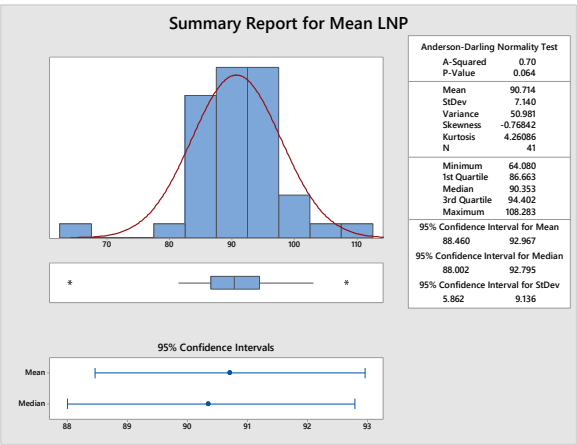

Figure 4: Summary report for the mean measurements of LNP across all locations in Ota

Result for the Analysis of Variance

The analyses of the means of the various measurements are presented in Table 2.

Table 2: Analysis of the Means

Factor	N	Mean	Standard Deviation	95% Confidence Interval
LNP_Morning	41	90.78	7.89	(88.16, 93.39)
LNP_Afternoon	41	90.64	9.31	(88.03, 93.26)
LNP_Evening	41	90.72	8.11	(88.10, 93.34)

The 95% confidence interval (CI) plot for the means is displayed in Figure 5.

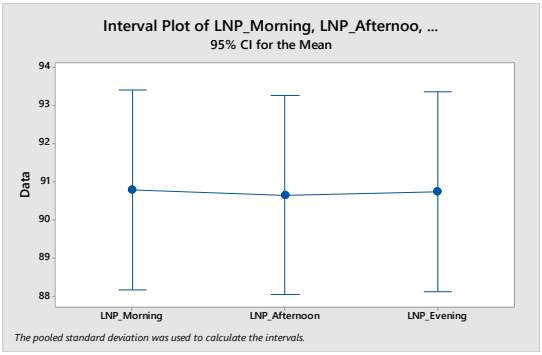

Figure 5: The 95% confidence interval (C.I) plot for the means

The result of the analysis of variance is presented in Table 3.

Table 3: Analysis of Variance (ANOVA) Table

Source	Degree of Freedom	Sum of Square	Mean Square	F-value	p-value
Factor	2	0.36	0.1805	0.00	0.997
Error	120	8585.85	71.5487		
Total	122	8586.21			

The result in Table 3 shows that the generated p-value is 0.997 which is far greater than the level of significance (0.05). Hence, there is no enough evidence to reject the null hypothesis, and it can, therefore, be concluded that there is no significant difference in the means of the noise level measurements taken in the morning, afternoon and evening. This result is further confirmed by Turkey's post-hoc test which is summarized in Figure 6.

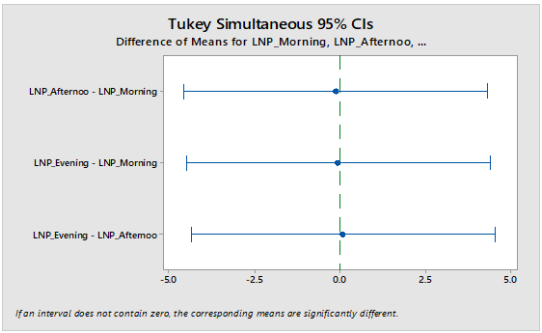

Figure 6: Summary of Turkey's post-hoc analysis

It can be observed in Figure 6 that all the intervals contained zero; this is an indication that there is no significant difference in the pair of each of the measurements considered.

Fitting of Probability Models

To determine the appropriate probability model that describes the mean noise pollution level in Ota metropolis, Easyfit (trial version) software was used to select distribution with the best fit. The Kolmogorov-Smirnov (KS) test of goodness of fit was used to select the best model. The software fitted sixty distributions to the dataset, but the best five was

reported in this research. The result is presented in Table 4.

Table 4: Fitted Distributions

Distributions	KS Statistic	Rank
Log-Logistic (3P)	0.06236	1
Burr	0.06846	2
Hypersecant	0.07131	3
Logistic	0.08415	4
Johnson SU	0.08629	5

From Table 4, the best-fitted model is the three-parameter Log-logistic distribution; this selection/decision is based on the Kolmogorov Smirnov statistic. A graph showing the best distribution fitted to the dataset on mean noise pollution level is presented in Figure 7.

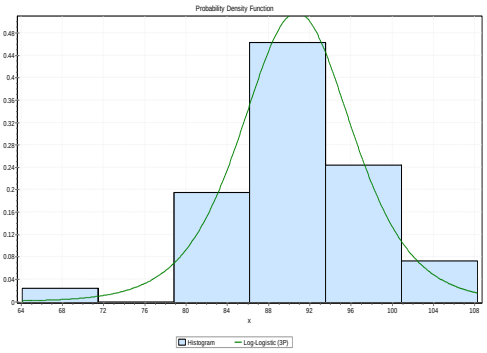

Figure 7: Graph of log-logistic distribution on the histogram of the dataset

In conclusion, further analyses of the noise pollution level in Ota metropolis has been provided in this research. The mean noise level in the morning was 90.78 which is higher than (though very close to) that of afternoon and evening with means 90.6 and 90.72 respectively. This is reasonable as more activities are expected during this time; pupils are going to school, workers going to the office, traffic at some junction and major bus stops. However, the analysis of variance result indicated that the time of the day (morning, afternoon and evening) have the same effect on the environment and populace. Also, the noise pollution level in Ota metropolis can be modelled using the log-logistic distribution as evident from the goodness of fit test. The model can now be used in predicting and managing noise pollution in that area. Furthermore, the model can be used in different geographical settings where noise pollution poses a perceived threat to the public health of the populace.

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The Link Between HIV Knowledge and Prophylaxis to Health Professionals

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Abstract

BACKGROUND: Healthcare workers have a high risk of professional exposure, especially in developing countries.

AIM: This paper aims to determine whether there is a link between knowledge and HIV prophylaxis on HIV prevention in Albanian healthcare system employees.

MATERIAL AND METHODS: This study was attended by professionals of the Albanian health care system who also attended second cycle studies at the Faculty of Medical Sciences (FMS) at the University of Medicine, Tirana. The study was conducted through a standard questionnaire with 24 questions, previously created by the Vojvodina Institute of Public Health in Serbia.

RESULTS: A group of 219 respondents participated in the study, of which 83.3% were women and 16.7% males. The risk of HIV transmission from syringe injection is > 75%, for 55.9% of the respondents. This result is statistically significant compared to other categories ($p < 0.01$). There is an increase in awareness of the use of gloves before manipulation and use of syringes, with increased work experience ($p = 0.01$). The use of specific containers for the elimination of syringes after manipulation is a more common practice by the most experienced professionals at work and results in a significant change ($p = 0.02$).

CONCLUSION: This study showed that there is not enough information from health professionals about potentially infectious fluids for HIV transmission. Younger professionals are less informed about HIV transmission and prophylaxis. These data indicate that there is a need for deepening of university curricula about the risks and exposure to biologically infectious fluids.

Introduction

During their daily professional practice, health professionals should avoid exposure to blood and other biological fluids containing viruses. This is a primary way to prevent the transmission of the immunodeficiency virus acquired (HIV) to health services [1]. Numerous studies have been carried out to determine what are the obstacles faced by health care professionals with regards to monitoring HIV patients. Some of these obstacles included: lack of knowledge about potentially infectious fluids, HIV virus carriers, negative feelings of professionals and response to HIV-infected patients, refusal or discrimination against these patients [2], [3], [4], [5], [6], [7], [8]. A study in nurses in Turkey has identified high-level negative attitudes and fears of HIV infection as a reason why they do not want to take care of HIV-infected patients [9]. Likewise, other studies have shown that there are barriers to healthcare systems in developing countries regarding medical care for HIV patients. Such practices are discriminatory and constitute a violation of the patient's right to medical care [10], [11], [12], [13], [14], [15], [16], [17].

This study aims to determine whether the

knowledge of Albanian healthcare professionals regarding HIV is at the right level. It also tries to determine whether there is a link between the level of knowledge and practical measures for the prophylaxis of HIV transmission to this category of professionals exposed to the virus, in their daily professional practice.

Material and Method

A cross-sectional study was conducted attended by Albanian healthcare professionals who also attend second cycle studies at the Faculty of Technical Medical Sciences (FSHMT) at the University of Medicine, Tirana, in March 2014. The study was conducted through a standard questionnaire with 24 questions, previously created by the Vojvodina Public Health Institute in Serbia [18].

The survey was voluntary and anonymous for students of the following branches: Nursing, Nursing-Midwife, Midwife and Laboratory Technician. The questionnaire was divided into three annexes: A-demographic and general data of respondents; B-specific questions regarding the ways of exposure to and transmission of HIV; C-Preventive measures against exposure. The information was recorded in the preformatted information collection database.

The data were analysed with the statistical package for Social Sciences (SPSS) version 20. To test the distribution of continuous variables, the Kolmogorov-Smirnov test was used. Descriptive statistics of continuous variables summarised as an average, and the standard deviation was presented. Categorical variables were presented as absolute frequencies and percentages. Chi-square and Fisher's exact tests were used to comparing the proportions between categorical variables and Pearson correlation to assess the relationship between work experience and the risk of HIV infection. The statistical tests were two-sided. Statistical significance was defined for $p \leq 0.05$.

Results

The study included 219 students practising the profession, of whom 83.3% were females and 16.7% males. The average age of the participants in the study was 30.5 ± 9.5 years. About the work profile, the nursing profession prevailed in most cases, 88.5%, followed by laboratory technicians (5.5%) and midwife professionals (4.6%).

Table 1: Job profile of participants in the survey

Work Profile	N	%
Nurse	193	88.5
Nurse – Midwife	2	0.9
Midwife	10	4.6
Student	1	0.5
Laboratory Technician	12	5.5

Participants with ≤ 5 working years prevail in the study with statistically significant changes as compared to the other categories ($p < 0.01$).

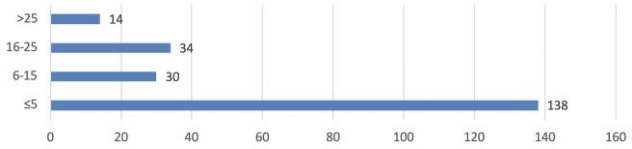

Figure 1: Categorization of respondents based on years of work

Most of the participants (55.9%) referred that transmission of the virus from an HIV-infected patient to a healthcare professional during a syringe injection accident was > 75%, with a significant change as compared to the other categories ($p < 0.01$). Whereas the remainder of the participants referred that the risk was: ≤ 25% (31.3%); 26-50% (7.1%); 51-75% (5.7%).

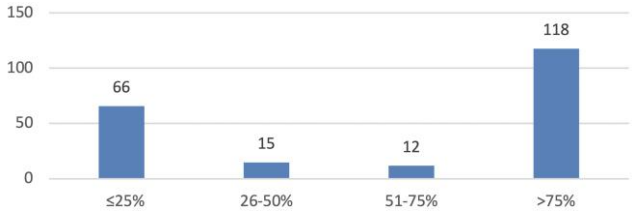

Figure 2: Number of respondents who answered the question: "To what extent can the virus of an HIV-infected patient be transmitted to a health professional during a syringe injection accident?"

Respondents were asked about which of the body fluids, besides blood can be considered as "more dangerous" for HIV transmission, they responded as per the following table.

Table 2: Number and percentage of respondents who determined biological fluids as dangerous for HIV transmission

Dangerous fluids	N	%
Breast milk	127	58.8
Saliva	55	25.5
Cerebrospinal fluid	44	20.4
Peritoneal fluid	20	9.3
Synovial fluid	15	6.9
Urine	11	5.1
Vomiting	10	4.6
Pleural fluid	4	1.9
Faeces	1	0.5
All together	7	3.2

In total, most participants referred as dangerous the breast milk (58.8%), saliva (25.5%) and cerebrospinal fluid (20.4%), with significant changes in other categories ($p < 0.01$).

Respondents were also asked about the implementation of procedures for HIV prophylaxis. Most participants correctly apply the procedures. This is also expressed as a sensible statistical significance

versus those who do not apply prophylactic measures. The exception is the use of protective glasses during manipulations with biological body fluids where most of the personnel (70%) refer not to use them.

Table 3: Prophylaxis measures

Procedures	Yes		No		P
	N	%	N	%	
Do you put the syringe cap back after its use?	171	85.9	28	14.1	< 0.01
Do you wear gloves before manipulating and using syringes?	167	83.5	33	16.5	< 0.01
Do you have plastic containers for throwing used syringes?	160	81.2	37	18.8	< 0.01
Do you use protective glasses during manipulation with body biological fluids?	54	30.0	126	70.0	< 0.01
If an injury has occurred to you, have you undergone examinations?	119	62.3	72	37.7	< 0.01
In the event of an accident, have you reported the case?	147	76.6	45	23.4	< 0.01

The implementation of prophylaxis procedures is related in some respects to the years of work of professionals. There is an increasing trend in wearing gloves before manipulation and use of syringes with increased work experience of the health professional (p = 0.01). Also, with increased work experience, there is a significant change in throwing used syringes in specific plastic containers (p = 0.02). Other procedures are not related to work experience.

Table 4: Procedures by years of work

Procedures		≤5y	6-15y	16-25y	> 25y	P
		n (%)	n (%)	n (%)	n (%)	
Do you put the syringe cap back after its use?	No	17 (12.3)	3 (10.0)	4 (11.8)	4 (28.6)	0.2
	Yes	107 (77.5)	27 (90.0)	27 (79.4)	7 (50.0)	
Do you wear gloves before manipulating and using syringes?	No	19 (13.8)	3 (10.0)	11 (32.4)	0	0.01
	Yes	104 (75.4)	26 (86.7)	21 (61.8)	13 (92.9)	
Do you have plastic containers for throwing used syringes?	No	31 (22.5)	1 (3.3)	4 (11.8)	1 (7.1)	0.02
	Yes	90 (65.2)	29 (96.7)	26 (76.5)	12 (85.7)	
Do you use protective glasses during manipulation with biological body fluids?	No	81 (58.7)	14 (46.7)	19 (55.9)	9 (64.3)	0.8
	Yes	31 (22.5)	12 (40.0)	9 (26.5)	2 (14.3)	
If an injury has occurred to you, have you undergone examinations?	No	43 (31.2)	9 (30.0)	11 (32.4)	7 (50.0)	0.6
	Yes	72 (52.2)	20 (66.7)	20 (58.8)	6 (42.9)	
In the event of an accident, have you reported the case?	No	24 (17.4)	5 (16.7)	10 (29.4)	5 (35.7)	0.3
	Yes	92 (66.7)	24 (80.0)	21 (61.8)	8 (57.1)	

Respondents were also asked about: "What are the first two actions to be done after syringe injection?" There is no significant difference between work experience and the first two actions to be done after syringe injection (p = 0.2.)

Table 5: What are the first two actions to be done after syringe injection?

	≤ 5y	6-15y	16-25y	> 25y
	n (%)	n (%)	n (%)	n (%)
Blood tests	4 (2.9)	2 (6.7)	4 (11.8)	1 (7.1)
Disinfection	63 (45.7)	14 (46.7)	23 (67.6)	12 (85.7)
Extrusion	19 (13.8)	12 (40.0)	19 (55.9)	4 (28.6)
Medication	6 (4.3)	2 (6.7)	0	1 (7.1)
You need to find the veins	1 (0.7)	0	0	0
Isolation	3 (2.2)	0	0	0
Contact with the doctor	6 (4.3)	0	1 (2.9)	0
Washing	15 (10.9)	0	4 (11.8)	4 (28.6)
Massage	2 (1.4)	0	0	0
Elisa test	0	0	0	2 (14.3)

Asked about how soon after syringe injection, with a high risk of transmission, should post-exposure prophylaxis (PPE) be initiated, respondents responded depending on years of work experience as shown in Table 6.

Table 6: How soon after syringe injection, with a high risk of transmission, should post-exposure prophylaxis (PPE) be initiated?

	≤ 5y	6-15y	16-25y	> 25y
	n (%)	n (%)	n (%)	n (%)
Urgent	62 (44.9)	16 (53.3)	20 (58.8)	5 (35.7)
5-30min	1 (0.7)	0	0	0
19 hours	1 (0.7)	0	0	0
24 first hours	5 (3.6)	1 (3.3)	2 (5.9)	0
Within 48 hours	0	0	2	2 (14.3)
After 72 hours	1 (0.7)	0	0	0
1-week	1 (0.7)	0	0	0
1-2 weeks	0	0	2 (5.9)	1 (7.1)
1-months	0	0	1 (2.9)	0
3 weeks-6 months	0	0	2 (5.9)	0
After 6 months	1 (0.7)	0	0	0
After doctor's diagnosis	0	1 (3.3)	0	1 (7.1)
After a long time	0	1 (3.3)	0	0
After HIV positive test	5 (3.6)	1 (3.3)	0	0
According to doctor prescription	0	0	1 (2.9)	0

In this case, it seems that there is enough knowledge about the time of PEP. There is no significant change according to work experience about the initiation of post-exposure prophylaxis (p = 0.1). It seems that even the staff with fewer years of work have a comparable knowledge with the experienced one.

Discussion

This study measured the knowledge of health professionals (HPs), who simultaneously attend their second cycle studies at the FSHMT, regarding prophylaxis, transmission ways, and post-exposure prophylaxis of HIV. Of the 219 health professionals surveyed, most were females, of the nursing profession and with less than five years of work experience. 55.9% of the respondents reported that transmission of the virus from an HIV-infected patient during a syringe injection accident to a health professional was > 75%. However, in prior studies of health professionals, the average HIV transmission risk after percutaneous exposure to HIV-infected blood is estimated to be approximately 0.3% (95% confidence interval [CI] = 0.2% to 0.5%) [19]. Regarding which of the body's fluids, besides the blood could be considered as "the most dangerous" for the transmission of HIV, they referred as dangerous fluids the breast milk (58.8%), saliva (25.5%) and cerebrospinal fluid (20.4%), Previous studies have determined that cerebrospinal, synovial, pleural, peritoneal, pericardial, and amniotic fluids are considered potentially infectious. However, the risk of HIV transmission from these fluids is unknown, and the potential risk to HPs from occupational exposure has not been assessed by epidemiological studies in healthcare facilities. Faeces, nasal secretions, saliva,

sweat, tears, urine, breast milk and vomiting are not considered potentially infectious if they do not contain blood. The risk of HIV transmission from these fluids and materials is extremely low [20], [21]. The answers to these two questions were used as indicators of knowledge on HIV transmission and showed insufficient knowledge of the respondents regarding the way the virus was transmitted. However, HP respondents reported the correct implementation of prophylaxis procedures. With the increase of the work experience of HPs, a growing trend of wearing gloves before manipulation and use of syringes and throwing of used syringes in specific plastic containers is noted. In many studies, various authors have determined that the implementation of some of these measures reduces the risk of HIV infection [22], [23]. In relation to the PEP, the two most important actions to be done according to the respondents are disinfection and extrusion and that they should be done as soon as possible. The interval within which the PPE should be started for optimal efficacy is not known, but animal studies have shown the importance of initiating PEP soon after exposure [24], [25], [26].

It has been 25 years since the diagnosis of the first HIV/AIDS case in Albania. Existing data from the studies conducted show that Albania does not have a generalised epidemic where Prevalence is P = 0.02 and Incidence I = 0.003. However, it has been noted a high incidence in recent years [27]. Despite the low number over the years, the increase in recent years has to be appreciated and for this reason, the awareness of the population, and in particular of the HPs, should be increased. A previous study conducted to HPs at the Mother Teresa University Hospital (QSUT), Tirana, Albania where the number of respondents was 443 in different job positions, concluded that the average occupational accidents rate in QSUT was 2,71, while in the European Union (EU) was 0.3 [28]. Also, there is no database in Albania to track professionals exposed to HIV-infected blood, to produce results regarding the development or not of HIV.

Despite the ongoing policies of the Ministry of Education and the Ministry of Health to improve university curricula and continuing education programs, there is a great need for new strategies to improve knowledge of the way HIV is transmitted and measures to be protected from potentially dangerous fluids as well, and PEP. The WHO states that blood infections among health care professionals appear in the form of professional exposure caused by percutaneous infections with 1000 HIV infections each year [29]. The current gravity of this problem is underestimated due to lack of information, underdeveloped monitoring systems or lack of data on the frequency of injuries in the HPs that work outside state public health institutions (long-term care, private offices and home health care). Similar studies have shown that there is a large number of HPs at risk of infection by HIV carriers. Thus these should be developed and evaluated with professionalism [30], [31], [32], [33].

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Impact of Pharmacist Intervention on Improving the Quality of Life of Patients with Type 2 Diabetes Mellitus

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Abstract

AIM: To analyse the characteristics, and analyse the impact of pharmacist intervention on quality of life (QOL) outpatients with type 2 diabetes mellitus (T2DM).

METHODS: This six-month analytical cohort study was conducted by assessing the patients' characteristics and their quality of life by distributing a questionnaire, and the 36-Item short form instrument to the patients with T2DM (n = 45) admitted to the Tertiary hospital in Tebing Tinggi. Patients who had mental disorders, HIV-AIDS, liver disease, stage 4 chronic kidney disease, and pregnant women were excluded from the study. The patients' quality of life was measured before and after interventions and analysed using the paired t-test. All analyses were performed using the Statistical Package for the Social Sciences (SPSS, version 22, Chicago, IL, USA) ($p < 0.05$ was considered significant).

RESULTS: The mean age of the patients was 61.96 ± 6.45 (years). Most (66.7%) of them were females. The mean QOL (in the score) of the patients: before the intervention, 61.07 ± 15.13; after the intervention, 70.15 ± 14.23, there was a significant difference between groups with and without interventions, $p < 0.001$.

CONCLUSION: Active contribution of pharmacists in the management of T2DM patients is urgent and important to improve the patients' QOL.

Introduction

Diabetes mellitus (DM) is a serious and chronic disease that occurs either when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin produced. The global prevalence of diabetes among adult has increased from 4.7% in 1980 to 8.5% in 2014. A more recent study indicated that as many as 422 million people live with diabetes in 2016 [1]. In Indonesia, there were over 10,276,100 diabetes cases (6.7% of total adult population) in 2017 [2].

Raise in blood glucose level can lead to many serious complications such. Type 2 diabetes called non-insulin-dependent or adult-onset diabetes results from the ineffective use of insulin by the body. When diabetes is not well managed, various complications will develop including diabetic retinopathy, diabetic nephropathy, diabetic neuropathy, and heart failure that affect the patients' quality of life (QOL). These conditions require complex management and multiple drug therapy, which in turn, may result in increased risk for the patients to experience drug-related problems (DRPs), readmissions, treatment costs, morbidity, and mortality [3]. The disease will accompany the patient's lifetime and worsen over time if not treated properly [4]. Many complicated factors are also associated with the successfulness of treatment of patients with T2DM including age, gender, educational level, socioeconomic status, the disease duration, multiple long-term complications of DM, the ability of the patients to cope with her or his diseases, adherence to the provided medications, and the provided healthcare. These issues are the challenges of the healthcare systems in the international as well as a national level [5], [6].

Management of the T2DM patients requires the active involvement of many healthcare providers, including a pharmacist. Pharmacists specialised in this growing chronic condition can have a significant and positive impact on the QOL of the patients as well as healthcare systems [7]. Awareness of healthcare providers on the need to assess and monitor the patients' QOLs as an important outcome in diabetes management has increased. The QOL is an important outcome since it influences the patient's self-care activities which can have a positive contribution to diabetes control [8].

Many pharmacist interventions programs have been established in various countries to enhance clinical outcomes and QOL. These programs were implemented by pharmacists, with the cooperation of physicians and other health care providers. Pharmacist interventions and the expanded role of pharmacists are associated with many positive diabetes-related outcomes, including improved clinical measures [9], improved patient and provider satisfaction [10], [11], and reduced the treatment cost [10], [12]. Subsequently, the pharmacist can contribute to an improvement in the QOL of patients with diabetes by informing and educating patients, answering their questions, and, at the same time, monitoring the outcomes of their treatment [13].

About the problems previously described, the present study was undertaken to analyse the impact of pharmacist intervention on QOLs of T2DM patients before and after educations.

Material and Methods

This six-month analytical cohort study was undertaken by assessing the patients' characteristics and their QOLs by distributing a self-designed questionnaire, and the 36-Item Short Form Survey (SF36) instrument [14] to the patients with T2DM (n = 45) admitted to a Tertiary hospital in Tebing Tinggi, Indonesia. The inclusion criteria were T2DM patients with age of 18 years or older and agreed to sign the informed consent. Patients had mental disorders, HIV-AIDS, liver disease, stage 4 of chronic kidney disease and pregnant women were excluded from the study. The study was approved by the Health Research Ethical Committee, Faculty of Medicine, University of Sumatera Utara, Indonesia. The required data were assessed from the three-month periods of admission with and without interventions. Thus, the overall study period was six months. The education provided to the patients comprised lifestyle changes (physical activity and eating habit), adherence to the prescribed medications, and how to use and to store the medications. The patients' characteristics assessed in this study comprised gender, age, education, occupation, duration of the disease, and utilisation of antihyperglycemic drugs from the patients' medical records. The QOL of each of the patients was assessed using the SF-36 questionnaire filled out by each of the patients in categories good, fair, bad and also divided at groups before and after the intervention to obtain their QOLs under the direction of the researchers.

At the beginning of the last three-month period of the study, leaflet contained materials regarding "Living healthy with diabetes", the guide suggested by American Diabetes Associaton [15] were also provided to the patients. The leaflet consists of how to take care of diabetes, healthy foods, physical activity, and medicine for diabetes. The researchers followed up the patients' outcome every admission (10 day period). Since the outpatients were insured by Indonesia Universal Health Coverage, they were asked to admit to the hospital every 10 days. At this time the treatment outcomes and laboratory examination were done. The patients' QOLs were recorded during each visit. Data required to analyse the patients' QOLs were collected at the last visit of each patient in each of the three months.

The patients' characteristics and the prescribed antihyperglycemic drugs provided to them were descriptively analysed. The significance of pharmacist intervention was analysed by comparing their QOLs before and after educations using the paired t-test. All analyses were performed using the Statistical Package for the Social Sciences (SPSS, version 22, Chicago, IL, USA) (p-value < 0.05 was considered significant).

Results

In this study, the target population obtained during the study period were 130 patients. Of these population, there were only 45 patients fulfilled the inclusion criteria; then these patients were used as a sample. Characteristics of the patients with T2DM are shown in Table 1.

Table 1: Characteristics of the patients with T2DM (n = 45)

		Percentage (%)
Age (Years)	46-55	13
	56-65	65
	> 65	22
Gender	Female	67
	Male	33
Education	University	38
	Senior high school	29
	Primary school	18
	Junior high school	15
Duration of the desease (years)	> 5	56
	> 1-5	31
	0-1	13

The mean age of the T2DM patients was 61.69 ± 6.45 (years). By age, more than half (65%) of them were in the age range of 56-65 years, twenty-two per cent of them were above 65 years old, and

thirteen per cent were in the age range of 46-55 years. Among the 45 patients, most (67%) of them were females. The T2DM patients had a different level of education. Most (38%) of them graduated from University, twenty-ninth per cent of them graduated from senior high schools. Less than a quarter (18%) of the T2DM patients graduated from primary school, and twenty per cent of them graduated from junior high school.

By duration of the disease, it was found that most of the patients (56%) have suffered from diabetes for more than 6 years. Nearly one third (31%) of them had suffered from diabetes for 1-5 years. Only thirteen per cent of them had suffered from the disease for 0-1 year.

The utilisation of antihyperglycemic drugs in the management of patients with T2DM before and after pharmacist intervention is listed in Table 2. As shown in Table 2, the four antihyperglycemic drugs widely provided to the patients with T2DM before pharmacist intervention in decreasing order were metformin 500 mg (47.3%), glimepiride 2 mg (28.4%), gliclazide (10.5%), and glimepiride 4 mg (4,2%). The same results were obtained in the group with intervention.

Table 2: Utilization of antihyperglycemic drugs in the management of patients with T2DM (n = 45)

Drug utilised	The proportion of the patients (%)	
	Before Pharmacist educations	After pharmacist educations
Metformin 500 mg	47.3	46.1
Glimepiride 2 mg	28.4	26.8
Gliclazide	10.5	11.9
Glimepiride 4 mg	4.2	7.5
Acarbose 50 mg	3.4	0
Glimepiride 3 mg	2.6	3.6
Glimepiride 1 mg	1.6	2.5
Insulin glulisine	1.5	1.0
Insulin glargine	0.4	0.5
Insulin lispro	0	0.1

The least prescribed antidiabetic drugs in the group before intervention were acarbose 50 mg (3.4%t), glimepiride 3 mg (2.6%), glimepiride 1 mg (1.6%), Apidra (1.5%), and Lantus (0.4%). Subsequently, the least frequently prescribed antihyperglycemic drugs provided to the patients with T2DM after pharmacist intervention in decreasing order were glimepiride 3 mg (3.6%), glimepiride 1 mg (2.5%), insulin glulisine (1%t), insulin glargine (0.5%), and insulin lispro (0.1%).

The QOLs of the patients with T2DM before and after pharmacist interventions is demonstrated in Table 3. There were only 36% of the patients with T2DM had good QOLs in the group before the intervention. However, the proportion of patients with a good category in the group with intervention has increased to 58%. Similarly, as much as twenty per cent of the patients had fair QOLs in the group before pharmacist intervention. The proportion of T2DM patients with fair category has increased to twenty-four per cent. Nearly half (44%) of the T2DM patients had bad QOLs in the group before the intervention while only eighteen per cent of the patients had bad QOLs in the group after intervention.

Table 3: The QOL of patients with T2DM (n = 45) before and after pharmacist education

Category	Before pharmacist intervention		After pharmacist intervention	
	Number of patients	Percentage (%)	Number of patients	Percentage (%)
Good (> 70)	16	36	26	58
Fair (60-70)	9	20	11	24
Bad (0-60)	20	44	8	18

Overall, the mean value of the patients' QOLs before pharmacist intervention was 61.08 ± 15.13. While the mean value of the QOL of the patients with T2DM after pharmacist intervention has improved to 70.15 ± 14.23. There was a significant difference between the patients' QOLs before the intervention and those with the intervention (p-value < 0.001).

Discussion

The present study found that the mean age of the T2DM patients was 61.69 ± 6.45 (years). Most of them (67%) were females. A study on T2DM patients conducted in Indonesia also revealed that most (66%) of T2DM patients were females [16]. Previous studies undertaken in India in the rural areas of Kumarapalayam and Alimosho general hospital, Nigeria revealed that the proportion of female T2DM patients were 60% and 72,4%, respectively [17], [18]. On the other hand, another study conducted in 2016 revealed that there was no significant difference in the prevalence of the disease between male and female [19]. It has been proved that the body mass index is an important contributor to the increase in the prevalence of diabetes [20]. Lastly, a large-scale prospective cohort study was undertaken in Spain also revealed that the incidence of T2DM increased with the increasing incidence of obesity [21].

By age, most of the T2DM patients were at the age range of 56-65 years and older. There was a similar study conducted in Helvetia primary health centre in Medan, Indonesia [16], in the rural areas of Kumarapalayam, India [17] and Alimosho general hospital, Nigeria [18]. This finding also supported the statement of the American Diabetes Association, that people at the age of 45 years or older are more prone to develop T2DM [22]. Ageing affects the pancreatic β cell sensitivity to glucose and delays the mediation of glucose uptake by insulin into the cells. Thus, the incidence of T2DM patients was high in older age [21].

The most frequently antihyperglycemic drugs provided to the patients with T2DM before and after pharmacist educations was metformin. According to the American Diabetes Association, metformin monotherapy should be started for a person initially diagnosed as having T2DM unless there are

contraindications [21]. This drug as first-line therapy has more beneficial effects on A1c, obese person, and cardiovascular mortality event if compared to sulfonylurea. Provision of metformin may be safe in patients with estimated glomerular filtration rate (eGFR) of 30 mL/min/1.73 m^2. The use of metformin as first-line therapy was supported by findings from a large meta-analysis [23], [24].

The next three antidiabetic drugs provided to the patients in decreasing order were glimepiride 2 mg, glucodex and glimepiride 4 mg. These drugs are included in sulfonylurea class whose mechanism of action to increase insulin secretion by pancreatic beta cells to have a hypoglycemic effect. A sulfonylurea is an option for adult patients with DM with normal weight who have never experienced ketoacidosis. This study is the same as with another study has been conducted, A previous study conducted in a tertiary care teaching hospital in Eastern India indicated that the most widely used antihyperglycemic agent was biguanide followed by sulfonylureas [25]. Another study proved that the most widely used antihyperglycemic agent was metformin followed by the sulfonylurea class of drugs [26].

Quality of life is the main health outcome in the treatment of T2DM [23]. Education and behavioural changes are required to manage the disease conditions properly and to improve the patients' QOLs. Lifestyle changes must incorporate careful dietary planning, appropriate use of antidiabetic drugs, and home blood sugar monitoring techniques for all DM patients [23]. In health care practice, therapeutic outcomes directly influence the physical, psychological and social domains of health. These factors will affect the overall QOL [27].

The present study proved that pharmacist intervention significantly improve QOLs of T2DM patients. Similar studies have been conducted by researchers in several countries. It has also been confirmed that clinical pharmacist mediated intervention on drugs, disease, diet, exercise, lifestyle modifications, and self-care practices in the management of diabetes has significant improvement of QOLs of patients with T2DM [27]. Additionally, a study undertaken toward T2DM patients in a military hospital, Myanmar proved that pharmacist intervention had a significant mean of QOL of the patients compared to those without intervention, $p < 0.001$. The researchers also reported that blood glucose concentration, body mass index, and waist circumference were significantly improved ($p < 0.05$) [28]. Eikenhorst reported in their systematic review and meta-analysis recruited from twenty-four studies from electronic databases from 2004 through 2017 revealed that pharmacist led-self-management interventions improved HbA1c value in the management of diabetes patients [29].

The present finding proved that enough provision of information related to the management of diabetes improved the QOLs of DM patients. Continuous education programs and counselling should be conducted for diabetic patients to emphasise and re-emphasize the importance of risk factor, prevention, medication, and behavioural changes [30]. The pharmacists' expanded roles in the healthcare sectors should be implemented to improve outcomes of the management of T2DM patients [31].

In conclusion, the present study highlighted that involvement of pharmacists in the management of patients with T2DM significantly improved QOLs of the patients. Metformin 500 mg was the most widely prescribed antihyperglycemic drug to the patients with T2DM. Improvement of the patients' knowledge about their disease, diet control, life style modification, and appropriate use of medications through education and medication counseling by clinical pharmacists have positive effects on the patients' clinical outcome.

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