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Basic Science

Hypoxia-preconditioned MSCs Have Superior Effect in Ameliorating Renal Function on Acute Renal Failure Animal Model

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Keywords: HP-MSCs; N-MSCs; ARF; BUN; Creatinin

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Abstract

BACKGROUND: Acute renal failure (ARF) is a serious disease characterised by a rapid loss of renal functions due to nephrotoxic drug or ischemic insult. The clinical treatment approach such as dialysis techniques and continuous renal enhancement have grown rapidly during past decades. However, there is yet no significant effect in improving renal function. Hypoxia-preconditioned mesenchymal stem cells (HP-MSCs) have positive effects on the in vitro survival and stemness, in addition to angiogenic potential.

AIM: In this study, we aimed to analyse the effect of HP-MSCs administration in improving renal function, characterised by blood urea nitrogen (BUN) and creatinine level.

METHODS: A group of 15 male Wistar rats weighing 250 g to 300 g were used in this study (n = 5 for each group). Rats were randomly distributed into 3 groups: Vehicle control (Veh) as a control group, HP-MSCs and normoxia MSCs (N-MSCs) as the treatment group. Renal function was evaluated based on the BUN and creatinine levels using the colourimetric method on day 5 and 13. The histological analysis using HE staining was performed on day 13.

RESULTS: The result showed there is a significant decrease in BUN and creatinine level ($p < 0.05$). The histological analysis of renal tissue also showed a significant decrease between Veh and treatment group ($p < 0.05$).

CONCLUSION: Based on this study, we conclude that HP-MSCs have a superior beneficial effect than N-MSCs in improving renal function in an animal model of gentamicin-induced ARF.

Introduction

Acute renal failure (ARF) is a serious disease characterised by a rapid loss of renal functions due to the renal damage by the nephrotoxic drug (cisplatin, cyclosporine A, gentamicin) and renal ischemia [1]. Depending on the severity and duration of renal dysfunction, ARF disease has high morbidity and mortality rate caused by massive retention of the nitrogenous compounds that indicated by the increase of blood urea nitrogen (BUN) and creatinine level [2]. The clinical treatment approach of ARF such as dialysis techniques and continuous renal enhancement have grown rapidly during past decades [3]. However there is yet no significant effect in improving renal function [4]. Therefore, the alternative treatments for ARF disease remain a major issue.

Stem cells transplantation has become an alternative and promising strategy in recent years in regenerating tissue damage including renal dysfunction of ARF. Among many kinds of stem cell, mesenchymal stem cells (MSCs) have several advantages distinguished to the others, such as their ease of isolation and harvest, no immunogenicity, abundant distribution and low tumour formation risk

[5]. MSCs are defined as a stromal cell that expresses specific markers such as CD44, CD73, CD90, CD105, and lack the expression of express hematopoietic markers, such as CD14 or CD11b, CD19 or CD79a, CD34, CD45 and HLA-DR [6]. Specifically, MSCs have multipotent differentiation capacity into various tissue lineages, including renal cells, in addition to self-renewal [7]. Several studies reported that transplanted-MSCs had been used to treat various types of injured tissues, including renal injury [8]. However, there are some limitations of MSCs transplantation [5], [6], [7] such as poor engraftment and low survival rate at injury area [9]. Therefore, it is critical to optimise the survival capacity of MSCs by modifying MSCs under certain stimulation.

HP-MSCs have positive effects on the *in vitro* stemness and survival capacity, in addition to angiogenic potential [10] that is mediated by hypoxia-inducible factors (HIFs), such as HIF-1α and HIF-2α [10], [11], [12]. HP-MSCs have a role in increased migration and engraftment capacities after transplantation into the injury area compared to N-MSCs as well as the extended lifespan [13], [14]. However, the role of HP-MSCs condition to improve the renal function of ARF remains unclear.

In this study, we aimed to analyse the effect of HP-MSCs administration in improving BUN and creatinine level (renal function marker) as well as histological appearances in an animal model of gentamicin-induced ARF.

Material and Methods

ARF Animal Model and Experimental Design

Fifteen male Wistar rats, weighing about 250-300 g, were used in this study (n = 5 for each group). Rats were housed in a 12h light-dark cycle cages at 24°C, with water and food ad-libitum. To generate ARF rat model, the rats were induced by gentamicin 140 mg/kg/day for 10 days, i.p., Rats were randomly distributed into 3 groups: Vehicle control (Veh) receiving NaCl injection, Hypoxia preconditioned-MSCs (HP-MSCs) receiving injection of 1 x 10^6 cell, and Normoxic-MSCs (N-MSCs) receiving injection of 1 x 10^6 cell intraperitoneally as treatment group respectively.

HUC-MSCs Isolation and Cultivation

Ethical concern was acquired by the institutional review board of the committee ethic of the medical faculty, Sultan Agung Islamic University of Semarang, Indonesia. Human umbilical cord-MSCs (hUC-MSCs) were isolated from umbilical cords obtained from donors with written informed consent. The isolation and expansion of hUC-MSCs were performed as described previously [15]. Briefly, the cords were chopped into small pieces. Then, cord pieces were cultured in Dulbecco`s Modified Eagle Media (DMEM) (Sigma-Aldrich, Louis St, MO) supplemented with 10% Fetal Bovine Serum (FBS) (Gibco™ Invitrogen, NY, USA) and 1% antibiotic/antimycotic (Gibco™ Invitrogen, NY, USA) at 37°C and 5% CO_2. The medium was renewed every 3 days. The cells were passaged with trypsin-EDTA after 80% confluence. The fourth passage cells were used for experiments.

Characterzation of UC-MSCs

HUC-MSCs-like were fixed with Cytofix™ fixation buffer (554655, BD Biosciences, Franklin Lakes, NJ, USA), and washed twice with stain buffer (554657, BD Biosciences). For the phenotype markers analysis, the cells were stained with phycoerythrin (PE) mouse anti-human CD44 (Clone G44-26, 555479; BD Biosciences), allophycocyanin (APC) mouse anti-human CD73 (Clone AD2, 560847; BD Biosciences), fluorescein isothiocyanate (FITC) mouse anti-human CD90 (Clone 5E10, 561969 BD Biosciences) and PerCP-Cy5.5.1 mouse anti-human CD105 (Clone 266, 560819, BD Biosciences) antibodies. Cells were stained with specific antibody for 30 minutes at room temperature, washed twice with stain buffer (554657, BD Biosciences) and examined with a BD FACSAria™ II flow cytometer (BD Biosciences) and BD FACSDiva™ software (BD Biosciences).

Differentiation Assay

HUC-MSCs-like at passage 4 were trypsinised and seeded at a concentration of 7.5 x 10^4 in a 24-well culture plate with standard medium. After 12 h incubation, hUC-MSCs were cultured in the medium of osteogenic differentiation containing DMEM (Sigma-Aldrich, Louis St, MO), supplemented with 10% FBS (Gibco™ Invitrogen, NY, USA), 10-7 mol/L/ 0.1 µM dexamethasone, 10 mmol/L β glycerophosphate, and 50 µmol/L ascorbate-2-phosphate (Sigma-Aldrich, Louis St, MO) in 5% CO_2 and at 37°C for 21 day incubation. The osteogenic differentiation analysis, cells were washed with PBS, and with Alizarin Red staining (Sigma-Aldrich Corp., St. Louis, MO, USA).

HP-MSCs Preparation

To prepare HP-MSCs, hUC-MSCs were cultivated under standard culture condition at fourth passage. After reached 80% confluence, the cells were transferred into a hypoxic chamber containing 5% O_2 and incubated for 24 h at 37°C CO2 5%, then collected for the following experiment.

Histological Analysis

Rats were sacrificed by cervical dislocation at day 13 after transplantation. Renal samples were collected after surgery and fixed in 4% paraformaldehyde. Sections (4 µm thick) were prepared from each group of renal tissue with standard protocols and processed by hematoxylin and eosin (H & E) staining. Then, the slides were analysed and scored with a semi-quantitative scale by an experienced technician evaluating changes found in ARF as the reference [16]. By previous reports [17], the tubular injury was assessed based on the level of tubular dilatation, loss of brush border, tubular necrosis and cast formation. The average histological score was used to analyse the renal tissue morphology.

Evaluation of renal function

Renal function was evaluated based on the BUN and creatinine levels using the colourimetric method (Quanti Chrom TM assay kits). Blood samples were collected from each rat (n = 5 for each group) on day 5 and 13 to compare the serum chemistry profile. Measurement of BUN and creatinine level was performed with the standard protocol.

Data Analysis

Data were presented as the means ± SD. All calculations were carried out using IBM SPSS 22.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. The statistical significance of the differences between the groups was assessed using one way-ANOVA and continued with Duncan post-hoc analysis. *P* values: **, $P < 0.001$.

Result

MSC characteristic

At the end of the expansion, the cells were arranged in monolayers with spindle-shaped and fibroblast-like cells. The surface markers of hUC-MSCs-like were analysed using Flow cytometry and revealed that these cells expressed high levels of CD44 (92.4%), CD73 (94.9%), CD90 (86.6%) and CD105 (96.8%).

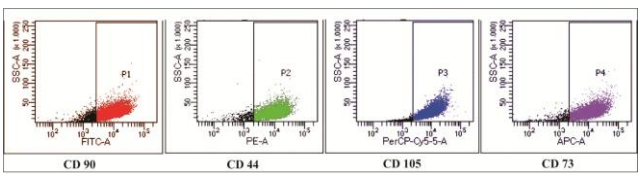

Figure 1: Immunophenotyping analysis of hUC-MSCs which was positive for CD44, CD73, CD90 and CD105

HUC-MSCs Isolation and Osteogenic Differentiation

HUC-MSCs isolation was carried out based on the plastic adherent capability under standard conditions (37°C, 5% CO2). The cells showed fibroblast-like (spindle shape) and peculiar morphology (Figure 2A). Osteocyte differentiations were determined using a standard protocol. The calcium deposits were confirmed by Alizarin Red. The positive osteogenic cells under the microscope were stained bright red.

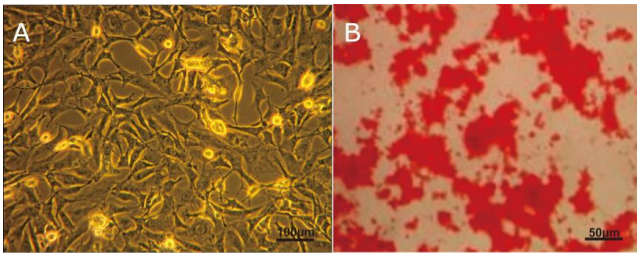

Figure 2: a) HUC-MSCs-like showed fibroblast-like or spindle-shaped characteristic (scale bar 100 µm); b) Osteogenic differentiation hUC-MSCs-like was evidenced by mineralised matrix formation using Alizarin red staining that showed by bright red colour (Scale bar 50 µm)

Renal Function Analysis

To ensure the ARF animal model, firstly we checked the level of BUN and creatinine of animal model before treatment for ARF confirmation. Then, after the treatment, the level of BUN and creatinine was analysed to compare the effect of HP-MSCs to N-MSCs in ARF animal model. The result showed that there was a significant decrease in BUN and creatinine level on day 5 and 13 between all treatment group than veh ($p < 0.05$). However, the result of HP-MSCs treatment is better than N-MSCs.

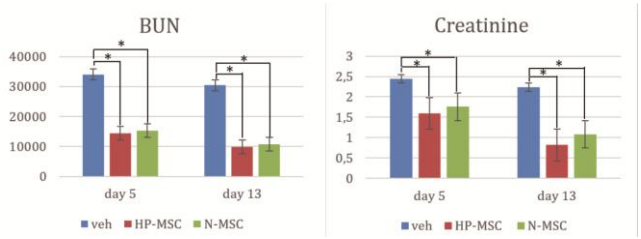

Figure 3: The result showed the highest decrease in BUN level is on HP-MSCs (9911.4 ± 1316.8) at day 13. The level of creatinine also significant decrease on day 13 in HP-MSCs (0.817 ± 0.038)

Histopathological analysis

The histological analysis was performed by HE staining. The average histological score was used to analyse the renal tissue morphology. The results showed that the histological features of renal tissues treated with HP-MSCs and N-MSCs groups showed normal renal cells compared to the vehicle group (Figure 4) in which HP-MSCs showed better renal cell repair.

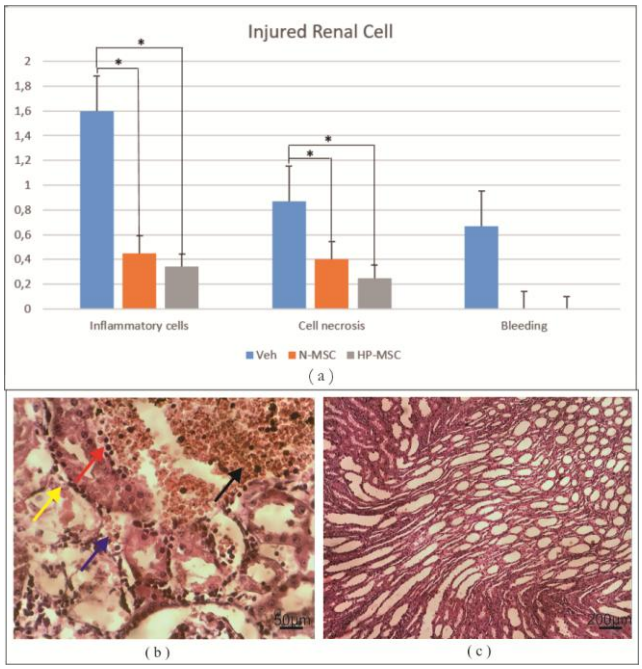

Figure 4: a) The histological analysis of renal tissue showed a significant decrease between and treatment group ($p < 0.05$). The HP-MCSs showed better result in decreasing inflammatory cells (0.333 ± 0.115) and cell necrosis (0.266 ± 0.305); b) Veh showed patchy necrosis and atrophy of distal and proximal tubules (yellow arrow), pyknotic nuclei (red arrow), bleeding (black arrow) and cell debris in the lumen (blue arrow); c) All treatment groups showed dense interstitial tissue infiltrate between tubules at corticomedullary junction

Discussion

ARF also was known as the acute renal injury is one of the serious renal diseases characterized by rapid deterioration of renal functions indicated by the accumulation of the nitrogenous compounds (urea and creatinine). Several studies reported that UC-MSCs as attractive candidates for renal repair of ARF [17], [18] due to the most of renal tissues structure such as nephrons are derived from mesenchymal that is similar source to MSCs, in addition to they have potential role for releasing the molecule differentiation signalling to both nephrons and collecting duct [18], [19]. However, the transplanted MSCs have poor engraftment and survivability in the injury area. To enhance their therapeutic capacity, the activated MSCs using hypoxic condition is needed to augment their homing and survival in renal injury sites. To analyse the effect of MSCs under hypoxic condition in ameliorating renal function of ARF we used gentamicin-injected rats as established ARF experimental animal model according to a previous protocol. In this study, we transplanted the HP-MSCs into ARF rat models intraperitoneally then analyse the BUN and creatinine level as well as histological finding on day 5 and 13. Our study is the first to administrate HP-MSCs to ARF animal model.

The biochemical result of pretreatment group showed marked impairment of the renal function of ARF that indicated by the plasma increase of BUN and creatinine level. The histological appearance also showed glomerular tuft shrinkage (damage) and haemorrhage; capsular space widened as well as pyknotic nuclei of the tubular cells (necrosis). These suggested that the all groups in this study were under ARF condition (BUN = 36.422 mg/dl and creatinine= 2.46 mg/dl).

In our study, we found that the level of BUN and creatinine were significantly decreasing at day 5 and 13 in all group ($p < 0.05$). These were supported by our morphology finding which shown that there is a significantly decrease in damage, necrosis, and hemorrhagic of the renal cell on day 13 in all treatment group (Figure 4). However, the BUN and creatinine level of HP-MSCs is lower compared to N-MSCs and morphological appearances as well. These suggested that the regeneration process of ARF that indicated by normal renal function has been well controlled in HP-MSCs group.

Active inflammation release several inflammatory molecules that triggering proteolytic degradation of the most growth factors of regeneration process [20] however they release several chemokine molecules for attracting the exogenous circulating HP-MSCs homing to damaged renal tissue of ARF. HP-MSCs homing to renal injury area involved specific attractant molecules such as VEGF, HGF, integrin α4 and integrin β1 forming very late antigen 4 (VLA-4), and stromal-derived factor-1 (SDF-1) [21] (Figure 5).

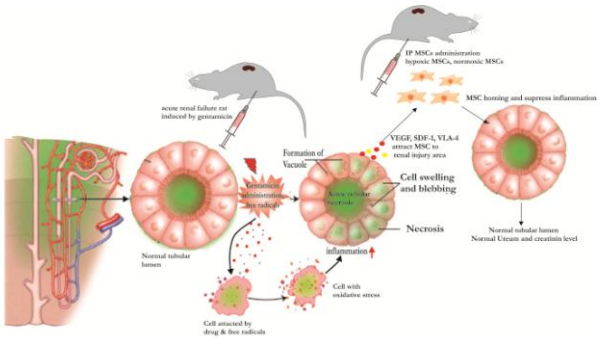

Figure 5: The homing mechanism of MSCs in renal failure. Model of oxidative stress-induced inflammation has been controlled post-HP-MSCs administration. The gentamicin induced-oxidative stress leads to inflammation in ARF. The interactions of ligands to MSCs receptor: (SDF-1 and HGF)-(CXCR4 and cMet) and (VCAM-1 and VEGF) – (VLA-4 and CD44) may trigger the binding of MSCs to target endothelial cells leading to the migration of MSCs to renal injury (homing phenomena). In this area, MSCs being activated and released anti-inflammatory molecules such as TGF-β, TSG-6, IL-10, IL-1ra, and PGE2 for controlling the inflammation process. Under-controlled inflammation may trigger the alteration of inflammatory to proliferation phase that characterised by the secretion of regeneration molecule (growth factor). These were correlated to the formation of renal cells (glomerulus and tubules) into a normal arrangement

HP-MSCs in renal tissue injury release anti-inflammatory molecule such as TGF-β and IL-10 for suppressing the inflammation process then accelerate

the shifting of inflammation to the proliferation phase [22]. In this proliferation stage, HP-MSCs release several molecules such as VEGF, PDGF and HGF to actively induce the endogenous stem cells and cells surrounding to repair and regenerate the damaged tissue of ARF, known as a paracrine mechanism. On the other side, HP-MSCs also may differentiate and transdifferentiate into several renal cells as well as exosomes mechanism by donating their crucial vesicle to accelerate the regeneration of ARF.

HGF has a crucial role as a mitogenic molecule to stimulate the proliferation of renal epithelial cell lines [23], including a rat visceral glomerular and proximal tubular cell in damage tissue of ARF by nephrotoxic drug administration (cisplatin, cyclosporine A, gentamicin) and renal ischemia [24], [25]. VEGF as a potent angiogenic factor and concurrently to PDGF have an essential role as potent mitogens in cell growth and regeneration of ARF [26]. All these cytokines play a role in the initiation phase of renal cell regeneration and prevention of apoptosis through Akt or ERK1/2 pathway activation [27]. This study has a limitation in which we do not analyse the molecule released by MSCs whether in inflammation or proliferation phase. Hence, the molecule anti-inflammation and regeneration released by MSCs as well as homing mechanism still unclear. We also do not analyse another soluble factor that may affect to the regeneration process.

In conclusion, HP-MSCs have superior beneficial effect than N-MSCs in ameliorating renal function and regeneration of ARF in an animal model of gentamicin-induced ARF.

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Basic Science

Thymoquinone, an Active Constituent of Black Seed Attenuates CCl4 Induced Liver Injury in Mice via Modulation of Antioxidant Enzymes, PTEN, P53 and VEGF Protein

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Keywords: CCl4; Hepatoprotective agents; PTEN; P53; Immunohistochemistry; Antioxidant enzymes; Aminotransferases

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Abstract

AIM: The present study was undertaken to evaluate the possible protective role of thymoquinone on CCl4-induced hepatotoxicity.

METHODS: The activities of liver function enzymes and antioxidant enzymes were measured. Haematoxylin-Eosin staining was performed to analyze the live tissue alterations. Additionally, expression pattern of different proteins was evaluated through immunohistochemistry staining.

RESULTS: The antioxidants enzymes activities were decreased significantly in the CCl4 induced group whereas recovery/increase of antioxidant enzymes was observed when thymoquinone was given to the mice. Moreover, thymoquinone administration significantly decrease the serum levels of alanine aminotransferase (ALT), alkaline phosphatase (ALP), and serum aspartate aminotransferase (AST). Liver tissue alterations were noted in CCl4 treated group whereas treatment with thymoquinone significantly prevented the CCl4-induced histological alteration. The expression of PTEN protein was high in CCl4 plus thymoquinone treated group while the loss of PTEN protein expression was observed in CCl4 treated group. Moreover, high expression of P53 protein was noticed in CCl4 treated the group as compared to CCl4 plus thymoquinone group. Difference in expression pattern of PTEN and p53 protein in CCl4 group and thymoquinone plus CCl4 treated group was statically significant ($p < 0.05$). Besides, expression of VEGF was high in CCl4 treated group as well as thymoquinone plus CCl4 treated group and difference in expression pattern was statically insignificant ($p > 0.05$).

CONCLUSION: Our results suggest that thymoquinone can protect CCl4 induced liver damage and could be a preventive drug in the development of novel therapeutic agents for liver diseases.

Introduction

Liver disease is one of the major global health problems regarding morbidity and mortality. In this regard, several factors including viruses and alcohol abuse show a role in the pathogenesis, but exact molecular mechanism involved in this respect is not fully explained. Moreover, the molecular routes causal the pathogenesis of acute liver injury is recognised to involve a complex interplay of oxidative stress, apoptosis, inflammation and another process [1], [2].

Carbon tetrachloride (CCl_4) is a well-known hepatotoxin that is usually used to induce liver injury in a large variety of laboratory animals [3], [4]. The overdoses of CCl_4 either orally or intraperitoneally, induce hepatic damage, including loss of architecture of hepatocytes, inflammation, congestion, degeneration and necrosis. Furthermore, exposure of CCl_4 shows role in the reactive oxygen species generation, which in turn decreases the antioxidant enzymes that show role in the detoxification of toxic materials. Additionally, CCl_4 reduces antioxidant enzymes which catalyse the decomposition of hydrogen peroxide to water and oxygen and which neutralise the reactive superoxide radical activity.

Intake of medicinal plants and its active component with antioxidant phytochemicals is confirmed to enhance the antioxidant level and inhibit the pathogenesis. In this view, medicinal plant or their constituents have proven their role in the inhibition of pathogenesis due to the rich source of antioxidant [5],

[6]. Thymoquinone is the principal component of *Nigella sativa* which is familiar as black cumin or black seed [7], [8] and its use in the cure of diseases, as well as inhibition of pathogenesis, has been described. Moreover, Ayurveda, Unani, Arabic and Chinese medicine have shown its importance in health management. Also, its role in cancer prevention has been noted through modulation of cell signalling pathways including angiogenesis, apoptosis and tumour suppressor gene [9]. However, its anti-tumour activity has been proven as it shows role in the cell death and tumour growth inhibitory activities and has been found to be associated with other tumorigenic processes [10], [11].

This study was undertaken to examine whether TQ protects against CCl_4-induced hepatotoxicity in mice.

Material and Methods

This study was conducted by the guidance of the ethical committee for animal handling at Qassim University. The experimental procedure was approved by the Bioethics Review Committee of the College of Applied Medical Science, Qassim University.

A total number of 24 young adult male albino mice were included in this study. The mice were housed in the animal house of the College of Applied Medical Science, Qassim University. The mice age was between the six and seven weeks and weighed between 23-28 g were included in the study. All mice were fed in the laboratory maintained at approximately 22°C with a 12-h light–dark cycle with free access to food and water. An acclimation period of 1 week was employed earlier to the experiments. Animal grouping and treatment plan are described in Table1.

Table 1: Grouping of animals and treatment plan

Group number	Experimental group	Treatment	Number of animal per group
1	Control	Normal mice administered with vehicle solution	8
2	Disease group	CCl_4 treatment group	8
3	Treatment groups	CCl_4 (0.04 cc of a 40 per cent solution of CCl_4) plus thymoquinone (10 mg/kg/day dissolved in a DMSO) treatment	8

The mice were randomly divided into 3 groups as follows:

Group 1: The first group is the untreated control group and was administered with olive oil (orally by gavage twice in a week until the last day of the experiment) which was used as a vehicle, and fed normal diet and water for 12 weeks.

Group 2: (Diseases control: CCl_4 control group): Mice were treated with CCl_4 (0.04 cc of a 40 per cent solution of CCl_4) in olive oil [12] orally by gavage three times in a week for 12 weeks.

Group 3: Thymoquinone plus CCl_4 treated group. Thymoquinone-CCL_4 treated group, mice received thymoquinone (10 mg/kg body weight/day) [13] starting one week before CCl_4 administration and continued throughout the experiment for 12 weeks.

Blood samples were collected and allowed to clot for 30 minutes and centrifuged to separate clear serum. The activities of enzymes such as alanine aminotransferases (ALT), aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Catalase, Superoxide dismutase (SOD) Glutathione peroxidase (GPx) and total antioxidant capacity activity was measured through ELISA kits, and the results were interpreted accordingly (Figure 1).

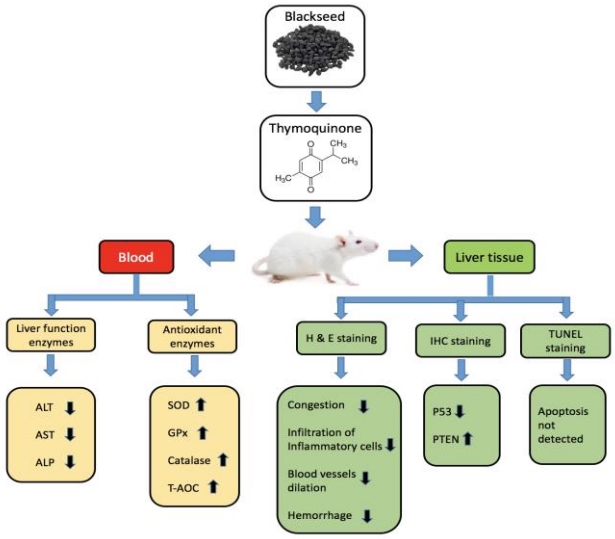

Figure 1: Implication of thymoquinone in the prevention of liver damage through modulation of biological activities

Liver tissues were collected from mice of each group and fixed in 10% buffered formalin. These tissues were processed through tissue processor to make a paraffin-embedded block. Sections were made from each tissue block and Hematoxylin-Eosin (H & E) staining was performed to analyse the live tissue alterations, and observation was noted under a light microscope.

Expression of different types of proteins including PTEN, VEGF and P53 was evaluated through immunohistochemistry as by previously described method [14]. Concisely, deparaffinization of the sections was made through xylene and rehydration on each section was performed. A blocking agent such as hydrogen peroxidase was used to block the endogenous peroxidase activity (Abcam, USA). Furthermore, nonspecific binding was blocked by a protein blocking agent (Abcam, USA). Monoclonal antibodies of PTEN, VEGF and P53 (Abcam, USA) were used as primary antibodies. Secondary antibody and tertiary antibody were used on each section for 90 minutes respectively. Finally, diaminobenzidine (DAB) step was performed on section according to the manufacturer's instructions,

and the sections were counterstained with haematoxylin. The cases were considered as positive for each marker when more than 5% of the cells showed positive expression or less than 5% expression was considered as a negative expression.

Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labelling was performed to assist in the detection of apoptotic cells in tissue sections. Apoptoticaly fragmented cellular DNA was identified by TUNEL assay according to kits guidelines (Apoptosis Detection Kit, Abcam, USA). Counterstaining with haematoxylin was made to evaluate and characterise the normal and apoptotic cells.

All values are expressed as mean ± SD. A level of $p \leq 0.05$ was taken as statically significant. Chi-square χ^2 test was used to make the correlation of marker with histopathological findings.

Results

Oral administration of CCl_4 to mice showed 2 (25%) mortality in CCl_4 treated group (Diseases group), whereas no mortality was seen in the other groups such as control group and CCl_4 plus thymoquinone treated group.

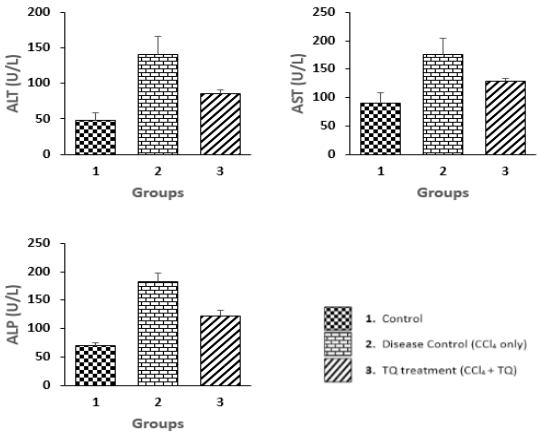

Figure 2: Effect of thymoquinone on serum liver function enzymes activity. Liver function enzymes were significantly reduced in CCl_4 plus thymoquinone treated group and was high in CCL_4 treated group ($p \leq 0.05$)

To study the therapeutic implication of thymoquinone (TQ) in liver toxicity, TQ was given to CCl_4-induced hepatotoxicity mice. The serum of ALT, AST and ALP enzymes activity was measured in different groups, and it was noticed that ALT, AST and ALP activity significantly increased in the CCl_4 treated group (Disease group) as compared to control group (Figure 2). Moreover, ALT, AST and ALP activity was significantly decreased in the group that received thymoquinone (CCl_4 plus thymoquinone treated mice group) ($p \leq 0.05$). This finding confirms that thymoquinone has a potential role in the liver protection through reduction of liver functions enzymes in CCl4 hepatotoxicity (Figure 2).

In this study, the activity of the antioxidant enzymes SOD, GPx, catalase activity and total antioxidant capacity were significantly decreased in the disease control group (group 2) as compared to the untreated control (Group 1) (Figure 3). Moreover, it was observed that thymoquinone significantly restore the antioxidant enzyme activity ($p \leq 0.05$) including SOD, GPx, catalase and total antioxidant capacity in CCl_4 plus thymoquinone group as compared to the disease control group (CCL_4 treated only) (Figure 3).

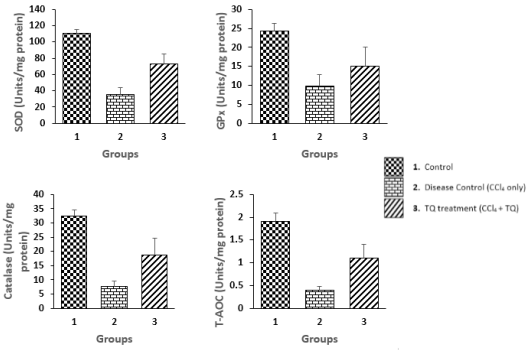

Figure 3: Effect of thymoquinone on SOD, GPx, Catalase and total antioxidant capacity (T-AOC) activity. Hepatic SOD, GPx, Catalase and total antioxidant capacity was high in thymoquinone plus CCl_4 treated groups as compared CCL_4 treated group only and this difference was statically significant) ($p \leq 0.05$)

Histopathological changes in liver tissues are presented in different groups of mice. The normal architecture of liver tissue was maintained in the control group (Figure 4a).

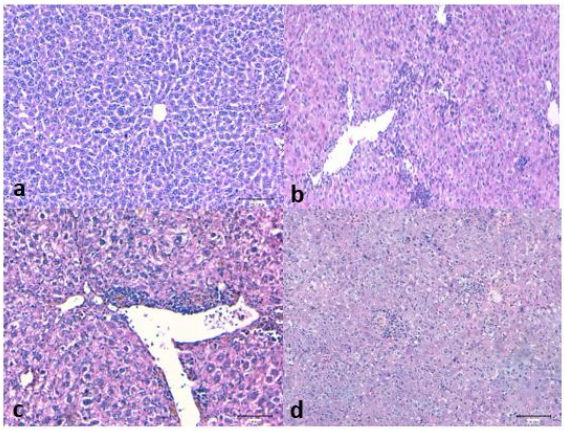

Figure 4 (a-d): a: Liver histology of control group showing the normal architecture of hepatocytes b: CCl_4 treated mice showing severe infiltration of inflammatory cells c: CCl_4 treated mice showing infiltration of inflammatory cells, and blood vessel dilation d: CCl_4 plus Thymoquinone treated mice showing less inflammatory cells and less congestion (Orig.MagX40)

Different types of liver tissue alterations were seen in CCl_4 treated group, and it was observed as severe congestion, infiltration of inflammatory cells, haemorrhages, fatty degeneration and blood vessel dilation (Figures 4b and c). Though these consequences were also noticed in the CCl_4 plus thymoquinone group, the incidence and severity of alterations were less than those in CCl_4 treated group (Figure 4d).

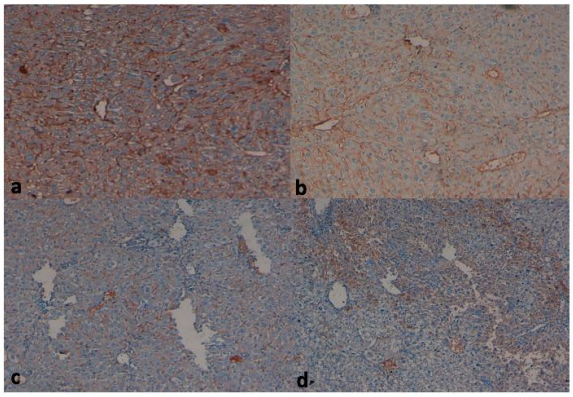

Figure 5: a) PTEN protein expression was detected in control cases and intensity of expression was high; b) PTEN protein expression was detected in CCl_4 plus thymoquinone treated group (Orig. X mag40); c) PTEN protein expression was detected in CCl_4 treated group, and the intensity of expression was low; d) PTEN protein expression was detected in CCl_4 treated group and intensity of expression was low (Orig. X mag40)

PTEN protein expression was examined in all the groups of mice and results were interpreted based on the expressional patterns. The loss of PTEN protein was noticed in CCl_4 treated group. The intensity of expression was high in the control group (Group 1) as well as in CCl_4 plus thymoquinone treated group (Group 3) as compared to CCl_4 treated group (Group 2) (Figure 5 a, b, and c).

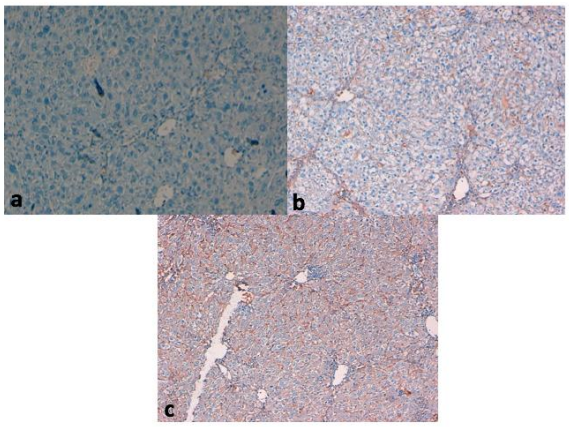

Figure 6: a) P53 protein expression was not detected in control cases; b) P53 protein expression was detected in CCl_4 plus thymoquinone group but expression intensity was low; c) P53 protein expression was detected in CCl_4 group, and intensity of expression was high (Orig. X mag40)

P53 expression was evaluated in all group of mice, and it was noticed that all 8 cases of the control group did not show any expression (Figure 6a). A higher expression of P53 protein was noticed in CCl_4 treated group (diseases control) (Figure 6b) as compared to CCl_4 plus thymoquinone group (Figure 6c). The difference in expression pattern among CCl_4 treated group and thymoquinone plus CCl_4 treated group was statically significant ($p < 0.05$)

The control group showed weak cytoplasmic VEGF expression (Figure7-a) whereas expression was high in CCl_4 treated group (Figure 7-b). Moreover, VEGF expression was also noted in thymoquinone plus CCl4 treated group (Figure 7-c). The difference in expression pattern between CCl_4 treated group and CCl_4 plus thymoquinone treated group was statically insignificant ($p > 0.05$)

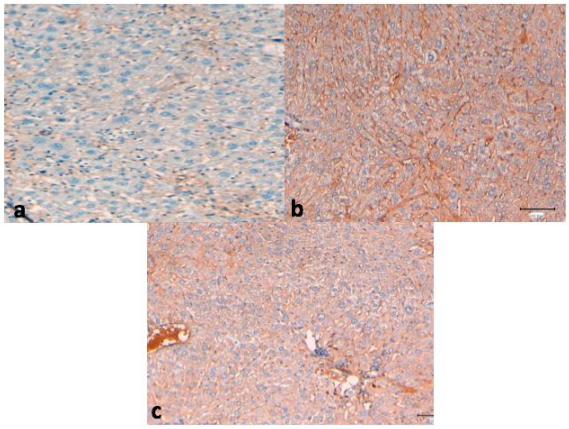

Figure 7: a) Weak VEGF protein expression was noted in control group; b) VEGF protein expression was detected in CCl_4 group; c) VEGF protein expression was detected in CCl_4 plus thymoquinone group (Orig. X mag40)

Apoptosis was not detected in any group including CCl_4 treated group (Figure 8a) as well as CCl_4 plus thymoquinone treated group (Figure 8b).

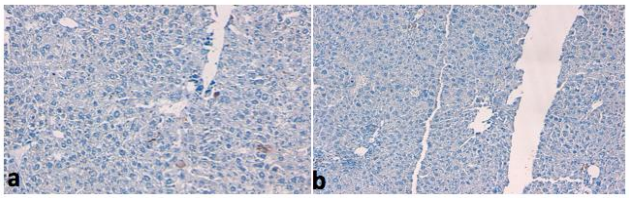

Figure 8: a) Apoptosis was not detected in CCl_4-treated group; b) Apoptosis was not detected in CCl_4 plus thymoquinone treated group (Orig. mag×40)

Discussion

The prevalence of liver pathogenesis is comparatively increasing, and the mortality and morbidity rates are significantly increasing in Saudi Arabia [15], [16]. The current study was based on a

mice model performed to examine the hepatoprotective effects of thymoquinone in repeated CCl_4 administration induced hepatotoxicity. CCl_4-induced acute liver injury in a murine model has been used for screening the hepatoprotective activities of drugs [17]. Thymoquinone (TQ) is an active constituent present in the black seed and broadly studied over the decades for its role in diseases cure without promoting any severe side effects. TQ has proven its role in the protection of organs against oxidative damage induced by a free radical generating agents [18]. TQ and a synthetic structurally-related TBHQ strongly inhibited iron-dependent microsomal lipid peroxidation in a concentration-dependent manner [19]. TQ importance has been discussed by earlier investigator [20], and TQ has proven role through modulation of diverse oncogenic transcription factors [21]. Moreover, another study was to investigate the effects of TQ on head and neck squamous cell carcinoma (HNSCC) cell lines. The result of the study revealed that TQ exhibited dose-dependent cytotoxicity via apoptosis in the investigated cell lines [22].

In the present study, TQ treatment showed significant protection against CCl_4-induced liver injury, which was noticed by the decrease of serum ALT, ALP and AST levels. Whereas serum activity of ALT, AST and ALP were significantly increased in the CCl_4 treated group (Disease control). This finding confirms that thymoquinone has a potential role in the liver protection through a reduction in liver functions enzymes. This result agrees with an earlier study [23] reported that serum ALT, AST levels, and SOD activity, as well as the serum and tissue MDA levels, were found to be higher in the acetaminophen group than in the control group. Whereas in the acetaminophen + TQ group, serum ALT, AST levels, SOD activity and the serum and tissue MDA levels were found to be lower as compared to that of the APAP group and such difference was statistically significant [23]. This result is consistent with the previous study, and it was reported that of exposure to sodium fluoride resulted in a change in liver function as designated by a significant increase in the activity of AST, ALT, ALP, LDH and the concentration of total bilirubin. Moreover, administration of TQ at a dose of 10 mg/kg protected the liver against sodium fluoride toxicity and improved its functioning as proven via the noteworthy decrease in these liver function biomarkers compared to the sodium fluoride group [24].

SOD, GPx is the major antioxidant enzyme-reducing superoxide and this way antioxidant enzyme prevent the pathogenesis. In this study, it was observed that thymoquinone, significantly restore the antioxidant enzyme activity including SOD, GPx and catalase in CCl_4 plus thymoquinone group as compared to the CCl_4 treated (disease control group). The activity of the antioxidant enzymes SOD, GPx and catalase were significantly decreased in the disease control group (Group 2) as compared to the untreated control (Group 1) (Figure 3). An interesting study reported that lead exposure significantly decreased reduced glutathione level and superoxide dismutase, glutathione peroxidase, catalase, and glutathione reductase activities in the renal tissue. Remarkably, supplementation with TQ significantly improved the affected antioxidant parameters [25]. Another finding result revealed that in the acetaminophen + TQ group, the GPx activity was found to be significantly higher compared to the acetaminophen group [23].

Liver tissue alterations were noted in CCl_4 treated group, and it was severe congestion, infiltration of inflammatory cells, degeneration and blood vessel dilation. Though these consequences were also noticed in the CCl_4 plus thymoquinone group, the severity of liver tissue alterations was less than those in the CCl_4 treated group. Earlier findings demonstrated that subchronic ethanol exposure caused severe steatosis, central vein congestion, and infiltration of inflammatory factors in focal portal space in the liver [26]. In this regard, other results demonstrated that the gentamicin-induced liver histological alterations, such as hydropic degeneration of hepatocytes, fatty changes, inflammatory cell infiltration and congestion of portal vein were successfully recovered by thymoquinone and curcumin treatment [27]. This finding revealed that TQ has potential effect in normalisation of architecture of liver tissue damage by CCl_4 induced hepatotoxicity.

PTEN is located on chromosome 10 and tumour suppressor gene is located in the 10q23 region encoding for a 403-amino acid multifunctional protein, which possesses lipid and protein phosphatase activities [28]. Additionally, the loss of PTEN gene expression causes abnormal activation of the PI3K/Akt and ERK pathways and, accordingly, leads to cancer cell proliferation and, finally, stimulates tumourigenesis [29]. In the current study, expression of PTEN protein was noted in all the animal groups. The intensity of the expression was high in the control group as well as in CCl_4 plus thymoquinone treated group as compared to CCl_4 treated group. Additionally, loss of PTEN protein expression was noticed in CCl_4 treated group as compared to control group as well as CCl_4 plus thymoquinone group. An interesting study based on TQ reported that TQ induces apoptosis in doxorubicin-resistant breast cancer cells through up-regulation of PTEN at transcription level and its treatment increased cellular levels of PTEN proteins, resulting in a significant decrease of phosphorylated Akt, a known regulator of cell survival [30].

P53 remains the most frequently mutated gene in several common human cancers, with mutations estimated to occur in 50% of all types of cancers [31]. Altered expression of P53 has been noticed in several types of tumors. P53 expression was evaluated in all groups of mice and it was noticed

that all cases of control group did not show any expression. A higher expression of P53 protein was noticed in CCl_4 treated group as compared to CCl_4 plus thymoquinone group. The difference in expression pattern among CCl_4 treated group and thymoquinone plus CCl_4 treated group was statically significant ($p < 0.05$). A recent study based on MCF-7 breast cancer cells confirms that thymoquinone can induce apoptosis in MCF-7 breast cancer cells through the up-regulation of P53 expression [32]. Another study reported that P53 higher expression was noticed in CCl_4 treated group as compared to control group and green tea extract (GTE) group. A supplementation of GTE with CCl_4 induced a significant reduction of P53 level [33]. Another study based on curcumin reported that STZ exposure significantly increased P53 protein levels and CUR attenuated this activation [34].

Vascular endothelial growth factor (*VEGF*) is a signalling protein that promotes the growth of new blood vessels. It is produced by several cell types including tumour cells [35], [36], macrophages [37] and its altered expression has been noticed in many tumours. In the current study, the control group showed weak cytoplasmic expression whereas expression was high in CCl_4 treated group. Moreover, VEGF expression was also noted in thymoquinone plus CCl_4 treated group. The difference in expression pattern between CCl_4 treated group and CCl_4 plus thymoquinone treated group was statically insignificant. Moreover, a finding demonstrated that administration of NAC (N-acetylcysteine) and ALA (α-Lipoic acid) and THQ (Thymoquinone) either alone or in combination along with acetaminophen down regulates flat-1 (VEGFR1) expression [38]. The potential anticancer activity of the combination of thymoquinone (TQ) and resveratrol (RES) against breast cancer in mice was evaluated, and the results demonstrated that combination therapy enhanced apoptosis, and decreased VEGF expression [39].

Understanding the mechanisms of apoptosis is crucial and helps in the understanding the pathogenesis of conditions as a result of disordered apoptosis [40]. Apoptotic bodies were not detected in any group including control, CCl_4 treated as well as CCl_4 plus thymoquinone treated group. In this regards, the previous study confirmed that apoptotic bodies were observed in cancer cases, while control cases did not show apoptosis [14].

In this study, a hepatotoxic agent such as CCl_4 causes liver hepatotoxicity as evidenced by the increase of liver function enzymes and also causes liver tissue alteration including infiltration of inflammatory cells, fatty degeneration and blood vessel dilation. The mice treated with CCl_4 plus thymoquinone shows role in the protection of the liver structure as the severity of fatty degeneration and blood vessel dilation and infiltration of inflammatory was less. Also, thymoquinone might play a role in restoring the liver antioxidant enzyme activity including SOD, GPx and Catalase. The intensity of PTEN expression was high well as in CCl_4 plus thymoquinone treated the group as compared to CCl_4 treated group. A higher expression of P53 protein was noticed in CCl_4 treated the group as compared to CCl_4 plus thymoquinone group and difference in expression pattern was statically significant. The protective effect of thymoquinone signifies a potentially preventive drug in the development of novel therapeutic agents for liver tissue alteration in the CCl_4 hepatotoxicity.

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Basic Science

Serum Iron Metabolism Variables in Clinically Healthy Persons

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Keywords: Iron homeostasis; Serum ferritin; Serum iron; Transferrin saturation; Chronic liver disease

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Abstract

BACKGROUND: In healthy persons, iron acquisition, trafficking and storage are strictly regulated processes due to the lack of a physiological pathway for the excretion of excess iron from the body. The liver, the duodenum and the bone marrow are involved in the regulation of iron metabolism.

MATERIAL: Subject to the testing were 60 healthy volunteers who took part in clinical trials.

METHODS: Case histories, physical check-up and demographic data including people's heights and weights, laboratory studies and tests using medical equipment.

RESULTS: None of the healthy persons were reported to have shown any deviation from the reference values for the serum markers of iron metabolism tested with the exception of hepcidin.

CONCLUSION: In healthy persons, there was only a positive correlation between iron level and IBC, and feedback between hepcidin serum levels and transferrin saturation.

Introduction

Iron homeostasis depends on a complex mechanism on the principle of feedback between the needs of the human body for iron and intestinal absorption. There is no physiological mechanism for the excretion of excess iron. The hepcidin hormone, discovered in 2001, is now considered to be the key regulator of iron homeostasis. The impaired regulation of hepcidin is the reason for many of the iron homeostasis disorders [1], [2].

In healthy persons, iron absorption, transport and storage are strictly controlled processes due to the lack of a physiological pathway for the excretion of excess iron from the body. The liver, the duodenum and the bone marrow are involved in the control of iron metabolism. Healthy elderly people have approx. 4 grams of iron in their bodies, about 3 grams of which are used to synthesize hemoglobin in the erythrocytes. When the erythrocytes get mature and die, their hemoglobin breaks down into its constituent parts-heme and globin. The heme-free iron is transferred from the transferrin to return to the heme synthesis cycle for erythropoiesis. These facts show that in a completely healthy adult, the body re-uses most of the available iron. Just 1 mg of iron is discharged from the body on a daily basis, mainly through the urine, faeces, enterocyte and epidermal desquamation in men, and through menstruation in women. Discharged iron is replaced by iron absorbed by the gastrointestinal tract through the food.

Material and Methods

Material

Subject to the testing were 60 healthy

volunteers involved in clinical trials None of them had any medical history, physical, laboratory, serological (HIV, HBV, HCV and acute HAV infection) testing as well as ECG and ultrasound data for current or past liver and gallbladder diseases, anemia, diabetes mellitus, cardiovascular, renal and other significant diseases, pregnancy, medication and toxic substance intake, including absolute alcohol over 20g daily, and any other conditions that could affect the results.

Methods

1. Case histories, physical check-up and demographic data.

2. Standard and specific disease-excluding laboratory blood and urine tests, immunological and virological studies.

3. Laboratory test for evaluation of iron exchange: - Serum iron (men: 12.5 – 26 mmol/L; women: 10.5 - 23 mmol/L); - TIBS (44 - 66 mmol/L); - transferrin saturation (Fe ÷ TIBC × 100% –20-40%); - serum ferritin (men: 20 – 280 mg/L women: 10 – 140 mg/L).

Case histories, physical check-up and demographic data including: height, weight, BMI [(kg/m^2 = body weight (kg)**/**Height (m^2)], waist circumference. The changes in body mass were calculated using the WHO-based BMI classification (Table 1) [2], [4].

Table 1: Assessment criteria for the changes in body mass according to the deviations from the WHO-based BMI [3], [4]

Group*	BMI (kg)/m2
Underweight by BMI	< 18.5
Normal weight by BMI	18.5 – 24.9
Overweight by BMI	25.0 – 29.9
Obesity by BMI	≥ 30.0
- Class 1	30.0 – 34.9
- Class 2	35.0 – 39.9
- Class 3	≥ 40

*****Other classes **–** morbid obesity (BMI - 40-50) and super obesity (BMI > 50).

Results

In none of the healthy persons we observed any deviation in the serum markers of iron metabolism tested from the reference values with the exception of hepcidin. Twelve of the investigated persons (20%) showed hepcidin below the lower reference value.

The mean values for the iron metabolism variables are given in Table 2.

The serum iron levels in healthy persons showed a positive correlation with IBC (Pearson correlation, r = 0.303, p = 0.019). There was also a reciprocal correlation between the serum hepcidin levels and transferrin saturation (Pearson correlation r = - 0.675, p = 0.0001).

Table 2: Serum iron metabolism variables in healthy people

	Iron metabolism variable	Healthy persons
Serum iron (mmol/L	Minimum	23.70
	Maximum	812,00
	Mean	18.30
	Median	16.80
	Standard deviation	4.66
IBC (mmol/L)	Minimum	10.80
	Maximum	30.2
	Mean	56.80
	Median	58.00
	Standard deviation	6.14
Ferritin (mg/l)	Minimum	45.00
	Maximum	73.5
	Mean	147.32
	Median	115.50
	Standard deviation	91.59
Transferrin saturation (%)	Minimum	68.00
	Maximum	397.00
	Mean	31.15
	Median	29.50
	Standard deviation	5.31
Hepcidin (ng/ml)	Minimum	21.00
	Maximum	40.01
	Mean	99.14
	Median	113.35
	Standard deviation	32.94

Relation of serum iron metabolism variables to sex, age and BMI

In the group of healthy persons, we identified that the serum iron, IBC and ferritin values were considerably higher with men than those with women (Table 3) and also that there was no significant differences in transferrin saturation (p = 0.143) and hepidine (p = 0.228) between both sexes.

Table 3: Serum iron metabolism variables (x± SD) relating to the sex of clinically healthy persons (Mann-Whitney)

	Variable	Healthy persons		
		Men	Women	P=
Serum iron (mmol/L	Mean	18.84	17.77	0.008
	Standard deviation	3.09	5.83	
IBC (mmol/L)	Mean	58.76	54.84	0.034
	Standard deviation	6.44	5.21	
Ferritin (mg/l)	Mean	152.83	141.80	0.002
	Standard deviation	72.14	108.62	
Transferrin saturation (%)	Mean	32.27	30.03	0.143
	Standard deviation	5.38	5.08	
Hepcidin (ng/ml)	Mean	94.80	103.47	0.228
	Standard deviation	34.26	31.54	

We identified no difference in the serum markers of iron metabolism of people under 45 years of age and those over 45 years old (Table 4). Furthermore, we could not prove any relation based on age.

Table 4: Serum iron metabolism variables (x± SD) for clinically healthy persons aged under 45 and those aged over 45 (Mann-Whitney)

	Variable	Healthy persons		
		< 45 r	> 45 r	P
Serum iron (mmol/L)	Mean	18.86	18.03	0.678
	Standard deviation	5.18	4.42	
IBC (mmol/L)	Mean	56.82	56.80	0.771
	Standard deviation	6.13	6.22	
Ferritin (mg/l)	Mean	127.10	157.43	0.327
	Standard deviation	66.05	101.25	
Transferrin saturation (%)	Mean	29.95	31.75	0.194
	Standard deviation	5.64	5.10	
Hepcidin (ng/ml)	Mean	108.72	94.35	0.111
	Standard deviation	26.24	35.15	

The average BMI value for the group of healthy persons was 23.01 ± 2.54 (ranging between 19.5 and 29.70), 50 (83%) of whom had normal body weight (BMI < 25) and the remaining 10 were overweight (BMI > 25 and < 30). No significant relation was identified between BMI and the serum iron metabolism variables (Table 5).

Table 5: Serum iron metabolism variables (x± SD) for clinically healthy persons, obese and not obese (BMI > 30 and < 30) (Mann-Whitney)

Variable	BMI group	Mean value	Standard Deviation	P =
Serum iron (mmol/L)	< 30	20.98	7.67	0.0001
	> 30	27.62	7.25	
IBC (mmol/L)	< 30	64.50	11.45	0.0001
	> 30	74.92	11.92	
Ferritin (mg/l)	< 30	194.68	192.83	0.0001
	> 30	355.93	231.10	
Transferrin saturation (%)	< 30	31.30	7.44	0.003
	> 30	35.38	7.24	
Hepcidin (ng/ml)	< 30	87.59	39.94	0.002
	> 30	64.65	39.15	

Discussion

In recent years, there has been an increased interest in the conditions and diseases accompanied by higher iron status and their evolution. Currently there are no sufficient comparative data available on the incidence and the characteristics of such disorders in people with chronic liver diseases compared to healthy people. For this purpose, we studied the serum iron metabolism in 60 healthy persons.

Iron is essential for a number of key biological processes including erythrocyte production, DNA synthesis and cellular respiration [3], [5], [6]. The normal iron content in the body of an adult man is 35-45 mg. iron per kilogram of body weight [3]. Most of the iron is combined with hemoglobin in the erythrocytes. The rest is distributed in the myoglobin in the muscles, in the tissue enzymes and the plasma transferin [3]. The liver parenchymal cells and the reticuloendothelial macrophages serve as a depot for storage of excess iron [3], [7]. In connection with its potential to take part in reactions as a transition metal, iron can be toxic in the cell [7], [1]. Iron-mediated generation of highly toxic Reactive Oxygen Species (ROS) plays a major role in the process leading to iron overload-related diseases [8].

Since there is no physiological pathway for the removal of excess iron from the body, iron acquisition, metabolism and storage must be strictly regulated [2], [6], [9]. A large number of new iron-regulating molecules, including iron transporters and soluble mediators, have been identified in recent years. Divalent Metal Transporter-1 (DMT1, also known as Nramp2) is a multifunctional transmembrane protein [4], [10], [11] responsible for the passage of non-food iron through the apical surface of the absorbing enterocytes in the duodenum. Ferroportin (also known as MTP1, Ireg1) transports iron into the blood. As a portable metal, iron undergoes reduction and oxidation reactions during this cell intake [7], [11]. Iron circulates in the plasma by binding to glyco protein, transferrin (Tf). The iron delivering Tf is absorbed by the cell to form compounds with transferrin receptor 1, TrfR [3], [7], [10].

Regulation of the iron metabolism involves many organs – the duodenum, the liver, the bone marrow. The identification of hepcidin-peptide emphasizes the importance of the liver in iron homeostasis. Hepcidin is an antimicrobial peptide that is isolated from human urine and blood [3], [6]. It is synthesized in the liver hepatocytes. Hepcidin synthesis in the liver is sensitive to iron levels-it increases in the presence of excessive iron and decreases in cases of iron deficiency [12], [13], [14].

Interestingly, the synthesis of hepcidin is also regulated by inflammatory signals and by the inflammatory cytokines IL-1 and IL-6 [1], [15]. The role of the Kupffer cells in the regulation of hepcidin levels in inflammation is disputable.

Hepcidin plays a major role in iron metabolism by inhibiting intestinal iron metabolism and iron release by the macrophages [7], by binding to ferroportin and inducing cellular penetration and degradation [15].

In conclusion, a better understanding of the molecular mechanisms that regulate the iron homeostasis can help develop therapeutic strategies and diagnostic methods for the detection of liver diseases in their early stages before they could turn into chronic diseases and cause irreversible liver damage.

In healthy persons there is only a positive correlation between iron level and IBC, and feedback between hepcidin serum levels and transferrin saturation.

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Clinical Science

Neurocognitive Function and Its Related Potentials in Children with Beta Thalassemia Major: An Egyptian Study

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Abstract

BACKGROUND: Repeated blood transfusions and hemolysis in β-Thalassemia major children lead to iron overload in various organs, including the brain which may cause neurodegeneration.

AIM: To evaluate intelligence quotient in children with β-thalassemia major and healthy counterparts and to assess risk factors that cause cognitive problems.

SUBJECTS AND METHODS: This case-control study was performed on 50 children aged 6-16 years old with β-thalassemia major as patients group and compared with 50 healthy children as a control group of matched age, sex, and social class. Cognitive functions were evaluated by using the Wechsler Intelligence Scale for Children. Serum ferritin and iron were measured by ELISA.

RESULTS: There were significantly lower mean performance and full-scale IQ scores of patients group in comparison with controls, whereas no significant differences between both groups as regards to a verbal IQ score. In thalassemic children, block design, comprehension and arithmetic were negatively correlated with age of disease onset, duration of illness and onset of chelation therapy. Serum iron and ferritin were negatively correlated with similarities and digit span. Serum iron levels were negatively correlated with performance IQ score.

CONCLUSION: Children with β-thalassemia major need to receive more academic attention and cognitive assessment to improve their IQ.

Introduction

β-Thalassemia major is a chronic hematologic disease of a genetic basis that is characterised by ineffective erythropoiesis, peripheral hemolysis, and severe anaemia [1]. It is the commonest chronic hemolytic anaemia in Egypt (85.1%), with a carrier rate about 9% to 10.2% from an examination of 1000 normal random subjects from different geographic areas of the country [2].

The affection of the nervous system in those patients can be attributed to many factors acting as several cumulative small injuries to the central nervous system over many years such as hemolysis and repeated blood transfusions which lead to a decrease in nitric oxide levels and iron overload in several organs, including the brain. Also, increased iron in the brain leads to oxidative stress and possible irreparable brain tissue damage, causing cognitive impairment [3], [4]. Thalassemic patients are dependent on regular blood transfusion to decrease complications of anaemia and expand bone marrow [5]. This repeated blood transfusion is associated with excessive iron absorption [6], iron overload, chronic hypoxic state; in addition to neurotoxicity due to lifelong chelating therapy (deferoxamine). All of these factors lead to brain dysfunction [7], [8]. Metafratzi et al., [9] stated that there was high iron deposition in the putamen, caudate nucleus, motor and temporal cortex of patients with β-Thalassemia major. These areas are extremely important for cognitive function as for implicit and explicit memory. Other risk factors for brain damage include transient ischemic attacks, asymptomatic brain infarcts and visual and auditory toxicity of deferoxamine [10]. In another study, Economou et al., [11] demonstrated subclinical involvement of central and peripheral neural pathways in β-Thalassemia major patients and recommended regular intellectual monitoring using the Wechsler

Intelligence Scale, for early detection of any intellectual dysfunction in young β-Thalassemia major patients [12].

Therefore, this study aimed to evaluate the neurocognitive function in β-thalassemia major patients and recognising the correlation between possible neurocognitive dysfunction and various clinical parameters as the age of disease onset, the frequency of blood transfusion, iron chelating drugs, and serum ferritin, iron, and haemoglobin concentration of the patients.

Subjects and Methods

Subjects

A descriptive case-control study was conducted in the outpatient Child Health Clinic in Centre of Medical Excellence, National Research Center, Egypt, throughout 8 months. One hundred children; 50 children with b-thalassemia major (25 boys, and 25 girls; mean age, 9.57 ± 1.33 years; range, 6-16 years), were enrolled into this study and referred from the Pediatric Clinical Hematology Outpatient Clinic at Ain Shams University Hospital in Egypt. They were compared with a group of 50 healthy children with matched age, sex, educational level, parental education, and years of schooling, and socioeconomic level, which were cognitively and neurologically normal, acting as healthy volunteers. Their past medical history and clinical examination were revised for confirmation of being and their first-degree relatives completely free of any chronic disorders, including thalassemia.

The inclusion criteria comprised of children diagnosed with β-thalassemia major aged between 6-16 years with regular blood transfusions and iron chelation treatment, formal education, and no fever. The exclusion criteria were as follows: (a) had history of major mental disorders with delayed milestone development; (b) had physical disabilities that could interfere with performance, such as deafness or blindness; (c) had received prior treatment with drugs known to be neurotoxic; or (d) had history of chronic medical illness other than thalassemia that could affect cognition. Children in β-thalassemia major group were receiving blood once a month regularly to maintain their haemoglobin concentration at more than 9 gm/dl level, and they were taking deferoxamine as medication.

Written informed consent was taken from all patients' guardians before participation in the study and after complete explanation of their task in the research. The consent was approved by The Ethical Committee of The National Research Center and Ain Shams University under the registration number 16358.

Methods

Clinical assessment and anthropometric measurements

Full medical history, complete data on transfusion and chelation regimens were taken from all patients. Clinical examination was performed with special emphasis on disease-related complications and chelation therapy-related side effects. Anthropometric measurements including weight, height, and head circumferences were assessed for all the studied subjects at the National Research Centre. Calculation of the body weight was done to the nearest 0.1 kg by a standard clinical balance. Measuring standing body height was done to the nearest 0.1 cm by using Holtain Stadiometer. Recalibration of all scales took place after each measurement following the recommendations of the International Biological Program [13]. Calculation of the subjects' body mass index (BMI) was done by using the formula of weight/height^2 (kg/m^2).

Laboratory investigation

After an overnight fast, venous blood samples were withdrawn from all participants and the separated sera were stored at −20°C. Routine laboratory investigations were done for thalassemic patients according to international standards, including complete blood picture, serum ferritin, and iron using enzyme-linked immunosorbent assay ELISA kit obtained from Glory Bioscience, (USA), according to manufacturer's manual.

Neuropsychological testing

All the patients and controls were subjected to IQ evaluation by using the Arabic version of the Wechsler Intelligence Scale for Children-Third Edition, (WISC-R) [14]. This test assesses the intelligence of children in three scales of full IQ, verbal IQ and performance IQ. Full-scale IQ is based on 10 tests incorporated in the verbal and performance (non-verbal) IQ scales. The administration time for the test was approximately 60 to 90 minutes. The child was allowed to complete the test in 2 separate sessions.

Verbal IQ is based on information, similarities, arithmetic, comprehension and digit span. The Comprehension subtest is a scale of the student's social knowledge and the depth of development of morals. Similarities subtest is a measure of logic, abstract thinking and verbal reasoning, while information is a scale of general knowledge, education, and long-term memory of his experience. Arithmetic and digit span subtests are measures of working, short, and long-term memory.

Performance (non-verbal) IQ is based on picture completion, coding, picture arrangement, block

design and object assembly. Block Design measures the ability to analyse and synthesise an abstract design, and production of the design from coloured plastic. The Picture Completion subtest is a measure of a student's capability of recognising closely related items. The Object Assembly subtest is a measure of the ability of visualisation of item parts of Mazes. The mazes subtest measures perceptual organisation, visual-motor coordination, and self-control.

Interpretation of IQ Score

The IQ was graded based on the following guidelines:

- 130 and higher: very superior;
- 120-129: superior;
- 110-119: high average;
- 90-109: average;
- 80-89: low average;
- 70-79: borderline;
- 69 and lower: extremely low.

Statistical Analysis

SPSS version 22 (SPSS Inc, Chicago) was used to enter, check and analyse data. Results were expressed as mean ± standard deviation (SD) for quantitative variables and as number and percentage for qualitative ones. Based on the calculated required sample size, our study included 50 subjects with β-thalassemia major. The marked differences in means for IQ between patients and controls and between males and females were analysed by unpaired Student t-test. Association between qualitative variables was done using a Chi-Squared test. Correlation between IQ and other parameters were investigated using Pearson coefficient of correlation. A p-value < 0.05 was considered significant and p < 0.01 was considered highly significant.

Results

The education level, school performance and disease complications of the two groups are shown in Table 1. In the present study, all candidates were between 6 and 16 years old. Both patients and control groups were matched as regards their age, sex, educational level, parental education, and socioeconomic status. Lower school performance, growth retardation, and positive family history of thalassemia were significantly more common in the patient's group compared to the control group. Thalassemic patients had disease complications in the form of splenectomy that was present in 32%, hemosiderosis in 46%, hypogonadism in 28%, and hypoparathyroidism in 16%.

Table 1: Education level, school performance and disease complications of the two groups

Variables		Patients group N (%)	Control group N (%)
Education level	Uneducated	4 (8%)	4 (8%)
	Read and write only	8 (16%)	13 (26%)
	Educated	38 (76%)	33 (66%)
School performance	Poor	18 (36%)	15 (30%)
	Average	22 (44%)	17 (34%)
	Above average	10 (20%)	18 (36%)
Disease complications	Positive family history	30 (60%)	0 (0%)
	Positive consanguinity	29 (58%)	0 (0%)
	Bone fracture	24 (48%)	8 (16%)
	Splenectomy	16 (32%)	0 (0%)
	Mongoloid facies	15 (30%)	0 (0%)
	Hemosidrosis	23 (46%)	0 (0%)
	Growth retardation	21 (42%)	6 (12%)
	Hypogonadism	14 (28%)	0 (0%)
	Hypoparathyroidism	8 (16%)	0 (0%)
	Hypothyroidism	25 (50%)	0 (0%)

In thalassemic patients group, the mean age at onset of symptoms was 7 ± 1.4 months, and the mean duration of illness was 9.89 ± 4.4 years. The mean frequency of blood transfusion per year is 4.08 ± 1.31times and range from 2 to 8 years. The mean duration of chelation therapy is 9.16 ± 4.43 years. Eighty-four per cent of the patients were on chelation therapy, in the form of deferoxamine as shown in Table 2.

Table 2: Clinical characteristics of the studied patients

Variables	Range	Patients group Mean ± SD
Age (years)	6-16	11.05 ± 3.83
Age at onset (months)	6-8	7 ± 1.4
Duration of illness (years)	2-15	9.89 ± 4.30
Duration of chelation therapy (years)	6-16	9.16 ± 4.43
The frequency of blood transfusion per year	2-8	4.08 ± 1.31

The mean and standard deviation of the age of children with β-thalassemia major was 9.57 ± 1.33, and that of the healthy children was 9.5 ± 1.31. In thalassemic patients group, the mean weight, and BMI (P < 0.01) were significantly lower compared to the control group. The mean serum levels of iron, and ferritin (P < 0.01) were significantly higher, while haemoglobin concentration (P < 0.01) was significantly lower compared to the control group as shown in Table 3.

Table 3: Comparison of anthropometric measures and laboratory investigations of patients group and control group

Variables	Control group Mean ± SD	Patients group Mean ± SD	t-test	P-value
Age (years)	9.5 ± 1.31	9.57 ± 1.33	0.36	0.24
Weight (kg)	44.18 ± 15.54	30.64 ± 11.15	-5.006	0.000**
Height (cm)	139.46 ± 15.07	134.62 ± 17.12	-1.5	0.137
BMI	22.18 ± 5.39	16.52 ± 2.69	-6.638	0.000**
Head circumference (cm)	52.62 ± 1.7	52. 28 ± 2.78	0.346	0.73
Hb concentration (gm/dl)	12.96 ± 1.12	11.024 ± 2.16	-3.334	0.001**
Serum ferritin (ng/ml)	142.44 ± 69.66	2453.36 ± 1297.12	12.580	0.000**
Serum iron (mcg/dl)	94.20 ± 32.16	233.09 ± 67.47	13.138	0.000**

*Significant difference at p < 0.05; **highly significant difference at p ≤ 0.01.

The mean performance IQ score (86.10 ± 20.13) of cases (P < 0.01) are significantly less than those of controls (100.98 ± 7.06), and the mean total IQ score (92.86 ± 17.72 and 101.42 ± 6.47, respectively; P < .05; whereas there is no significant difference among patients group and control group as regards to verbal IQ score (100.10 ± 16.49, and

102.30 ± 6.98, respectively; P > 0.05). The mean scores of comprehensions, similarities, digit span tests (verbal IQ subsets) of patients group are significantly lower than those of controls. The object assembly, coding, and mazes (performance IQ subsets) of patients group are significantly lower than those of controls. Comparison of verbal, performance, and full IQ scores and its subsets between patients group and control group are presented in Table 4.

Table 4: Comparison of IQ scores and its subsets between patients group and control group

Variables	Control group (N = 50) Mean ± SD	Patients group (N = 50) Mean ± SD	t-test	P value
Verbal IQ scores	102.30 ± 6.98	100.10 ± 16.49	-0.869	0.387
Information	9.72 ± 3.57	9.96 ± 1.71	-0.429	0.669
Comprehension	12.50 ± 4.86	10.70 ± 1.94	2.433	0.017*
Arithmetic	7.60 ± 3.37	7.54 ± 2.27	0.104	0.917
Similarities	11.60 ± 3.61	10.16 ± 1.83	2.513	0.014*
Digit span	5.84 ± 2.98	4.76 ± 1.66	2.239	0.027*
Performance IQ scores	100.98 ± 7.06	86.10 ± 20.13	-4.932	0.000**
Picture completion	8.02 ± 2.06	7.70 ± 3.01	-0.622	0.536
Block design	6.88 ± 3.27	6.28 ± 1.45	-1.186	0.239
Object assembly	6.82 ± 0.87	5.70 ± 3.41	-2.252	0.027*
Coding	10.20 ± 2.88	7.68 ± 4.78	-3.194	0.002**
Mazes	10.32 ± 1.88	8.98 ± 4.22	-2.053	0.043*
Full IQ scores	101.42 ± 6.47	92.86 ± 17.72	-3.209	0.002**

*Significant difference at p < 0.05; **highly significant difference at p ≤ 0.01.

A significant difference exists between males and females groups as regards digit span and mazes (P < 0.01). No significant difference present among male and female patients in other verbal performance, full IQ scores, and other IQ subtests (P > 0.05) as demonstrated in Table 5.

Table 5: Comparison between males and females as regard to IQ scores in patients group

Variables	Males Mean ± SD	Females Mean ± SD	t-test	P value
Verbal IQ scores	101.08 ± 17.71	99.12 ± 15.48	0.417	0.679
Information	10.00 ± 4.12	9.44 ± 2.97	0.551	0.584
Comprehension	13.32 ± 5.09	11.68 ± 4.57	1.199	0.236
Arithmetic	8.12 ± 3.32	7.08 ± 3.04	1.094	0.280
Similarities	11.76 ± 3.88	11.44 ± 3.4	0.310	0.758
Digit span	6.68 ± 3.08	5.00 ± 2.68	2.059	0.045*
Performance IQ scores	87.04 ± 20.42	85.16 ± 20.21	0.327	0.745
Picture completion	8.12 ± 3.47	7.28 ± 2.46	0.988	0.328
Block design	6.72 ± 3.62	5.84 ± 2.88	0.950	0.347
Object assembly	5.80 ± 3.77	5.60 ± 3.07	0.206	0.838
Coding	7.52 ± 4.16	7.84 ± 5.41	0.234	0.816
Mazes	10.16 ± 4.54	7.80 ± 3.57	2.042	0.047*
Full IQ scores	93.76 ± 18.82	91.96 ± 16.89	0.356	0.723

*Significant difference at p < 0.05.

Three patients (6%) were superior, (8%) of patients with high average full IQ scores, (34%) average, (22%) patients with low average, and (14%) patients with extremely low full IQ scores as shown in Table 6.

Table 6: Distribution of IQ grades among the patient's groups

Variable	Very superior	Superior	High average	Average	Low average	Borderline	Extremely low
Full IQ scores	0 (%)	3 (6%)	4 (8%)	17 (34%)	11 (22%)	8 (16%)	7 (14%)

In thalassemic children, block design (one of performance IQ subsets), and comprehension and arithmetic (some verbal IQ subsets) were negatively correlated with age of disease onset, duration of illness and chelation therapy. No significant correlation is found between IQ scores (Verbal, performance, and full-Scale) and frequency of transfusion as shown in Table 7.

Table 7: Correlation between cognitive variables with some clinical variables

Variables		Age of disease onset	Duration of illness	Duration of chelation therapy	The frequency of blood transfusion/ year
Verbal IQ score	Pearson correlation	0.052	0.037	0.053	-0.089
	P- value	0.719	0.799	0.717	0.537
Information	Pearson correlation	0.007	-0.021	-0.011	0.014
	P- value	0.962	0.887	0.938	0.925
Comprehension	Pearson correlation	-0.406**	-0.379**	-0.382**	-0.023
	P- value	0.003	0.007	0.006	0.877
Arithmetic	Pearson correlation	-0.374**	-0.387**	-0.366**	-0.099
	P- value	0.007	0.006	0.009	0.493
Similarities	Pearson correlation	0.114	0.090	0.102	-0.127
	P- value	0.431	0.533	0.480	0.379
Digit span	Pearson correlation	-0.161	-0.214	-0.205	0.182
	P- value	0.265	0.136	0.153	0.207
Performance IQ score	Pearson correlation	-0.159	-0.196	-0.180	-0.017
	P- value	0.271	0.174	0.212	0.909
Picture completion	Pearson correlation	0.000	-0.095	-0.087	0.162
	P- value	0.998	0.512	0.549	0.261
Block design	Pearson correlation	-0.411**	-0.475**	-0.459**	-0.034
	P- value	0.003	0.000	0.001	0.815
Object assembly	Pearson correlation	-0.077	-0.137	-0.128	-0.132
	P- value	0.596	0.344	0.376	0.361
Coding	Pearson correlation	-0.133	-0.210	-0.196	-0.205
	P- value	0.357	0.144	0.172	0.153
Mazes	Pearson correlation	0.027	0.006	0.005	0.108
	P- value	0.851	0.967	0.975	0.457
Full IQ score	Pearson correlation	-0.092	-0.117	-0.099	-0.045
	P- value	0.525	0.418	0.493	0.755

**Highly significant difference at p ≤ 0.01.

Anthropometric measures as weight, and height were positively correlated with some verbal IQ subsets as comprehension and arithmetic as well as block design (one of performance IQ subsets). BMI is positively correlated with performance IQ and some of its subsets as block design and coding as well as some verbal IQ subsets as comprehension and arithmetic. Head circumference was positively correlated with arithmetic (verbal IQ subset), and coding (performance IQ subset). There were significant negative correlations between serum ferritin levels and comprehension, similarities, and digit span (some of the verbal IQ subsets). Serum total iron was negatively correlated with a performance IQ score, similarities, and digit span (some of the verbal IQ subsets). Haemoglobin concentration was positively correlated with digit span (verbal IQ subset), and object assembly (performance IQ subset) as shown in Table 8.

Discussion

Multiple risk factors contribute to cognitive impairment in β-thalassemia major patients [15]. Duman et al., [12], Vichinsky et al., [16] denoted the involvement of the nervous system in b-thalassemia major patients. They attributed the neurological problems to several causes as iron overload, bone marrow expansion, chronic hypoxia and desferrioxamine neurotoxicity in thalassemia patients.

In our study, a positive family history of β-thalassemia major was present in 60%, and positive consanguinity in 58%. Disease complications in thalassemic patients were found in the form of splenectomy that was present in 32%, growth retardation in 26%, hemosiderosis secondary to long term blood transfusion in 46%, hypogonadism 28%, and hypoparathyroidism in 16%. These findings were in agreement with Egyptian study done by Raafat et al., [17] who reported growth retardation in 42%, hemosiderosis in 46%, hypogonadism in 22%, and hypoparathyroidism in 6%, hypothyroidism in 4%, cardiac complication in 6%.

Ferritin is a protein that stores iron and exists in all tissues including the brain. Serum ferritin level is a good marker for assessing body iron stores [18]. In our study, the mean levels of serum iron, and ferritin were markedly higher, while haemoglobin concentration was significantly lower in thalassemic patients group in comparison to control group. These results come in agreement with the previous study by Fadlyana et al., [19] who assessed patients with beta-thalassemia major, registered at 2 different centres of Rawalpindi and Islamabad. They stated that the majority of patients demonstrated very high ferritin levels, in which 76% of patients had values above 250 mg/L. The high level of ferritin in beta-thalassemia can be attributed to repeated blood transfusions, ineffective erythropoiesis, and increased gastrointestinal iron absorption which causes iron overload in the body.

Thalassemia can affect growths in the fetal, infancy, pre-puberty and puberty periods [20]. The principal cause of growth disorders in β-thalassemia major patients are influenced by many factors and still debated [21]. High serum ferritin levels and iron overload in puberty were reported to cause short stature and delayed body growths in thalassemia major patients [22]. Iron overload can prohibit bone metabolisms leading to growth disorders [23], [24]. In our present study, as thalassemia is a chronic form of anaemia, the weight difference is expected compared to control. The anthropometric measures as weight and BMI were affected and significantly lower in thalassemic patients compared to controls ($P < 0.01$).

Our study analyzed the intelligence quotient of β-thalassemia major patients and healthy peers in terms of full, verbal and performance scales of IQ using 10 subscales of Wechsler IQ test for children and demonstrated that β-thalassemia major patients had marked lower performances and full-Scale IQ scores compared to controls; while, there was no difference in Verbal IQ between patients and controls. These results were compatible with Egyptian study done by Raafat et al., [17] who found marked lower performances and full-scale IQ scores and no apparent variations in verbal IQ scores in thalassemic patients' group in comparison to control group. In another study, Economou et al., [11] studied the IQs of children with β-thalassemia major using WISC III and said that those children had higher scores on the verbal scale than Full and performance scales, and claimed that β-thalassemia probably had increased impairments in cognitive performance. These results about higher verbal scale scores are also similar to our results.

Our results partly came in agreement with those of other studies that reported impairment of full-scale IQ (including both verbal and performance components) in children with β-thalassemia major. Duman et al., [12] had evaluated cognitive function in 20 children with β-thalassemia major and 21 healthy controls and found that Full-Scale IQ, verbal IQ, and performance IQ ($P < 0.05$) were markedly lower in the patients. In another study, Monastero et al., [25] assessed cognitive function in 46 β-thalassemia major patients and 46 controls of matching age, sex, and education and found that the β-thalassemia major patients, particularly those having signs of hemosiderosis, were profoundly affected on all neuropsychological tests. However, our results were not in agreement with Vichinsky et al., [16] as regards the verbal scale. Conversely, Karimi et al., [26] stated that there is no significant difference in IQ between patients of β-thalassemia major and controls. Again, Khairkar et al., [27] reported normal IQ in β-thalassemia major patients.

In our present study, the mean scores of comprehensions, similarities, digit span tests (verbal IQ subsets) of patients group are markedly lower than those of controls. The object assembly, coding, and mazes (performance IQ subsets) of patients group are significantly lower in comparison to those of controls. These findings agreed with Homayouni et al., [28], who reported that the verbal IQ subsets of β-thalassemic children were significantly lower than that of healthy group in terms of information, arithmetic, comprehension, digit span, and the performance IQ subsets as picture completion, symbol search and mazes subscales of β-thalassemic children were significantly lower than that of healthy controls.

In our study, three patients (6%) were superior, (8%) of patients had high average full IQ scores, (34%) average, (22%) patients with low average, and (14%) patients with extremely low full IQ scores. Canatan et al., [29] reported that academic problems were found in 60% of a sample population of thalassemic children. These findings suggest that there is a little caring about the quality of education of those children.

Economou et al., [11] who demonstrated no association between gender of the patients and abnormal IQ in β-thalassemia major. In our study, there was a marked difference between male and female groups as regards digit span and mazes ($P < 0.01$); but, there was no marked difference between gender of the patients and other verbal IQ, performance IQ, and full-scale IQ subtests.

This study discovered a positive correlation

between weight and height with some verbal IQ subsets as comprehension and arithmetic as well as block design (one of performance IQ subsets). Body mass index (BMI) is positively correlated with performance IQ and some of its subsets as block design and coding as well as some verbal IQ subsets as comprehension and arithmetic. Head circumference was positively correlated with arithmetic (one of the verbal IQ subsets), and coding (one of performance IQ subsets). To our knowledge, the association between anthropometric measures as weight, height, BMI, head circumferences and abnormal IQ scores in the patients of β-thalassemia major has not yet been investigated.

In our study, the influence of age of onset of symptoms, frequency of blood transfusion/ year, duration of illness, and chelation therapy and the effect of concentration of haemoglobin, serum iron, and ferritin levels on IQ scores of the patients were also examined. The reasonable clarification for lower mean performance IQ in our study, in comparison to that of verbal IQ, comes from the study of Ai et al., [18] on 171 Chinese children who found that children with low haemoglobin concentration had marked lower scores in performance IQ but not in verbal IQ. They explained this by that the low Hb concentration could have affected the brain areas that are responsible for performance IQ components during the critical stage of development in early childhood. This explanation can be applied to patients of β-thalassemia major who suffered from severe anaemia in their early childhood.

In our study, there were significant negative correlations between serum ferritin levels and comprehension, similarities, and digit span (some of the verbal IQ subsets). Serum total iron was negatively correlated with a performance IQ score, similarities, and digit span (some of the verbal IQ subsets). Haemoglobin concentration was positively correlated with digit span (verbal IQ subset), and object assembly (performance IQ subset). These findings agreed with Duman et al., [12] and Vichinsky et al., [16] who found a marked negative correlation between serum ferritin concentrations with some cognitive function. These findings can be attributed to marked erythropoiesis and hemolysis. On the contrary with our study, Shehata et al., [30] stated that there is no important correlation between IQ (verbal, performance, and full-scale) and age of onset of transfusion, onset of chelation therapy, chelation compliance, and serum ferritin levels. Besides, there was no marked correlation between IQ and chelation therapy type.

In our study, abnormal block design (one of performance IQ subsets), and comprehension and arithmetic (some verbal IQ subsets) were negatively correlated with age of disease onset, duration of illness and onset of chelation therapy in thalassemic children. No significant correlation is found between IQ scores (verbal, performance, and full-Scale) and frequency of transfusion. Our results were not compatible with Economou et al., [11] who claimed no significant relation between abnormal IQ in β-thalassemia major patients and serum ferritin levels, the age of onset of symptoms, the onset of transfusion, the onset of chelation therapy, chelation compliance, type of chelation therapy, and disease complications.

In conclusion, this study concluded that performance IQ, not verbal IQ is significantly affected in the studied children with β-thalassemia major being affected by long duration of illness, and the onset of chelation therapy, and high serum ferritin and iron levels. Cognitive assessment is easy to be done, and β-thalassemia major children with learning problems need to receive more academic attention in order to improve their performance IQs.

Recommendation: Intelligence quotient tests should be a part of routine comprehensive care of β-thalassemia major patients especially cases of learning difficulties to detect cases that suffering from verbal or performance dysfunction and to be subject to the learning skills development program.

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Clinical Science

The Effect of Lower and Higher Calorie Meal on the Parameters of Ventricular Repolarization in Healthy Subjects

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Abstract

BACKGROUND: Cardiovascular modulation following meal consumption has been known. Little and conflicting data is present regarding electrocardiographic QT and QTc intervals after a meal, and status of ventricular repolarization following meal is not known comprehensively.

AIM: To inquire the electrocardiographic status of ventricular repolarisation thoroughly after lower and higher calorie meal consumption in a comparative manner.

METHODS: A group of 61 healthy individuals were studied before and after lunch. They were divided into two groups according to the calorie consumed (higher calorie and lower calorie; median 1580 and 900 kcals, respectively). Calorie consumed was estimated using dietary guidelines. Data was collected from 12-lead ECG both in a fasted state and 2nd postprandial hour for each participant. Parameters of ventricular repolarization, namely, JTp, Tp-e, QT, QTc intervals and their ratios, as well as RR intervals, were compared between fasted and postprandial states for every participant.

RESULTS: Tp-e and QTc intervals, and Tp-e/QTc ratio do not significantly change after both higher- and lower-calorie meals. JTp and QT intervals significantly shorten in both groups, regardless of the calorie consumed. While JTp shows a positive correlation with RR interval both before and after a meal in lower calorie intake group, no correlation was found with RR interval after a meal in higher calorie group. Logistic regression analysis revealed that higher calorie intake during a meal is a predictor for greater shortening in JTp and QT, compared to lower calorie meal.

CONCLUSION: Our study may guide future studies on ventricular repolarisation, particularly those conducted on various disease conditions or drug effect of cardiac electrophysiology.

Introduction

A vast realm of significant bodily modulations regarding cardiovascular and neuroendocrine systems has long been recognised following meal consumption. The mesenteric arterial system requires a boost in its blood flow for digestive and absorptive purposes during the postprandial period compared to the previous fasting state [1]. To attain this, accordingly, the heart responds to a meal by increasing its output, rate and stroke volume [2], [3], despite confounded findings regarding the latter [4]. Moreover, both glucose and insulin levels escalate, together with alterations in autonomic nervous and other relevant endocrine systems [4], [5], [6].

Electrocardiographically, ventricular repolarisation is represented by QT interval, and its prolongation was suggested to be associated with higher risk of ventricular arrhythmias in a variety of clinical settings [7], [8], [9]. Moreover, the QT interval can be divided further into three parts as such: QRS width; JTp interval, which stands for the time period between the end of QRS and the peak of the T wave; and T peak to T end interval (Tp-e interval), which stands for the time period in between the peak and the end of the T wave. In general, JTp comprises the major part of a QT interval and can roughly be

regarded as an early phase of ventricular repolarization, whereas Tp-e interval may represent the late phase of ventricular repolarization. Repolarization in the cardiac ventricles commence very soon after the onset of ventricular activation. Hence QT interval provides insight about the overall repolarization status of the ventricles. Tp-e interval, on the other hand, appears as an index for transmural dispersion of ventricular repolarisation [10] and is associated with varying durations of the action potential in endocardium, myocardium and epicardium. Just as is the case in QT interval, evidence suggests that prolongation of Tp-e interval has been associated with the ventricular arrhythmogenic potential of different degrees [11], [12], [13].

More recently, effect of food intake on some electrocardiographic parameters appeals more and more to the investigators, since accumulating evidence points out that meal consumption of varying composition and calories are likely to have effect on QT and QTc intervals [14], [15], [16], [17], [18]. This especially holds true when it comes to the development of new drugs and drug regiments in thorough QT/QTc studies, where food intake is a likely challenge in anticipation of drug-related proarrhythmic potential [17], [19]. Previous studies have yielded conflicting results on the effect of food intake on QT and QTc. There appears to be no data regarding changes in such different parts of QT interval as JTp, Tp-e, JT and their ratios with each other, namely, Tp-e/QT, Tp-e/QTc, Tp-e/JTp, Tp-e/JT and JTp/JT following meal consumption.

In the present study, we aimed to evaluate these ventricular repolarisation parameters comparatively between lower- and higher-calorie meal consumption in healthy individuals.

Methods

Study design and participants

Our longitudinal study included a total of 61 (36 men and 25 women; aged between 18 and 50) consecutive and healthy individuals who admitted to our cardiology out-patient clinic for a routine cardiologic check-up between May 2018 and July 2018. All the participants were in an overnight fasted state for their anticipation of a probable routine blood chemistry screen. Thorough physical examinations, and electrocardiographic (ECG) and transthoracic echocardiographic scans were implemented in each of the participants. Further, each was subjected to a comprehensive interrogation for any past and/or present medical disease, and smoking and alcohol habits. Body-mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters. Blood samples were obtained through venipuncture for routine laboratory analysis. Then, the participating individuals were asked to eat their lunch as ordinarily as in their previous daily lives, but to abstain from caffeine-containing and alcoholic beverages as well as smoking. Two hours after the onset of their lunch [17], they were asked to re-admit to the out-patient polyclinic to have their postprandial ECG recordings taken. Subsequently, the participants were interrogated about what they had eaten at lunch, recording the composition and the volume of the food consumed, and then we calculated the estimated total calorie intake. Among the exclusion criteria were renal, gastrointestinal or hepatic dysfunction, alcoholism, any known History of cardiac disease, malignancy, diabetes mellitus, and medication use of any kind, endocrine pathology, hypertension, supplementation therapy of any kind, and having a complex meal that prevents appropriate estimation of energy content of the relevant meal. Informed consent was obtained from every participant, and the local committee approved our study (Kirsehir Ahi Evran University Ethical Committee, No: 2018-35/135, Date: 27.04.2018).

Calculation of basal metabolic rate and estimated caloric intake

The daily required total calorie intake to maintain the study participants' basal metabolic rate was calculated specifically by the use of Harris and Benedict equation [20], and the preliminary result obtained by the equation was multiplied by the physical activity level to obtain the ultimate result. As for the physical activity level, it was estimated using the Baecke Questionnaire [21]. Moreover, the estimation of the energy content of meals by the composition and the volume that the participants consumed at lunch were done using ''Turkey Dietary Guidelines'' [22].

ECG measurements

In a supine position following at least 10 minutes rest, 12-lead ECGs were obtained from each of the participants 1-2 hour before the lunch and 2 hours after the onset of lunch using a standard ECG system (Nihon Kohden, Tokyo, Japan) at a paper speed of 50 mm/s. All of the ECG papers were scanned and transferred to the digital media, and the digital records were analysed under x300% magnification in a personal computer. RR interval, QT interval, JT interval and JTp interval and Tp-e interval, together with such ratios as Tp-e/QT, Tp-e/QTc, Tp-e/JT, Tp-e/JTp and JTp/JT were calculated using the precordial lead V3 [23], [24], [25]. There are two commonly used methods of measuring Tp-e interval: tangent method and tail method [26], [27]. We used in our study the tangent method, which refers to the time interval between the peak of the T wave and the point

where the tangent of the steepest down-slope of the T wave intersects with the isoelectric line (T wave offset) [26]. Respective QT, JT and JTp intervals were measured as follows: from the Q onset to T wave offset; from the S wave offset to T wave offset; and, from the S wave offset to the peak of T wave. Although variable methods were applied as methods of correction for heart rate in different studies [14], [15], [16], [17], the decision of heart rate correction for QT and JTp intervals in the present study was made to implement the Bazett's formula [28].

On the other hand, no incentive was felt in favour of heart rate correction for Tp-e interval, as Tp-e was suggested to be heart-rate independent [29]. Moreover, three consecutive beats were averaged to obtain the ultimate measurement for each parameter. All the ECG parameters were assessed by a single experienced cardiologist blinded to the study design to avoid inter-observer variability, especially in the detection of S wave offset [30].

Statistical analysis

Statistical analysis of the study data was performed using SPSS Version 22.0 (SPSS Inc., Chicago, IL, USA). Numbers, percentage, mean ± standard deviation, median, minimum (min), maximum (max) and 25-75 percentiles were used for the descriptive statistics. Independent sample t-test and Mann-Whitney U test were used for the comparison of the demographic characteristics between the two groups. Wilcoxon Signed Ranks test was utilised to compare differences of ECG parameters of interest between fasted and postprandial states in lower and higher calorie-intake groups. Moreover, Spearman's rank correlation analysis was used to determine the correlation of the ECG parameters with RR interval, and multivariate linear logistic regression analysis (variables at a $p < 0.10$ significance level in the univariate analysis) was applied to evaluate the independent association of calorie intake (high-low calorie) with the ECG parameters of interest. A p-value < 0.05 was accepted to be statistically significant.

Results

Demographic Characteristics

Baseline demographic characteristics of the patients are presented in Table 1. Higher calorie intake group was composed of 35 participants [15 female (42.8%)], whereas lower calorie intake group was composed of 26 participants [10 female (38.4%)]. Both groups were similar regarding age, gender and blood analysis. BMI (kg/m^2), however, was only slightly greater in the lower calorie intake group compared to the higher calorie intake group, which was statistically significant [24.5 (23.8-25.2) vs 23.8 (23-24.7), respectively; $p = 0.045$]. As for the echocardiography, both systolic and diastolic functions, as well as cardiac dimensions were normal in both groups.

Calculated Basal metabolic rate and Estimated Caloric Intake

One-third of the caloric intake required for the maintenance of the basal metabolic rate, correspondingly to lunch, by Harris and Benedict formula was similar in both groups [860 Kcal (830-880) vs 850 Kcal (820-860), respectively; $p = 0.415$]. Moreover, median calorie consumption at lunch was estimated to be 900 Kcal (850-930) for the lower calorie intake group, while it was 1580 Kcal (1500-1670) in the higher calorie intake group ($p < 0.001$).

Table 1: Demographic characteristics of the study groups

	Lower Calorie Intake Group (median, IQR 25-75) (n = 26)	Higher Calorie Intake Group (median, IQR 25-75) (n = 35)	p
Age (years)	35.5 (22-43)	34 (25-41)	0.502
Gender, female, n(%)	10 (38.4%)	15 (42.8%)	0.730
BMI, kg/m2	24.5 (23.8-25.2)	23.8 (23-24.7)	0.045
Total Cholesterol (mg/dL)	178 (165-189)	187 (170-203)	0.113
Hemoglobin (g/dL)	14 (13.5-14.5)	14.1 (13.2-14.6)	0.832
ALT (U/L)	18 (15-21)	18 (16-20)	0.861
Glucose (mg/dL)	84.5 (81-88)	89 (82-92)	0.111
WBC (10^3/uL)	7.8 (6.4-8.6)	7.9 (6.5-9)	0.366
C-reactive protein (mg/dL)	0.09 (0.05-0.4)	0.19 (0.09-0.29)	0.290
E/E'	5.7 (5.4-6.0)	5.8 (5.3-6,2)	0.563
LVEF (%)	64.5 (60-67)	64 (61-68)	0.583
LVEDD (mm)	45 (43-48)	45 (44-49)	0.596
Left atrial diameter (mm)	34 (32-36)	34 (33-37)	0.513
IVS thickness (mm)	9.4 (9.1-10)	9.3 (9.1-9.8)	0.918
PWT (mm)	8.9 (8.7-9.2)	9.0 (8.7-9.3)	0.558
Calorie Consumption (Kcal)	900 (850-930)	1580 (1500-1670)	< 0.001
(Calorie required for BMR)* (Kcal)	860 (830-880)	850 (820-860)	0.415

Values were given in median (25-75 IQR). E/E' = the ratio of transmitral early filling velocity to tissue Doppler early diastolic lateral mitral annular velocity. ALT = alanine aminotransferase; LVEDD = left ventricle end-diastolic diameter; LVEF = left ventricle ejection fraction; IVS = interventricular septum. PWT = posterior wall thickness; BMR = basal metabolic rate. BMI = body-mass index; WBC = white blood cell count; * Calorie corresponding to one-third of total daily basal metabolic rate, expected to consume at lunch.

Electrocardiographic Repolarization Parameters

Comparison of the ECG parameters of ventricular repolarization is presented in Table 2. Respective JTp, JTpc and QT intervals significantly decreased in the postprandial 2nd hour compared to the fasting state in both of lower and higher calorie intake groups [194 ms (177.2-209.9) vs 179.9 ms (161.6-189.5), $p < 0.001$; and, 201 ms (190.2-217.9) vs 179.7 ms (157.2-193.2], $p < 0.001$], [203 ms (196-211.1) vs 198.8 ms (180.1-208.6), $p = 0.003$; and, 216.6 ms (206.9-232.9) vs 205.7 ms (189.2-226), $p = 0.004$], [361.2 ms (345.52-378.57) vs 351.8 ms (334.72-369.30), $p = 0.001$; and, 388 ms (364-404) vs 364 ms (340-378), $p < 0.001$]. Respective Tp-e

interval, QTc interval and Tp-e/QTc ratio, however, did not change postprandially compared to the fasting state in both of the lower and higher calorie intake groups [76.05 ms (66.60-79.92) vs 75.25 ms (65.50-82.75), p = 0.440; and, 71.9 ms (63.6-79.2) vs 73.5 ms (67.4-80), p = 0.051], [391.5 ms (373.95-403.50) vs 391.5 ms (378.75-411.65), p = 0.139; and, 412 ms (391-428) vs 419 ms (399-430), p = 0.148], [0.195 (0.178-0.205) vs 0.193 (0.170-0.209), p = 0.77; and, 0.172 (0.155-0.190) vs 0.178 (0.158-0.192), p = 0.987]. Furthermore, respective Tp-e/QT, Tp-e/JTp and Tp-e/JT ratios increased significantly, whereas JTp/JT ratio decreases significantly in the postprandial 2nd hour both in lower calorie and higher calorie intake groups [0.198 (0.183-0.226) vs 0.214 (0.188-0.228), p = 0.004; and, 0.183 (0.163-0.202) vs 0.197 (0.182-0.225), p < 0.001], [0.376 (0.338-0.451) vs 0.404 (0.372-0.479), p < 0.001; and, 0.345 (0.296-0.397) vs 0.400 (0.347-0.469), p < 0.001], [0.273 (0.252-0.311) vs 0.288 (0.271-0.324), p < 0.001; and, 0.257 (0.228-0.284) vs 0.286 (0.258-0.319), p < 0.001], [0.726 (0.688-0.747) vs 0.711 (0.675-0.728), p < 0.001; and, 0.742 (0.715-0.771) vs 0.713 (0.680-0.741), p < 0.001]. Lastly, RR interval decreased significantly due to increase in hearth rate in the postprandial period in both of the lower and higher calorie intake groups [882 ms (787.5-967.1) vs 779.2 ms (703.2-892.5), p < 0.001; and, 880 ms (778-974) vs 742 ms (682-808), p < 0.001].

Table 2: ECG parameters of ventricular repolarisation compared between the two groups

		Fasting ECG Parameters	Postprandial ECG parameters	P
Lower Calorie Intake Group	Tp-e (ms)	76.05 (66.60-79.92)	75.25 (65.50-82.75)	0.440
	JTp (ms)	194 (177.27-209.90)	179.95 (161.62-189.55)	< 0.001
	JTPpc (ms)	203 (196-211.15)	198.85 (180.10-208.67)	0.003
	QT (ms)	361.2 (345.52-378.57)	351.8 (334.72-369.30)	0.001
	QTc (ms)	391.5 (373.95-403.50)	391.5 (378.75-411.65)	0.139
	Tp-e/QT	0.198 (0.183-0.226)	0.214 (0.188-0.228)	0.004
	Tp-e/QTc	0.195 (0.178-0.205)	0.193 (0.170-0.209)	0.77
	Tp-e/JTp	0.376 (0.338-0.451)	0.404 (0.372-0.479)	< 0.001
	Tp-e/JT	0.273 (0.252-0.311)	0.288 (0.271-0.324)	< 0.001
	JTp/JT	0.726 (0.688-0.747)	0.711 (0.675-0.728)	< 0.001
	RR interval (ms)	882 (787.5-967.17)	779.2 (703.25-892.50)	< 0.001
Higher Calorie Intake Group	Tp-e (ms)	71.9 (63.6-79.2)	73.5 (67.4-80)	0.051
	JTp (ms)	201.1 (190.2-217.9)	179.7 (157.2-193.2)	< 0.001
	JTPpc (ms)	216.6 (206.9-232.9)	205.7 (189.2-226)	0.004
	QT (ms)	388 (364-404)	364 (340-378)	< 0.001
	QTc (ms)	412 (391-428)	419 (399-430)	0.148
	Tp-e/QT	0.183 (0.163-0.202)	0.197 (0.182-0.225)	< 0.001
	Tp-e/QTc	0.172 (0.155-0.190)	0.178 (0.158-0.192)	0.987
	Tp-e/JTp	0.345 (0.296-0.397)	0.400 (0.347-0.469)	< 0.001
	Tp-e/JT	0.257 (0.228-0.284)	0.286 (0.258-0.319)	< 0.001
	JTp/JT	0.742 (0.715-0.771)	0.713 (0.680-0.741)	< 0.001
	RR interval (ms)	880 (778-974)	742 (682-808)	< 0.001

Values were given as median (IQR 25-75).

Correlations and Predictors

The correlation of RR interval with some ECG parameters of ventricular repolarisation is shown in Table 3. The JTp interval both in fasted and postprandial states in lower calorie intake group exhibits a significant correlation with respective RR intervals [(r = 0.708, p < 0.001; fasted state) and (r = 0.576, p = 0.002; postprandially)]. On the other hand, only fasted state JTp interval shows a statistically significant correlation with fasted state RR interval in the higher calorie intake group, while no statistically significant correlation was evident between the postprandial JTp and RR intervals in the same group [(r = 0.440, p = 0.008; fasted state) and (r = 0.213, p = 0.219; postprandially)].

Table 3: Spearman's Rank Correlation analysis comparing fasted-state and postprandial JTp and QT intervals with relevant RR intervals

		Fasting State RR interval (ms)		Postprandial RR interval (ms)	
		p	r	p	r
Lower Calorie Intake Group	Postprandial JTp (ms)			0.002	0.576
	Fasting JTp (ms)	< 0.001	0.708		
	Postprandial QT (ms)			< 0.001	0.644
	Fasting QT (ms)	0.001	0,605		
Higher Calorie Intake Group	Postprandial JTp (ms)			0.219	0.213
	Fasting JTp (ms)	0.008	0.440		
	Postprandial QT (ms)			0.001	0.534
	Fasting QT (ms)	0.002	0.512		

As for the QT interval, it shows a statistically significant correlation with RR interval in respective fasted and postprandial states in both of the groups [(r = 0.605, p < 0.001; fasted state) and (r = 0.644, p = < 0.001; postprandially); the lower calorie intake group], [(r = 0.512, p = 0.002; fasted state) and (r = 0.534, p = 0.001; postprandially); the higher calorie intake group].

Further in logistic regression analysis, greater increase in caloric intake at lunch was found to be predictor of greater decrease in JTp and QT intervals (β = 1.045, p = 0.034; β = 1.036, p = 0.018; respectively). Such an association, however, did not show up with the other ECG parameters of ventricular repolarization (Table 4).

Table 4: Multivariate linear logistic regression analysis

Variables	B	Exp (B)	p	95% CI	
				Lower	Upper
JTp (ms)	0.44	1.045	0.034	1.003	1.088
QT (ms)	0.36	1.036	0.018	1.006	1.067
Tp-e (ms)	-0.025	0.976	0.647	0.878	1.084
QTc (ms)	-0.001	0.999	0.942	0.947	1.025
Tp-e/QTc	-3.193	0.041	0.852	0	146

Discussion

The very first finding of our study indicated that Tp-e interval, QTc and Tp-e/QTc did not change significantly in the postprandial 2nd hour compared to the fasted state, irrespective of the total calorie consumed during a meal. On the other hand, QT, JTp and RR intervals decreased significantly in the postprandial 2nd hour compared to the fasted state in both of the higher and lower calorie intake groups, which was compatible with the findings of previous studies [15], [17], [19].

RR interval has long been known to decrease

following meal consumption since postprandial state pertains to hyperemia in the mesenteric vasculature to meet digestive requirements [1], [2], [31]. As stated in the introduction section, QT interval can roughly be divided into two as the early phase of the ventricular repolarisation represented by JTp interval and late phase of the ventricular repolarisation represented by Tp-e interval. Although QT interval is known to be heart rate dependent, the same does not hold true for the Tp-e interval. Hnatkova et al., [29] reported in their study that only a very small portion of Tp-e interval ($T_{95\%}$-Te) was affected by heart rate changes, hence that omission of heart rate correction of Tp-e does not incur any inaccuracy in the absence of dramatic heart rate changes. Accordingly, most of the heart rate dependency of QT interval is associated with the heart rate dependency of JTp interval [29]. According to the Spearman's correlation analysis in the current study, JTp interval exhibited a positive and significant correlation with RR interval both in fasted and postprandial states in lower calorie intake group. Thereby, shortening of JTp postprandially in lower calorie intake group cannot be ascribed to anything other than a normal response to the postprandial heart rate increase. Note, however, that postprandial JTp does not show any correlation with postprandial RR interval in higher calorie intake group, despite the persistence of positive correlation between fasted-state JTp and fasted-state RR intervals. Aside from heart rate increase, it may be reasonable to assume that this condition in part is due to more pronounced neuroendocrine and cardiovascular activation incited by higher calorie intake than metabolic requirements which particularly affects the early phase of the ventricular repolarisation in healthy subjects [31], [32]. Contrary to the more specific early phase of ventricular repolarisation, global ventricular repolarisation represented by QT interval still exhibits positive correlation with RR interval during fasted and postprandial states in both of the groups.

Previous studies yielded conflicting results concerning the effect of food intake on QT and QTc. Cirincione et al., [19] reported in their study that QT and QTcF intervals are subjected to the most dramatic decrease in 2-3 hours after a meal. The heart rate increase was also the most prominent 2 hours after the meal. Although they utilized Fridericia'sformula [33] for heart rate correction, we preferred to use the Bazett's formula due to lack of precise consensus regarding the most eligible formula to apply in clinical settings and to the fact that most of the currently-available formulas for heart rate correction yield almost equivalent results in resting heart rates within the range of 60-90 beats/min [34]. Taubel et al., [17] showed in their study that the maximum decrease in QT interval was 2 hours after the onset of a standardised breakfast composed of 617 kcal. However, they applied different correction formulas on QT for heart rate which yielded as follows: QTcF interval with maximum shortening at 2.5 hours; QTcIP interval with maximum shortening at 3.5 hours; and, QTcB with an initial slight increase, then returning to the baseline at 2 hours after the onset of breakfast. Nagy et al., [15] conducted a study inquiring RR interval, QT and QTcB at postprandial 1 hour and reported a decrease in postprandial QT at 1 hour. However, QTcB, they suggested, increased significantly 15 minutes after the meal, then gradually decreased to reach such a level that was still significantly greater than the baseline value at 1 hour. Sciot et al., [14] reported a significant increase in heart rate, a matched decrease in QT interval, but no difference either in QTcF or in QTcI intervals. The absence of change in QTcB in our study seems compatible with the findings of Taubel et al., [17] Multivariate linear logistic regression analysis revealed that higher calorie consumption during meal acts as a predictor for greater JTp and QT interval shortening, but not for the Tp-e and QTc intervals and Tp-e/QTc ratio.

The main strength of our study is that, apart from previous studies, we evaluated such recently more appealing parameters of ventricular repolarisation as Tp-e, JTp, QT, QTc intervals, and Tp-e/QTc ratio in a comparative manner after meal consumption between higher and lower calorie intake groups of healthy subjects. Moreover, to our knowledge, this study is the first in this regard.

Our study is likely to provide a new framework for further studies about ventricular repolarization, particularly those conducted on various disease conditions or drug effect of cardiac electrophysiology.

This study should be evaluated in light of some limitations. First, we did not provide a fixed lower and higher calorie meal for the study participants and relied solely on the information provided by them. Secondly, our study population is relatively small, and further studies with greater participation may reveal different results. Thirdly, we could not estimate the macronutrient portion (proteins, glucose, lipids) of the meals consumed, which may have exerted effects to some extent on the repolarisation parameters of interest.

In conclusion, Tp-e interval, QTc interval and Tp-e/QTc ratio do not change significantly at a 2nd postprandial hour, regardless of the amount of the calorie consumed. However, QT interval shortens, due almost solely to JTp interval shortening. Further, JTp interval shortening following higher calorie meal may not only be associated with increased heart rate but also higher calorie consumption during the meal, possibly through unproportional escalation in neuroendocrine and cardiovascular response. Lastly, higher calorie consumption at meal represents a predictor for greater JTp and QT shortening. We believe that the current study may provide a new insight for further studies about ventricular repolarization parameters, particularly those conducted on various disease conditions or drug effect on cardiac electrophysiology. Also, our study is

very likely to increase the awareness about the effect of the timing and the calorie of the meals consumed on the assessment of the parameters of ventricular repolarisation among the researchers in their future studies regarding noninvasive electrophysiology. However, further large-scale studies are warranted to justify our study results.

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Lambdacism, Rhotacism and Sigmatism in Preschool Children: Frequency and Distribution

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Abstract

BACKGROUND: Speech sound appears first in the child's speech development and is the primary means of expression. Articulation disorders can hinder the comprehensibility of children's speech. The speech, in turn, can limit the child's inclusion in the social and educational environment.

AIM: To establish frequency and distribution of lambdacism, rhotacism and sigmatism or their combination in preschool children and the frequency and distribution of these articulation disorders among boys and girls.

MATERIAL AND METHODS: A retrospective analysis of data from preventive examination for early diagnosis of impairments of hearing, speech and sounds in preschool children has been carried out. In the selected sample, only the data for children diagnosed with lambdacism, rhotacism, sigmatism or their combination are analysed. The data is statistically examined, represented in tables and figures and analysed descriptively.

RESULTS: In the analysed sample, the greater presence of lambdacism was observed before rhotacism and sigmatism. Most commonly, these three types of articulation disorder appear alone, as isolated cases, instead of a combination of two out of the three impairments. They are more common in boys than in girls.

CONCLUSION: Timely diagnosis and rehabilitation of lambdacism, rhotacism and sigmatism or their combination in preschool children will enable easier and faster integration of the children in the social and educational environment without leaving lasting consequences.

Introduction

While most children are ready for verbal communication at preschool age, some lack speech and linguistic abilities equal to their peers [1]. Such abilities can limit the children's inclusion in the social and educational environment [2]. Due to this, speech and linguistic abilities play an important role in human society and are crucial for establishing verbal communication.

Speech is formed and developed as a need for realising the language system, while language forms and develops as a result of speech [3]. The basic elements of speech are the sound, word and sentence. The sound takes a primary position since it appears first in the child's speech development and is the primary means of expression. Children should be encouraged and trained for proper articulation of sounds, which lays the foundation for speech.

Speech production is a complex motor skill where movements of organs involved in the articulation are precisely coordinated in time and space [4]. To produce a highly structured and limited flow of acoustic energy, over 50 muscles must quickly change the shape and position of the speech organs in the vocal tract [5]. Deviation or disturbed function of these organs can lead to impaired speech. Articulation disorders are difficulties with the way sounds are formed and strung together, usually characterised by substituting one sound for another, omitting a sound, or distorting a sound [6]. The speech is primarily unintelligible and difficult to understand. Various causes such as impaired hearing, orthodontic anomalies, auditory perception disorders and imitation, motor dyspraxia and lazy tongue can lead to late or impaired development of normal articulation.

Depending on which sound or group of sounds the child has difficulty with, the following types of articulation disorders can be differentiated: sigmatism (inability or difficulty in pronouncing the

sounds: /S/, /Z/, /Sh/, /Zh/, /Ch/, /Dzh/, /C/, /Dz/, /Kj/, /Gj/), kapacism (inability or difficulty in pronouncing the sound /K/), gammacism (inability or difficulty in pronouncing the sound /G/), lambdacism (inability or difficulty in pronouncing the sounds /L/ and /Lj/), rhotacism (inability or difficulty in pronouncing the sound /R/ sound), tetacism (inability or difficulty in pronouncing the sound /T/ sound), deltacism (inability or difficulty in pronouncing the sound /D/), etacism (inability or difficulty in pronouncing the sound /E/) and tetism (replacement of sounds /S/, /C/, /Sh/, /Ch/, /Kj/ and /K/ with sound /T/ and replacement of sounds /Z/, /Zh/, /Dzh/ and /Gj/ with /D/).

A large number of sounds are similar in different languages, some even identical, but the words are not, so languages differ in words (among other things) and can be learnt. Still, the terms for articulation disorders of certain sounds or group of sounds are universal, i.e., internationalised. However, that does not necessarily mean that impairment of a certain sound in one language will be considered impairment in another. This is so because every language has its phonology. For example, rhotacism, i.e., the pronunciation of the French /R/ is not preferred in the Macedonian language, but it is the norm in the French language. Some research shows that the most common types of articulation disorders include sigmatism, lambdacism and rhotacism, either alone or in combination [7], [8], i.e. the most common error sounds are [S] [L] and [R] [6].

Considering that speech development in children begins with the first word and ends between the ages of 6 and 8, [9] this research aims to examine the condition of articulation of sounds from the Macedonian language in preschool children. At the same time, the frequency and distribution of irregular articulation of sounds from the type of lambdacism, rhotacism and sigmatism, either alone or in combination, will be determined, as well as the frequency and distribution of the same among the males and females.

Material and Methods

To carry out the research, a retrospective analysis was performed of the data collected through previously conducted preventive examinations by a team of experts (a specialist doctor orthodontist and clinical speech therapist) from the Center for rehabilitation of hearing, speech and voice-Skopje. The data is based on the assessment of speech of preschool children made by the team of experts during spontaneous speech and model speech. By model speech, test words and sentences are first examined where there is a common presence of a certain sound or sounds, which are suspected of their pathology and, naturally, of the isolated sound itself. In words, the sound is in different positions (initial, medial and final). The observation of the visible organs participating in articulation established the presence of visible deviations and quality of the oral system. The preventive examinations were conducted for each child individually during a period of 9 months (March 2018 – November 2018). Namely, examinations are carried out by the Center for rehabilitation of hearing, speech and voice-Skopje with the aim of early diagnosis of impairments in the development of hearing, speech and voice in children aged 4 to 6.

For the research, a random sample of data (n = 738) was selected from the data obtained through the preventive examinations which the Center for rehabilitation of hearing, speech and voice-Skopje has at its disposal. The sample consisted equally of data from males (n = 369) and females (m = 369). The children are with normal intellectual development, without mental retardation or impaired hearing. The data comes from five preschools in five municipalities on the territory of the city of Skopje, Republic of Macedonia. The data from the selected sample was analysed in detail only for children diagnosed with lambdacism, sigmatism and rhotacism or their combination (n = 289).

By means of a descriptive method, the following parameters were analyzed: age, gender, type of articulation disorder, i.e., deviation in the pronunciation of sounds in the Macedonian language /R/; /L/ and /Lj/; /S/, /Z/, /Sh/, /Zh/, /Ch/, /Dzh/, /C/, /Dz/, /Kj/, /Gj/.

Statistical analysis

The obtained data are statistically examined, represented in tables and graphs and analysed descriptively. The categorical (attributive) variables are represented with absolute and relative numbers. The numerical (quantitative) variables are represented with the mean, minimum values, maximum values and standard deviation.

The statistical analysis of the data obtained through the research was done in the programs Statistica for Windows 7.0 and SPSS 17.0. To compare the analysed variables between the male and female respondents, non-parametric (Pearson Chi-square test) and parametric tests (Student's t-test) were used. The statistical significance was defined at level $p < 0.05$.

Results

With the aim of obtaining a clearer picture for the research, the results of the analysed data are divided into two groups: results of the total sample (n

= 738) and results of the sample with articulation disorders (n = 289).

Total sample

To meet the purposes of our research out of the total set of data, children with lambdacism, rhotacism or sigmatism were first identified; these, appearing either alone, i.e., isolated as one articulation disorder or a combination of two out of the three (sigmatism – rhotacism, rhotacism – lambdacism or sigmatism – lambdacism). Out of the analyzed sample (n = 738), 39% of the children (n = 289) were with the abovementioned disorders, where more common was the presence of an isolated articulation disorder, 29% (n = 211), than a combination of two out of the three, 10% (n = 78) (Figure 1).

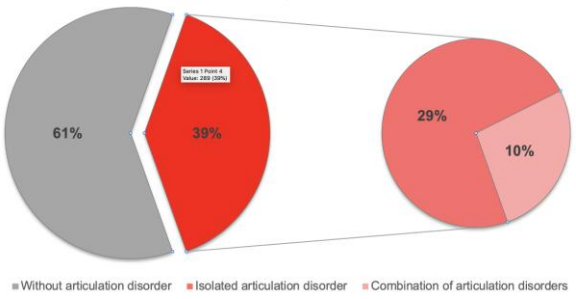

Figure 1: Distribution of articulation disorders and the form in which they appear

According to the type of articulation disorder about the total analysed sample, the most common is lambdacism with 18.2% (n = 134). The combination of rhotacism and lambdacism is present in 7.6% (n = 56) of the children, while 6.6% (n = 49) have rhotacism only. Sigmatism as an articulation disorder is present in 3.8% (n=28), while the combination of sigmatism and lambdacism in 2.6% (n = 19). The least common combination is sigmatism and rhotacism, only present in 0.4% of the children (n = 3) (Figure 2).

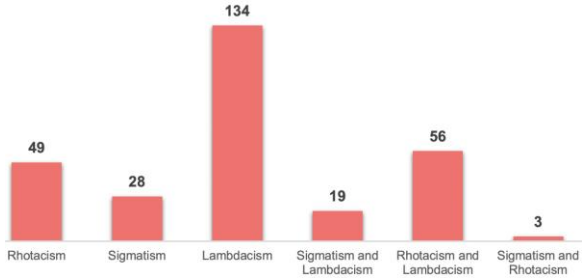

Figure 2: Frequency of the articulation disorders according to the type

For the purposes of cumulative representation of the articulation disorders, when the isolated types of articulation disorder (lambdacism; rhotacism; sigmatism) are supplemented with the data from the respective combinations (sigmatism – rhotacism; rhotacism – lambdacism; sigmatism – lambdacism) where the isolated type of articulation disorder is included, again the most common is lambdacism, followed by rhotacism and sigmatism. More precisely, if the data on rhotacism – lambdacism and sigmatism – lambdacism is added to the data for lambdacism, then the occurrence of lambdacism is 28.4%. Moreover, if the data on sigmatism – rhotacism and rhotacism – lambdacism is added to the data for rhotacism, then the occurrence of rhotacism is 14.6%. Finally, if the data on sigmatism – rhotacism and sigmatism – lambdacism is added to the data for sigmatism, then the occurrence of sigmatism is 6.8%.

About the children's gender, out of the total analysed sample, 43% of the boys had one articulation disorder (lambdacism; rhotacism; sigmatism; sigmatism – rhotacism; rhotacism – lambdacism; sigmatism – lambdacism) and 35% of the girls. The difference in the distribution of children with or without an articulation disorder between the males and females was confirmed as statistically significant (p=0.019) (Table 1).

Table 1: Frequency of lambdacism, rhotacism, sigmatism, sigmatism – rhotacism, rhotacism – lambdacism or sigmatism – lambdacism according to the gender

Articulation disorders	Male		Female		*p-value*
	n	(%)	n	(%)	
With	160	(43)	129	(35)	p = 0.019
Without	209	(57)	240	(65)	

p (Pearson Chi-square test)

Only the data for respondents where articulation disorder is established is taken into consideration for further analysis.

Respondents with an articulation disorder

The respondents (n = 289) where presence of one articulation disorder was established (lambdacism; rhotacism; sigmatism; sigmatism – rhotacism; rhotacism – lambdacism; sigmatism – lambdacism) were of a mean age of 5.39 ± 0.64 years, where the youngest respondent was 4, while the oldest 6. 55% were males, while 45% were females. The mean age of the males was 5.44 ± 0.61 years, while the females 5.33 ± 0.67. The difference was statistically proven as not significant (p = 0.15).

Among the males, articulation disorders more commonly appear alone (68%) than in combination (32%). Among the females, this difference is even more highlighted, i.e., the articulation disorders appear more alone (79%) than in the combination of two out of the three (21%).

Lambdacism as an articulation disorder appears more alone (64%) than in combination with rhotacism or sigmatism (36%). The distribution of respondents according to gender and about lambdacism (either alone or in combination with rhotacism or sigmatism) reveals that this condition is more noticeable in the males (60.8%) than the females (39.2%) (Table 2).

Table 2: Distribution of respondents with lambdacism (either alone or in combination with rhotacism or sigmatism)

Gender	Lambdacism		Rhotacism - Lambdacism		Sigmatism - Lambdacism		Total	
	n	%	n	%	n	%	n	%
Male	77	57.5	35	62.5	15	78.9	127	60.8
Female	57	42.5	21	37.5	4	21.1	82	39.2
Total	134	100	56	100	19	100	209	100

Rhotacism as an articulation disorder appears more often in combination with lambdacism or sigmatism (55%) than alone (45%). The distribution of respondents according to gender and about rhotacism (either alone or in combination with lambdacism or sigmatism) reveals that this condition is equally present in the males and females (Table 3).

Table 3: Distribution of respondents with rhotacism (either alone or in combination with lambdacism or sigmatism)

Gender	Rhotacism		Sigmatism - Rhotacism		Rhotacism - Lambdacism		Total	
	n	%	n	%	n	%	n	%
Male	18	36.7	1	33.3	35	62.5	54	50.0
Female	31	63.3	2	66.7	21	37.5	54	50.0
Total	49	100	3	100	56	100	108	100

Sigmatism as an articulation disorder appears more often alone (56%) than in combination with lambdacism or rhotacism (44%). The distribution of respondents according to gender and about sigmatism (either alone or in combination with lambdacism or rhotacism) reveals that this condition is more noticeable in the males (60%) than the females (40%) (Table 4).

Table 4: Distribution of respondents with sigmatism (either alone or in combination with lambdacism or rhotacism)

Gender	Sigmatism		Sigmatism - Lambdacism		Sigmatism - Rhotacism		Total	
	n	%	n	%	n	%	n	%
Male	14	50.0%	15	78.9%	1	33.3%	30	60.0%
Female	14	50.0%	4	21.1%	2	66.7%	20	40.0%
Total	28	100%	19	100%	3	100%	50	100%

Discussion

Vast research on articulation disorders, i.e., the appearance of lambdacism, rhotacism and sigmatism, either alone or in combination, has been carried out by some authors. Each of them has their approach towards research, analysis and presentation of results. When comparing results for articulation disorders, what is important is the fact that every language has its phonology of sounds, which means that what is considered an articulation disorder of a certain sound in one language does not necessarily mean a disorder in another. For these reasons, the possibility of making a partial or complete comparison of the obtained results from our research with other similar studies where the same methods and size of the examined sample were used is limited. However, a limited comparison for certain results was still possible. The results of our research are consistent with some other research, though different at times.

In our research, atypical articulation (lambdacism; rhotacism; sigmatism; sigmatism – rhotacism; rhotacism – lambdacism; sigmatism – lambdacism) was established in 39% of the children from the total analysed sample (n = 738). Stanković-Milićević et al., [10] established atypical articulation in 31.96% of the examined children in research conducted on a sample of 316 respondents, all aged 5. The most common type of atypical articulation in their research was lambdacism; then followed by rhotacism and sigmatism, while the least common were tetacism and deltacism. These results also overlap with our research with the only difference that Stanković-Milićević et al., included atypical articulation tetacism and deltacism as well.

According to our research results for the total examined sample (n = 738) and in relation to the frequency of occurrence of a certain articulation disorder, either alone or in combination, most of the children had lambdacism (28.4%), then rhotacism (14.6%), and the least number of children had sigmatism (6.8%). Junuzović-Žunić and Ibrahimagić [11] researched with 1.600 respondents who speak Bosnian/Croatian/Serbian language, aged 3 to 7, and from both genders. According to their research, sigmatism as an independent disorder, but in combination with lambdacism or rhotacism as well, is the most widespread disorder with a frequency of 17.6% compared to the other articulation disorders. In a sample of 76 children studied by Filić, Kolundžić and Vidović [7], most of the children had sigmatism, lambdacism and rhotacism. Farago et al., [8] suggest that the most common articulation disorders are sigmatism, rhotacism and lambdacism. Jurišić [12], on the other hand, researched phonological-articulation disorders of 31 children, who speak two languages and are aged 6 to 11. Her research revealed that sigmatism and rhotacism were the two most common disorders in the examined sample. Sigmatism is present in 19.35% of the respondents, and if the combined disorders are added to it, then sigmatism is present in 26% of the respondents. Rhotacism is present in 12.9% of the respondents, that is, 16% of the respondents when the rhotacism is supplemented with the combined articulation disorders. According to Filipova, Levenska and Ikadinović-Talevska [9], who studied a sample of 324 respondents, the most common in the speech of preschool children from the municipality of Negotino (Republic of Macedonia) is the articulation disorders. Their results show that the most present are lambdacism, then combined articulation problems, i.e., speech problems with more sounds. Apart from the research of Filipova, Levenska and Ikadinović-Talevska, the key difference with the other studies is that while in our research the most common articulation disorder is lamdacism that is not the case in the others.

According to our research results and about

the total examined sample (n = 738), the articulation disorders, lambdacism, rhotacism and sigmatism, more commonly appear alone as isolated articulation disorders than in the combination of two out of the three disorders. The research of Junuzović-Žunić, Banović and Bratovčić [13] conducted on a smaller sample of respondents compared to our research found that respondents, aged 4 to 6, most often had an articulation disorder from a combined type (sigmatism – rhotacism; sigmatism – lambdacism; sigmatism – lambdacism – rhotacism) with a frequency of 34.4%. Alić, Radić and Kantić [14], who analysed a sample of 150 respondents, aged 3-10, have established a different type of articulation disorders. Most of the respondents in their research had a combination of articulation disorders (32.67%). The respondents diagnosed with only one articulation disorder often had sigmatism, lambdacism and rhotacism. These results differ from the results of our research, where more common was the occurrence of isolated articulation disorders with 29% than in combination 10%.

Our research results in relation to the total examined sample (n = 738) show that the occurrence of one articulation disorder, either alone or in combination, (lambdacism; rhotacism; sigmatism; sigmatism – rhotacism; rhotacism – lambdacism; sigmatism – lambdacism) is more common between boys (43%) than between girls (35%). According to Vila and Opsenica [15], most studies suggest that delay in speech development; articulation disorders and similar conditions occur more often in males than females. This is consistent with the results of our research, but not with those of Stanković-Milićević et al., [10], who established that the frequency was higher among the girls, totalling at 35%, while 28.84% among the boys.

It is worth pointing out that future research should include a larger number of respondents so that the results obtained can have greater relevance. Then, the frequency of certain types of articulation disorder should be investigated and analysed about the respondents' age, and if and how the respondents' place of residence (urban or rural area) affects articulation disorders.

The results of our and similar research greatly benefit clinical practice. They influence the awareness of the importance of timely diagnosis and rehabilitation of lambdacism, rhotacism, sigmatism or other types of articulation disorder in preschool children, which finally will enable easier and faster integration of the children in the social and educational environment without leaving lasting consequences.

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Clinical Science

Angioedema - Our Experience Focused On Socio-Demographic, Etiological and Clinical Characteristics of the Condition and Its Management

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Keywords: Angioedema; Triggering factors; Clinical features; Underlying disorders

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Abstract

BACKGROUND: Angioedema (AE) is acute oedema of the skin and mucous surfaces, involving the respiratory and gastrointestinal tracts. AE could be a life-threatening medical condition. Regardless of its growing clinical importance, many aspects of its aetiology and pathogenesis remain poorly understood. Its incidence, demographic characteristics, diagnosis and therapy, need further investigation.

AIM: This study reports our experience with angioedema concerning its social and demographic characteristics, aetiology, clinical features, diagnosis and treatment outcomes. Study design: Eighty-eight patients with AE were enrolled. The study is a retrospective analysis of patients treated in our Clinics.

METHODS: All participants were asked on a voluntary basis to fill out a specially designed questionnaire on the day of their discharge. Other important data sources included: patients' history and notes during the hospital stay, information from previous admissions, etc. Parametric and non-parametric statistical methods were used for data processing. Data analysis was performed using SPSS (SPSS Inc., IBM SPSS Statistica) version 20.0

RESULTS: Based on our results, AE affects more frequently patients over 50 years of age, regardless of their sex. Urban residents prevail, among them - more commonly working women. Non-steroidal anti-inflammatory drugs (NSAIDs), hormones and antibiotics were among the most common triggers – in 8%, 4.5% and 11.4% of the cases respectively. In 9.09% of the patients, food-induced AE was seen, the most common foods reported, were – nuts, eggs and egg products. The common sites of involvement were periorbital area and lips. In only 9.1% of the patients, oedema progressed to spread to the upper respiratory tract. Cardiac conditions were the most frequent underlying disorders – 33%, of the patients, auto-immune thyroiditis was the second most common- 14.8%, followed by musculo- skeletal disorders (10.2%) and diabetes (4.5%) Family history of allergy was seen in 8.4% of the patients, the most frequent allergic disorder, reported, was asthma. In patients with HAE, family history was present in 2.9% of the patients.

CONCLUSIONS: All patients received therapy with steroids and antihistamines, resulting in resolution of symptoms and no invasive procedures were necessary. Based on our results, the diagnosis of AE is often difficult and delayed and requires specialist evaluation. If recognised on time and adequately treated, the outcomes are favourable.

Introduction

Angioedema (AE) usually presents with oedema of the deep layers of the skin, located predominantly on the eyelids, lips, tongue, pharynx and larynx. However, it may also affect other parts of the body, including the extremities. Regardless of its growing clinical importance, many aspects of its aetiology and pathogenesis remain poorly understood. It is the objective of this study to present a detailed picture of the incidence, aetiology, triggering factors and pathology of AE. An attempt is made to describe the complexity and the many different aspects of the clinical presentation, the sociodemographic and clinical characteristics, the causal factors and the current diagnostic criteria as well as the therapeutic approaches. The analysis is made, based on our more than 5-years experience with the condition. A comparison is drawn between our diagnostic and therapeutic approach to these patients against the

established criteria, based on data in the available literature.

Henrich Quinke [1] first described the clinical picture of AE in 1882. Though it is known that there had been some earlier descriptions – by Marcelo Donatti in 1586 [2] and by John Milton [3] in 1876. In 1888, W. Osler [4] reported the first cases of the hereditary form of the disease; he coined the term "hereditary angioneurotic oedema. In 1961, Lepou et al. discovered the C1 esterase inhibitor (C1-INH) and demonstrated that it increases vascular permeability in guinea pigs. In 1962, Landerman [5] suggested that the reduced levels of kallikrein could trigger the formation of oedema. Donaldson and Evans [6], [7] were the first to describe family members of AE patients with reduced C1-INH levels and respective elevation of C1 esterase concentration. In our country, the first case of AE was reported at the first National Allergy Conference in 1976 [8]. Throughout 30 years, at the National Allergy Clinical Center, a database was developed, including approximately 39 affected families – 120 patients [8]. In the last decades, new forms of acquired AE have been described, and significant progress has been made in clarifying the aetiology, pathogenetic mechanisms, and the genetics of the disease. Serious advances have been achieved in the management of AE. Due to accumulated knowledge, current diagnosis of AE requires the definition of its type. Attempts to classify AE types have been made after it was first described. At present, there is an international consensus that AE could be hereditary (HAE) or acquired (AAE). However, the international task group [9] on AE, analysing and summarising the data, introduced a new classification of AE in 2014. Based on this classification if AAE responds to antihistamines, it is termed histaminergic AE – (IH-AAE). In this case, exposure to allergens should be sought. Another form is the idiopathic non- histaminergic AAE (InH-AAE). It responds well to prophylaxis with tranexamic acid. It was first described by Cicardi et al., [10]. There is an AAE form related to the use of angiotensin-converting enzyme inhibitors (ACEI-AAE). This enzyme plays a crucial role in the metabolism of bradykinin. It is seen in < 0.5% of hypertensive patients and is more common in Africans [11]. Acquired AE with C1INH (C1-INH-AAE) deficiency has also been defined. In this form, there is no family history or established gene mutations. It seems related to depletion of C1INH and is seen mainly in lymphoproliferative disorders. In hereditary AE-two phenotypes are described – type I with quantitative reduction of C1-INH (C1-INH-HAE type I), and type II – with normal C1-INH level, but it is non – functional-(C1-INH-HAE type II) [12]. Another form of HAE is characterized by normal C1INH level and accompanying mutation of factor XII (FXII-HAE). The likely etiology in this case is a missense mutation of the factor XII gene [13]. However, there are patients, non – carriers of this mutation who exhibit AE. This form is of unknown aetiology and is termed U-HAE [14]. AE, as seen from the classification as mentioned above, has many subtypes. The key to its understanding is that fact, that histamine and bradykinin are the most recognized vaso-active mediators known to be critical in the pathological process; most cases of AE are primarily mediated by 1 of these 2 mediators. The final result is increased capillary permeability, leading to plasma extravasation and accumulation in the deeper layers of the skin and submucosa Figure 1.

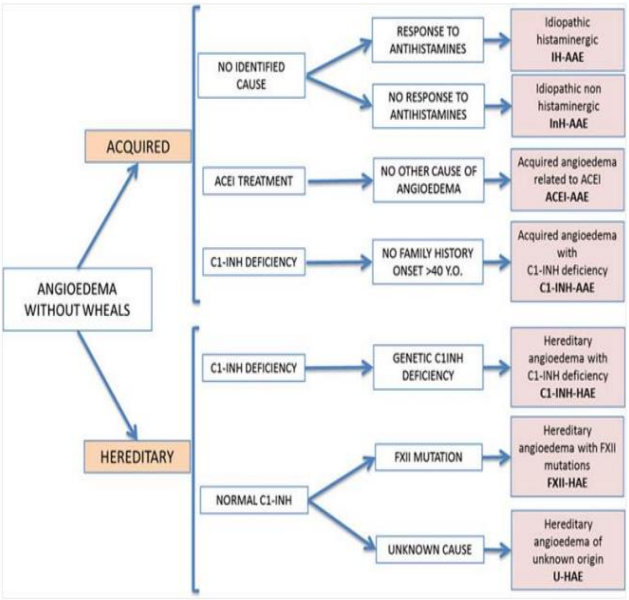

Figure 1: Classification of angioedema

Material and Methods

The study was conducted at a Clinic of Allergy and Occupational Diseases. It encompassed all patients with AE, admitted and treated at the Clinics from January 1, 2010, to December 31, 2014.

Analyzed variables were categorized as 1. factorial – age, sex, place and district of residence, social status, occupation; 2. resultative – diagnosis at admission and discharge, disease outcome, previous admissions, etiology, other underlying medical conditions, family history, location of the edema and its dynamics, diagnostic criteria, laboratory tests, treatment and therapeutic outcomes.

Two basic methods of medical sociology were applied during data collection: medical interview and analysis of documents.

The study medical questionnaire was developed before the conduction of the study. The first variant was initially tested with 20 patients. This allowed further detailization and clarification of the content of questions. Some questions were added, and others were deleted from the questionnaire.

The questionnaire included 56 open-ended,

semi-open-ended and closed questions. They were grouped into 5 categories as follows:

- social and demographic characteristics of the patients;
- hospital treatment;
- aetiology;
- clinical features and diagnosis;
- laboratory diagnosis.

Document analysis was performed based on patients' hospital papers. Data were entered in a specialised database.

All participants were asked on a voluntary basis to fill out the questionnaire on the day of their discharge. Other important data sources included: patients history and notes during the hospital stay, information from previous admissions, etc.

Primary information was translated, coded and entered into a specialised data base. At a second stage, data were grouped, aggregating factorial and resultative variables to allow summarisation and analysis, thus meeting the study objective. The following statistical methods were used:

- Descriptive statistical methods: extensive variable analysis and graphic analysis
- Analysis of variance – for quantitative data processing. To test the normality of the distribution, Kolmogorov-Smirnov λ criterion was applied. U criterion was used to compare the two sample means. Statistical significance and evidence in support of the null hypothesis were assumed at p = 0.05.
- Parametrical tests were applied for quantitative variables. Depending on the parameters of interest both the classic and Fisher's exact tests were used. To calculate percentages, Van der Vaerden test was applied.
- Correlation analysis was used to test the relationships between factorial and resultative variables. Regression analysis was applied to assess quantitative relationships.
- Non- parametric tests – non-parametrical tests were used to test hypothesis not meeting the criteria for normal distribution. Used tests included: Pearson's squared test, Kolmogorov-Smirnov test, Wilcoxon- Mann – Whitney and Kruskal-Wallis tests.
- Dispersion analysis (Tukey's) tests were applied to allow comparison among multiple pairs of means.
- The method of the least squares was used to equalise dynamic data lines (for the period January 1, 2010, and February 1, 2017) and find the best fit for the data sets.
- The graphic analysis allowed visualisation of the observed relationships and processes.

Data procession was performed using SPSS (SPSS Inc., IBM SPSS Statistica) version 20.0 and Microsoft Office 2010 platform.

Results

In the studied period, 88 patients with suspected AE on admission were treated at the Clinics of Allergy and Occupational Disorders. Regarding their socio-demographic characteristics, it was found out that: most patients with AE were over 50 years of age, the condition affected equally males and females, the sex differences being statistically insignificant in all age groups. Results are presented in Table 1.

Table 1: Distribution of the studied contingent according to age group and sex in AE

Age group	Males			Females			Total	
	Number	%	Sp	Number	%	Sp	Number	%
Up to 30 years of age	7	20.00	6.40	4	7.55	4.15	11	12.50
Between 30-40 years of age .	9	25.71	7.39	9	16.98	5.16	18	20.45
Between 40-50 years of age.	3	8.57	4.74	11	20.75	5.58	14	15.91
Over 50 years of age	16	45.71	8.42	29	54.72	6.84	45	51.14
Total	35	100.00	-	53	100.00	-	88	100.00

Regarding the place of residence, most patients were urban residents, however, among urban residents; females were predominant (61.7%). Regarding socio-economic status, working females were prevailing (53.6%), the percentage of students was least-0.9%. The occupational characteristics of the study population were also addressed as occupational exposure could play a role as a trigger in AE. Of the study contingent, 9 patients were medical professionals. Of them, women, assistant nurses were predominant (30.5%), most of the men worked in the hospital administration (70.8%). Finally, some seasonal distribution of the cases was established – the number of admitted patients with AE peaked in early spring and autumn.

Among cases with HAE, emergency hospital admissions predominate, whereas other AE cases, attended as either emergency patients or as planned admissions. (46% and 54% respectively) This fact illustrated the need for detailed diagnostic work up, laboratory tests and planned long term treatment necessary in AE as well as the need to manage acute attacks of AE. The median duration of the hospital stay was 3 days.

Our study demonstrates that 34.1% of our patients have no history of taking medication before

the onset of AE, however, drug-induced AE could be suspected in 42.05% of the cases. Based on our data the most common therapeutic agents implicated in the aetiology of AE were: NSAID, including aspirin (8% and 3.9%) hormones (estrogen – 4.5) and antibiotics – 11.4% Table 2.

Table 2: Etiology of drug-induced AE

Medication	Based on the patient's history						Total	
	No			Yes				
	Number	%	Sp	Number	%	Sp	Number	%
NSAID	81	92.0	2.89	7	8.0	2.89	88	100
Acetyl-salicilic acid and derivatives	74	84.1	2.51	14	15.9	3.90	88	100
Analgesics, anti-pyretics, anaestetics	81	92.0	2.89	7	8.0	2.89	88	100
Anti-platelet agents	86	97.7	1.60	2	2.3	1.60	88	100
Antibiotics	78	88.6	3.39	10	11.4	3.39	88	100
Radio-contract dyes	88	100	-	0	0	0.0	88	100
Psychoactive agents	86	97.7	1.60	2	2.3	1.60	88	100
Beta-blocking agents	87	98.9	1.11	1	1.1	1.11	88	100
Herbs	87	98.9	1.11	1	1.1	1.11	88	100
Cytostatics	87	98.9	1.11	1	1.1	1.11	88	100
Hormones	84	95.5	2.21	4	4.5	2.21	88	100
Transfusion of plasma, plasma substitutes and colloid solutions	88	100	-	0	0	0	88	100
Local therapeutic agents	82	93.2	2.68	6	6.8	2.68	88	100
Homeopathic agents	88	100	-	0	0	0	88	100
Vitamins	88	100	-	0	0	0	88	100
Latex products	88	100	-	0	0	0	88	100
Synthetic polymers and plastics	84	95.5	2.21	4	4.5	2.21	88	100
Other	62	70.5	4.86	26	29.5	4.86	88	100

It is worth noting that in some cases, the triggering medication could not be identified.

Based on the results from our study, food-induced AE could be suspected in 9.09% of the cases. Nuts, eggs and egg products, as well as sea foods, were most frequently involved. Insect bites, radio contract dyes and physical agents were not commonly implicated in the aetiology of AE, based on the results of our study. Interestingly, stress and sustained trauma as triggers of AE, in our research were identified in 30% of the cases. However, repeated trauma did not result in an increased frequency of edemas. The relationship of AE with other underlying medical conditions, especially allergic and auto-immune have been addressed in other research works. Our study demonstrated that in AE patients, most frequently cardiac conditions were identified (33%). Auto-immune thyroiditis was the second most common-14.8%, followed by musculo-skeletal disorders (10.2%) and diabetes (4.5%) Family history of allergy was seen in 8.4% of the patients, the most frequent allergic disorder, reported, was asthma. In patients with HAE, family history was present in 2.9 % of the patients.

According to the findings in our research, half of the patients were diagnosed at the specialised Clinics of Allergic Disorders, in 20% of the cases, the primary care physician established the diagnosis, the remaining-were diagnosed at the Emergency Centers-15.9%. Most patients were diagnosed after having recurrent attacks-43.2%, only 5% were diagnosed after the first attack. The diagnosis of AE was based on the internationally established criteria – history, clinical symptoms and allergic status; laboratory tests were performed in 54% of the patients. Clinically, AE most frequently affected the face, including the lips and eyelids (43.2%). The oedema was located on the lips only in ¼ of the patients, the involvement of the eyelids was even less frequent-13.6%. Facial oedema, progressing to involve the upper respiratory tract was seen only in 9.1%. It is worth noting that prodromal symptoms were seen in the majority of cases – 84.1% of our cases. Prodromal symptoms are presented in Table 3.

Table 3: Signs and symptoms associated with AE

Symptoms	Based on the patient's history						Total	
	No			Yes				
	Number	%	Sp	Number	%	Sp	Number	%
Itching	29	33.0	5.01	59	67.0	5.01	88	100
Redness	66	75.0	4.62	22	25.0	4.62	88	100
Pain	86	97.7	1.59	2	2.3	1.59	88	100
Rash	42	47.7	5.32	46	52.3	5.32	88	100
Breathlessness	81	92.0	2.88	7	8.0	2.88	88	100
Hoarseness	78	88.6	3.38	10	11.4	3.38	88	100
Nasal congestion	85	96.6	1.93	3	3.4	1.93	88	100
Running nose	85	96.6	1.93	3	3.4	1.93	88	100
Wheezing	86	97.7	1.59	2	2.3	1.59	88	100
Other	69	78.4	4.39	19	21.6	4.39	88	100

In most cases, AE is accompanied by other symptoms as follows: 30.7 % of the patients present with at least two accompanying symptoms, 23.9%-report three symptoms and 20.5% had only one accompanying symptom. Another important issue, regarding AE is the evolution and course of oedema as it influences the choice of treatment approaches. Based on our research, in 71.6% of the cases, the oedema is self-limiting and does not progress to involve other body parts; oedema expands in 15.9% of the cases; in 12.5% of the patients, its duration was more than 24 hours regardless of the administered therapy. In 35.2% of the patients, AE is the only presentation and is rarely associated with other allergic and auto-immune disorders.

In our study patients, the diagnosis was based on the established diagnostic criteria. Laboratory tests were performed in 54%. All patients received anti-histamines and steroids with a very good therapeutic effect. The results are presented in Figure 2.

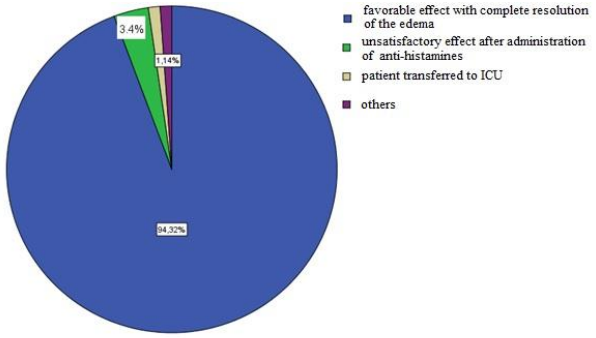

Figure 2: Treatment outcome in angioedema

All patients in our study received treatment. In 95.4% of them, it resulted in complete resolution of AE after the application of anti-histamines and steroids. In 73.3% of the patients, favourable therapeutic results were seen after the third hour.

Laboratory tests were performed in 23.9 % of the patients to exclude HAE. Diagnostic laboratory tests were: the level of C1esterase inhibitor, the level of functional C1INH and the level of C4. In all cases, the tests were within the reference levels. Skin allergy prick tests were performed in 52 patients, and IgE levels were tested in 86 patients. Allergy to pollens was noted in 13.6% of the patients, to occupational allergens in 8.0%; the remaining 4.5% had documented allergy to domestic dust, mites and pets. Specific IgE was detected in only one patient.

Discussion

Regarding the social and demographic characteristics, our research showed that most admitted patients were over 50years of age. This fact seems related to the finding that medication was the most common triggering factor for AE with NSAID being predominant. No significant differences based on sex were reported unlike other studies (Cohen et al.) that found female predominance [15]. However, male predominance has also been reported [16], [17]. The likely explanation for female predominance is that estrogens have been found to exacerbate type III HAE. In our study, no sex differences were established. However, hormones were the third most common triggering factor for AE. A total of 88 patients were diagnosed and treated for AE at the Clinics in the studied period. Among them, urban residents were 53.3%, of them working women were predominant. More cases were registered in the early spring and autumn. It is known from the literature that AE has been frequently associated with other allergic and autoimmune disorders. Our study provides further evidence of this finding – the most common accompanying allergic disorder in our patients was atopic asthma followed by allergic rhinitis.

Regarding autoimmune disorders, thyroiditis was the second most common co-morbidity. Based on the European guidelines, an attempt was made to establish the triggers, however, even at discharge the aetiology remained unclear in 81.61% of the cases, and 13.79 % of the patients had accompanying urticaria. The detailed analysis of the triggering factors found out that our data was similar to data from the literature [18], 42.05% of our patients have a history of medication intake-aspirin, (15.9%), other NSAIDs (8%), antibiotics (11%), analgesics (8%), hormones(4.5%). Rarely there is a history of intake of more than 3 different medications. Most patients do not associate the AE with food, however, some (9.09%) report that certain foods could trigger AE – most frequently nuts (5.7%) eggs (4.5%) and fish and seafood – 1.1%. This incidence is lower compared to literature data [19]. Allergic reaction to food can cause IgE mediated hypersensitivity and release of mediators from mast cells, followed by accumulation of mononuclear cells and neutrophils as part of systemic anaphylaxis. However, food allergy can occur in cases of direct mast cell mediator release.

Interestingly, available literature data show a relationship between sustained trauma and AE in 54% of the cases [20]. In our research, there is a relation between trauma and AE but only 30% of the cases. Another finding is the association of AE and urticaria with other co – morbidities as those with immune etiology [20], [21], [22], [23], [24] – a history of atopic asthma – in 26 patients (33.3%), allergic rhinitis – in 4 patients and other allergic conditions in 17 patients (eczema atopic dermatitis etc.). In our study, cardiac conditions were the most common comorbidities (33.3%), followed by auto-immune thyroiditis (14.8%) and musculoskeletal disorders (10.2%). In these patients, AE seems related to the use of ACEI and NSAIDs. The percentage of accompanying auto-immune disorders is similar to that established by other authors [25]. A family history of HAE was established in only 0.9% of the cases, which is less than the established incidence at the population level. There was a family history of allergic disorders (8.4%), most commonly asthma – 56%.

Many authors working in the field of AE, report that the diagnosis of the disease usually takes time. According to a large study in Spain [25], the median time between the first attack and the diagnosis of AE is 13 years. In our study, we document that in most cases, the diagnosis requires evaluation by a specialist. More rarely it is diagnosed by the GP and at the Emergency Centers. Regarding the clinical features of AE, our findings were similar to those reported by other authors [26]- oedema involved most frequently the face, the lips and the e eyelids. The course was self-limiting in 71. 6% of the cases, rarely progressed to involve other body parts – 15% and the duration of the symptoms was more than 24 hours in 12.4%. The international group on AE has reported similar findings. Most patients present with accompanying symptoms (84.1%). This fact has been documented by other authors [26]. Based on the results from our study, these symptoms are – itching (67%), rash (52.3%) and erythema in the oedema zone (25%). Compared to other authors, the percentage of redness is slightly higher – 48 %. Most patients were admitted and treated at the Clinics after they had had more than three attacks (43.2%). This finding confirms that the diagnosis of AE is often delayed, similarly to other reports in the literature.

In our study, the diagnosis was based on history, clinical features and allergy status. Laboratory tests were performed in 54% of the patients. The C1, C1INH and C4 levels were tested as well as skin prick tests and specific IgE. At least two diagnostic procedures were used. All our patients received treatment – steroids and anti-histamines, which resulted in resolution of symptoms. Specific HAE therapy was not applied. No invasive procedures

(intubation) were necessary.

In conclusion, this study presents an overview of AE. We attempt to analyse the socio-demographic characteristics of AE as well as its most important etiological and clinical features. Based on our result, this is not an uncommon medical condition. The diagnosis is often difficult and delayed and requires specialist evaluation. If recognised on time and adequately treated, the outcomes are favourable.

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Cesarean Section and Development of Childhood Bronchial Asthma: Is There A Risk?

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Abstract

BACKGROUND: Asthma is a chronic inflammatory disease of the airways that results from complex interactions between multiple environmental and genetic influences. In recent years, studies have observed an increase in caesarean section rates, and have suggested a strong association with the rapid increase in the incidence of childhood asthma that cannot be explained by genetic factors alone. In this case-control study, we investigate the association between the developments of childhood asthma with the mode of delivery. We also explored the relationship between mode of delivery and control of asthma.

METHODS: Two groups (509 pediatric patients in total) were assessed between January 1, 2017, and January 1, 2018. Part of these patients, 257 (50.4%) were asthmatic children visiting specialised clinics, and 252 (49.6%) controlled cases selected from a primary health care clinic from the same institution (control group).

RESULTS: The Chi-square test revealed a significant association between cesarean sections and bronchial asthma (OR, 1.483 [95% CI, 1.013–21.71]; P = 0.042). However, the adjusted OR from our binary logistic regression model revealed this association to be insignificant (adjusted OR, 1.417 [95% CI, 0.885–2.269]; P = 0.804). The value of the chi-square of the model shows that the overall model is statistically significant at 1%. The Nagelkerke R square indicates that 34.9% of the variation in having asthma is explained by the risk factors included in the model.

CONCLUSION: We do not believe that the rise in cesarean sections explains the increase in childhood bronchial asthma – at least not in our population. We also found no association between the mode of delivery and asthma control. We encourage further research into this topic, namely to recruit a larger number of patients, and to adjust for the significant risk factors found in our study.

Introduction

Asthma is a common chronic inflammatory disease of the airways resulting from complex interactions between multiple environmental and genetic influences. In children, several risk factors for asthma have been identified, such as male sex, atopy, allergens, infections, secondhand smoke, and perinatal factors including reduced lung function in early infancy, maternal age, prematurity, and in utero exposure to antibiotics. It is well documented that asthma is more common in children with other atopic diseases, such as atopic dermatitis or allergic rhinitis [1]. In recent years, numerous studies have observed a significant increase in cesarean section rates, and have suggested a strong association with the rapid increase in the incidence of childhood asthma that cannot be explained by genetic factors alone [2], [4]. One explanation for this association is the 'hygiene hypothesis', which states that reduced exposure to bacteria causes dysregulation of the immune system by altering the neonatal gut microflora [5]. Compared to normal vaginal deliveries, during which neonates are exposed to the mother's microflora through the birth canal. This exposure is absent in cesarean section deliveries. In turn, this alters the neonatal cytokine response patterns that can consequently result in changes in the stability of Th1/Th2 (T helper) cells, thereby increasing the risk of developing chronic inflammatory conditions [6]. The association between mode of delivery and chronic inflammatory diseases such as inflammatory bowel disease and celiac disease has been extensively investigated [7], [8], [9]. Other studies have also studied the association between the mode of delivery and bronchial asthma [2], [3], [4], [10], [11], [12], [13], [14], [15], [16], [17].

Although several studies have discussed the association between the mode of delivery and atopic diseases, their findings have been inconsistent. This might be attributed to a failure to adjust for confounding risk factors of atopy; for example, in a cohort that did adjust for family history of atopy, children born by cesarean section were two times more likely to have atopy than those born by normal vaginal delivery [10]. Other confounders are affecting the association, such as sex, have also not been accounted for. Identifying risk factors for bronchial asthma is particularly useful in recognising the influence of the mode of delivery and its effect on children's immune systems.

Finding new independent risk factors is essential in understanding the pathophysiology of asthma, which in turn, may improve its treatment and prevention. For instance, this study has the potential to affect a mother's choice in undertaking an elective cesarean section. Additionally, it might prompt paediatricians to screen for asthma among children by cesarean section. In this study, we investigated the association between the developments of childhood asthma with the mode of delivery. To our knowledge, this is the first study of its kind in our local population. We also explore the relationship between mode of delivery and asthma severity (control of asthma).

Methods

Patient selection

This is a case-control study involving children aged 2–14 years, selected from the pediatric asthma clinic, allergy immunology clinic, and the pulmonology clinic at King Abdulaziz Medical City, King Khalid National Guard Hospital (a tertiary hospital), Jeddah, Saudi Arabia. Ethical approval was obtained from our local institutional ethics committee, and informed consent was obtained from the parents before recruiting children into the study. Two hundred and fifty-seven children with a definite diagnosis of asthma (according to the criteria set by the Global Initiative for Asthma, GINA) were invited to join the study. Asthma was diagnosed if the child had a history of recurrent intermittent episodes of a cough; chest tightness; shortness of breath; bilateral wheezing occurring at night, with activity, or when exposed to cold, smoke, fumes or dust; and/or if pulmonary function tests indicated that the child had an airflow obstruction (i.e. if the forced expiratory volume [FEV] in 1 second [FEV1)] was reduced to less than 80% of that predicted; if the FEV_1/forced vital capacity [FVC] ratio was less than 0.85 [85%]; or if a low dose of inhaled steroids, and bronchodilators as needed, demonstrated clinical improvement during a trial period of 2–3 months with symptoms worsening on cessation of treatment) [18], [19]. Children with chronic lung diseases, e.g. cystic fibrosis, congenital cystic adenoid malformation, bronchopulmonary dysplasia, and ciliary dyskinesia, were excluded, as well as children with congenital and chromosomal anomalies. Cases in which the child's family was unable to provide the birth or perinatal history were also excluded. Two hundred and fifty-seven age-matched, non-asthmatic pediatric patients who presented to the primary health care clinic or general pediatric clinic were concurrently enrolled as control subjects. These children were seen in the hospital for other, unrelated problems. Control patients with a history of allergic rhinitis or eczema were not excluded from this study.

Data collection and investigations

The parents of children enrolled in the study provided the following data about their children: demographic data; risk factors of bronchial asthma, including parental history of atopy, defined as current allergy to house dust mites or pets, current hay fever or ever having asthma; diagnosis of other atopic diseases, e.g. atopic dermatitis, allergic rhinitis, and conjunctivitis (based on history alone) [19]; parents' smoking habits; and presence of furry pets in the home. Prenatal and perinatal risk factors were also provided, including smoking during pregnancy; maternal exposure to indoor or outdoor allergens (dust or grass); birth order; maternal history of taking any of the following during pregnancy: antibiotics, non-steroidal anti-inflammatory drugs (NSAIDs), or beta-blockers; maternal age; maternal history of gastroesophageal reflux disease (GERD) or obstructive sleep apnea; and gestational age. Parents also disclosed the mode of delivery of their child (normal vaginal delivery, emergency cesarean section, or elective cesarean section), and the mode of infant feeding categorized as no breastfeeding, partial breastfeeding (introduction of formula or weaning before 6 months), and exclusive breastfeeding (breastfeeding only for at least 6 months). Control of asthma was assessed using GINA guidelines and was categorised as controlled asthma, partly controlled asthma, and uncontrolled asthma [190].

Statistical analysis

Data were analysed using IBM SPSS software (version 23). Descriptive analysis of the characteristics of the control and asthma groups was performed. Univariate analysis, which compared differences between the control and asthma groups, was done using Student's t-test, the Chi-squared test, and Fisher's exact test (where appropriate). A P-value of < 0.05 was considered to be statistically significant. Binary logistic regression was used to assess the independent relationship between asthma and cesarean section. Confounding factors included in the final model are listed in Table 3. Results are

expressed as odds ratios (OR) and 95% confidence intervals (CI).

Results

Demographic characteristics of the study population

The two groups of patients, amounting to 509 pediatric patients in total, were assessed between January 1, 2017, and January 1, 2018. Of these, 257 (50.4%) patients visiting the pediatric asthma clinic, allergy immunology clinic or pulmonology clinic at King Abdulaziz Medical city was diagnosed with bronchial asthma. Two hundred fifty-two control cases (49.6%) were selected from a primary health care clinic or the general pediatric clinic in the same institution. In univariate analysis, cases and controls were similar with regards to weight, height, and body mass index. However, there was a significant difference in age between asthma and control groups (mean, 7.55 years in the asthma group versus 6.8 years in the control group; P = 0.014). Most (62.3%) of the asthma cases were males.

On the other hand, in the control group, there was no significant difference in sex distribution. Patients' main demographic and clinical characteristics are provided in Table 1. Factors associated with a higher risk of developing bronchial asthma were: a history of atopy in case patients, a family history of atopy (such as atopic dermatitis, allergic rhinitis, conjunctivitis), maternal age (calculated by mean), order of child (calculated by mean), maternal use of NSAIDs during pregnancy, maternal diagnosis of GERD, and premature birth (see Table 1).

Table 1: Demographic characteristics of the study population

Characteristics (N = 509)	Children without asthma (N =252, 49.6%)	Children with asthma (N = 257, 50.4%)	Odds ratio	95% confidence interval	*P*-value
Demographics					
Age mean (SD)	6.80 (3.3%)	7.55 (3.56%)			0.014
Height mean (SD)	116.45 (19.5%)	119.70 (20.40%)			0.052
Weight mean (SD)	23.24 (12.7%)	25.64 (13.71%)			0.080
Body mass index mean (SD)	16.12 (3.8%)	16.65 (3.90%)			0.138
Sex					
Male	127 (50.4%)	160 (62.3%)	1.624	1.142–2.309	0.007
Female	125 (49.6%)	97 (37.7%)			
Asthma risk factors					
FHx of atopy	80 (31.5%)	122 (47.5%)	1.966	1.370–2.819	0.000
Hx of atopy	43 (16.9%)	125 (48.6%)	4.647	3.087–6.996	0.000
Parent smoking	91 (35.8%)	76 (29.6%)	0.752	0.519–1.090	0.132
Pets	25 (9.8%)	40 (15.6%)	1.688	0.991–2.877	0.052
Prenatal risk factors for asthma					
Maternal age mean (SD)	27.83 (5.9)	30.05 (6.44)			0.000
Maternal smoking	4 (1.6%)	0 (0.00%)	0.984†	0.969–1.000	0.060*
Exposure to smoke	88 (34.6%)	72 (28.00%)	0.734	0.504–1.069	0.106
Order of child mean (SD)	2.73 (2.7%)	3.46 (2.36%)			0.001
Medication use					
Antibiotics	20 (7.9%)	17 (6.6%)	0.829	0.424–1.621	0.302
NSAIDs	45 (17.7%)	21 (8.2%)	0.413	0.238–0.717	0.001
Beta-blockers	2 (0.8%)	0 (0%)	0.992†	0.981–1.003	0.247*
Maternal history					
GERD	93 (36.6%)	48 (18.7%)	0.398	0.26–0.596	0.000
OSA	26 (10.2%)	25 (9.7%)	0.945	0.530–1.685	0.848
Natal risk factors					
Preterm	21 (8.3%)	45 (17.5%)	2.355	1.358–4.084	0.002
Mode of delivery					
Emergency cesarean section	38 (15.0%)	44 (17.1%)	1.174	0.731–1.885	0.506

*These have at least 1 cell with an expected count of less than 5. Therefore, for these odds ratios, we relied on the *P*-value of Fisher's Exact Test instead of Pearson's Chi-square: †Risk estimate for the cohort.

Cesarean sections and the risk of developing asthma

The Chi-square test revealed a significant association between cesarean sections and bronchial asthma (OR, 1.483 [95% CI, 1.013–21.71]; P = 0.042); Table 2. However, the adjusted OR from our binary logistic regression model revealed this association to be insignificant (Adjusted OR, 1.417 [95% CI, 0.885–2.269]; P = 0.804); Table 3. The value of the chi-square of the model shows that the overall model is statistically significant at 1%. The Nagelkerke R square indicates that 34.9% of the variation in having asthma is explained by the risk factors included in the model.

Table 2: Univariate analysis of cesarean section delivery and bronchial asthma

	Children without asthma (N = 252, 49.6%)	Children with asthma (N = 257, 50.4%)	Odds ratio (95% confidence interval)	*P*-value
Children born by cesarean section	66 (26.2%)	88 (34.2%)	1.483 (1.013-21.71)	0.042
Children born by natural delivery	186 (73.8%)	169 (65.8%)		

The overall percentage of classification ability of the model is 74.8%. According to our regression model, other risk factors include a family history of atopy, history of atopy, use of NSAIDs during pregnancy, a diagnosis of GERD during pregnancy, gestational age, duration of breastfeeding and maternal age were found to be significant (Table 3).

Table 3: Binary logistic regression model for asthma risk factors

Variables in the equation

	B	S.E.	Wald	df	Sig.	Exp (B)	95 % CI for Exp (B) Lower	95 % CI for Exp (B) Higher	Exp (B)$^{-1}$
FHxA*	0.675	0.231	8.549	1	0.003	1.964	1.249	3.088	
HxA*	1.750	0.248	49.634	1	0.000	5.753	3.536	9.360	
PS	–0.288	0.225	1.628	1	0.202	0.750	0.482	1.167	
Pets	0.643	0.330	3.783	1	0.052	1.902	0.995	3.634	
		18087.36							
MS	–20.8785		0.000	1	0.999	0.000	0.000	0.001	
MES	–0.369	0.243	2.303	1	0.129	0.692	0.430	1.113	
OC	0.113	0.061	3.446	1	0.063	1.120	0.994	1.263	
AB	0.124	0.437	0.080	1	0.777	1.132	0.481	2.664	
NSAIDS*	–1.027	.354	8.411	1	0.004	0.358	0.179	0.717	0.9737
		26285.76							
BB	–21.3222		0.000	1	0.999	0.000	0.000	0.001	
GERD*	–1.228	0.260	22.311	1	0.000	0.293	0.176	0.487	0.8143
OSA	–0.265	0.376	0.496	1	0.481	0.767	0.367	1.604	
GA*	1.212	0.332	13.328	1	0.000	3.362	1.753	6.445	
C-sec	0.348	0.240	2.104	1	0.147	1.417	0.885	2.269	
DBF*	0.041	0.012	11.240	1	0.001	1.042	1.017	1.067	
MA*	–0.036	0.011	11.979	1	0.001	0.964	0.945	0.984	27.7

*Significant risk factor; FHxA: family history of atopy; HxA: history of atopy; PS: parent smoking; Pets: owning pets; MS: maternal smoking during pregnancy; MES: maternal exposure to smoke; OC: order of child; AB: antibiotics taken during pregnancy; NSAIDs: non-steroidal anti-inflammatory drugs taken during pregnancy; BB: beta-blockers taken during pregnancy; GERD: gastroesophageal reflux disease diagnosis during pregnancy; OSA: obstructive sleep apnea; GA: gestational age; C-sec: cesarean section; DBF: duration of breastfeeding; MA: maternal age. The overall percentage of classification is 74.8%. The Nagelkerke R square is 0.349. The chi-square of the model is 153.998.

Cesarean sections and the control of bronchial asthma

Among the 88 asthmatic children born by cesarean section, 34.1% had their asthma under control, 31% had it partially controlled, and 42.1% did not have it under control. On the other hand, among

the children born by natural delivery, 65.9% had their asthma under control, 69% had it partially controlled, and 57.9% had uncontrolled asthma. The results of the Chi-square test shows that there is insufficient evidence to suggest an association between the control of asthma and mode of delivery (χ^2 = 1.449, P = 0.485) (Table 4).

Table 4: Univariate analysis of cesarean section delivery and control of asthma

	Controlled asthma (N = 135, 52.5%)	Partly controlled asthma (N = 84, 32.7%)	Uncontrolled asthma (N = 38, 14.8%)	*P*-value
Children born by cesarean section	46 (52.3%)	26 (29.5%)	16 (18.2%)	0.485
Children born by natural delivery	89 (52.7%)	58 (32.7%)	22 (14.8%)	

Discussion

Many previous studies have investigated the association between cesarean section delivery and bronchial asthma in children, but the topic remains controversial. In our study, as illustrated in our regression model, delivery by cesarean section was not independently associated with childhood bronchial asthma. And consistent with other reports, our data suggest that any relationship between cesarean section and bronchial asthma may be explained by the influence of other confounding variables and not the mode of delivery itself [10], [11], [12], [13], [14]. However, some other studies have found a significant association, including two meta-analyses [2], [3], [4], [15], [16], [17]. with pooled ORs of 1.18 and 1.20 [15], [16]. Discrepant findings may be attributed to some factors. Firstly, the studies included in the meta-analysis were from high-income countries, and this might have affected their results; a large cohort study in Brazil found no evidence of an association between mode of delivery and risk of wheezing [11]. Perhaps the children in our population have alternative sources of microbiological exposure, other than normal vaginal delivery. Secondly, several of the studies that did find a positive association did not account for the many confounding risk factors, such as duration of breastfeeding, use of NSAIDs, or a diagnosis of GERD during pregnancy [2], [3], [17]. To our knowledge, our study is the first to investigate the effect of mode of delivery on the control of asthma (asthma severity). This association was explored to determine whether children born by cesarean section have a deregulated immune system that causes their asthma to be more sensitive to triggers, thus more difficult to control. However, our results show no association between control of asthma and cesarean section.

Our study has a few limitations. Firstly, we did not investigate the relationship between cesarean section and atopy because only a few of our patients had undergone relevant objective investigations, such as IgE levels or allergy skin testing. Secondly, we were not able to determine whether the children in our study born by cesarean section were subject to premature rupture of membranes, which would have exposed them to maternal microflora. Thirdly, since our study is retrospective, data regarding cofounding risk factors may have been affected by recall bias. Nevertheless, the parents of the patients included in this study were confident of the mode of delivery of their children. Finally, limited statistical power means we could not separately investigate emergency cesarean section rates versus scheduled cesarean sections.

In conclusion, we do not believe that the rise in cesarean sections explains the increase in childhood bronchial asthma – at least not in our population. We also found no association between the mode of delivery and asthma control. We encourage further research into this topic, namely to recruit a larger number of patients, follow a cohort study design, and to adjust for the significant risk factors found in our study.

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The Relationship between the Type of Cleft and Nasal Air Emission in Speech of Children with Cleft Palate or Cleft Lip and Palate

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Abstract

BACKGROUND: Cleft palate, due to damage of the soft palate, leads to dysfunction, i.e., inappropriate closure of the velopharynx during speech production, thus resulting in velopharyngeal insufficiency which characterises with hypernasal speech and nasal air loss/emission during speech production.

AIM: To establish the relationship between the type of cleft according to the Veau classification and the degree of nasal air emission in the speech of patients with cleft using auditory-visual perceptual assessment procedures.

MATERIAL AND METHODS: A group of 40 patients with irregular speech aged 4 to 7, out of which 20 with cleft palate or cleft lip and palate, participated in the research. The Veau classification was used to classify the cleft severity, while an indirect instrumental examination was conducted with the See-Scape instrument to detect nasal air emission during the speech.

RESULTS: The respondents with cleft palate or cleft lip and palate of higher Veau class had a greater degree of nasal air emission during the speech. There is a positive, statistically significant correlation between the results obtained with the Veau classification of cleft lip and palate, and the degree of nasal air emission. The value of Spearman's coefficient of correlation is R = 0.46, and the calculated p-value is p = 0.04.

CONCLUSION: A more severe cleft type is associated with an increased degree of nasal air emission during the speech, and vice versa.

Introduction

Cleft lip and/or palate, depending on the form in which they appear, generate many problems, such as difficulties while feeding, nursing and swallowing, impaired hearing, orthodontic anomalies, speech disorder, impaired resonance, aesthetic and psychosocial disorders, social and professional isolation and the like [1]. Cleft lip and palate affect all oral functions, and the consequences are especially evident in a speech [2].

Children with cleft lip and palate have pathology in verbal communication, and the reason for that is the incomplete closure of the velopharyngeal sphincter, i.e., the soft palate, lateral walls and posterior wall of the pharynx, which means the oral cavity is not separated from the nasal cavity during speech and swallowing [3]. In the speech, the closure of the velopharyngeal sphincter separates the oral from the nasal cavity, which prevents nasal air loss in the production of all sounds, except for the nasal consonants /M/, /N/ and /Nj/. The incomplete closure during speech production results with velopharyngeal insufficiency which characterises with hypernasal speech and nasal air emission [4]. If there is no closure of the velopharyngeal sphincter, the child will lack the adequate aerodynamic conditions for the adoption of normal articulation during speech development. Thus, the dysfunctional velopharyngeal sphincter, i.e., cleft palate or cleft lip and palate affect the development of speech and compensatory mechanisms greatly during articulation.

Articulation is not a mechanism the child is born with; it is adopted through the process of learning over time when the speech-language system is

formed. The conditions for the development of regular speech are anatomical, well-functioning speech and hearing organs and a proper speech model. If these conditions do not exist, the resulting adoption of compensatory mechanisms leads to a pathological and completely incomprehensible articulation. The most noticeable articulatory mistakes in the speech of children with cleft palate occur during the production of consonants that require high pressure in the oral cavity [5]. The child's speech can only be understood by his parents, while social contacts are encumbered. The speech disorders impair the individual and disturb their emotions deeply as well. Bearing in mind that speech is an indispensable tool for expressing and conveying thoughts and the most perfect means of communication, it is important that the impairments are resolved from the very beginning, which asks for inclusion of a multidisciplinary specialist team and team approach [6].

The basis of impaired speech lies in hypernasality, i.e. nasal air emission, or directing part of the air through the nose during the speech when the intraoral pressure, which is most important for speech, cannot reach its necessary value. Hypernasality is one of the most typical characteristics of children with cleft [7], [8]. As it is known, nasal air emission often occurs with hypernasality, but it can occur with normal resonance, too [9]. There are four types of nasal air emission [10]: inaudible nasal air emission, audible nasal air emission, nasal rustle (nasal turbulence) and nasal emission typical for phonemes. Apart from the last one, the three other types of nasal air emission are often and commonly accompanied by severe, moderate or mild hypernasality.

Therefore, establishing a link between hypernasality, i.e., nasal air emission during speech and the type of cleft palate can provide useful information for future diagnostic and therapeutic purposes in the field of speech disorders.

The relationship between the degree of anatomical disruption of the primary and the secondary palates, on which the Veau classification for cleft is based, and nasal air emission in the speech of children with a cleft can be established using perceptual and instrumental (direct and indirect) examinations. Since it is primarily a matter of dealing with a young population from a very early age, it is important to consider non-invasiveness and simplicity during implementation, but fast and precise diagnosis, too. These are just some of the advantages and benefits of perceptual examinations and some other instrumental indirect examinations as well.

Still, the last tool in the chain of procedures for treating speech disorders is not diagnostics. The final correction from an anatomic, morphological and functional aspect is what matters. The success in resolving the existing problem depends on an efficient and appropriate treatment, but palatoplasties as well.

Material and Methods

Material

To meet the established aims, the clinic and paraclinical examinations were conducted in the Center for rehabilitation of hearing, speech and voice – Skopje, during the period from January to December 2016. The patients included in the research were a total of 40, aged 4 to 7, out of which 22 (55%) were females and 18 (45%) males. According to the established diagnosis (congenital anomaly: Palatoschisis or Cheilognatopalatoschisis; irregular speech: Rhinolalia or Dyslalia), the respondents were divided into two groups. The first comprised 20 children with cleft palate or cleft lip and palate and irregular speech Rhinolalia (experimental group). These respondents have already undergone surgery for cleft correction. The second group (control group, comprised 20 children with no cleft, but with irregular speech Dyslalia.

Methods

For this paper, one perceptual and one indirect instrumental examination were conducted. The respondents from the experimental group underwent a clinical, i.e., perceptual examination with the Veau classification for determining the type of cleft [11], which is normally used as an objective measure for determining the severity of the clinical picture of the cleft [12]. Considering the fact that some of the methods for instrumental examination characterize with invasiveness, and having in mind the age group of the patients included in the research (aged 4 to 7), paraclinical examination was chosen (instrumental examination) to carry out the quantitative determination of the degree of nasal air emission by means of the See-Scape instrument [13], [14].

Veau classification of cleft lip and palate

The degree of anatomical disruption of the primary and the secondary palates, on which the Veau classification of cleft lip and palate is based, affects speech greatly. Having that in mind, to determine the type of cleft in our paper the Veau classification was used, which is a widely used system [11]. With the application of extraoral and intraoral examination (Figure 1), it was established whether our respondents had a cleft, and, if so, the same was classified as:

Class I – The cleft only includes the soft palate;

Class II – The cleft includes the hard and soft palate and is limited to the secondary palate;

Class III – The cleft is a completely unilateral cleft lip and palate;

Class IV – The cleft is bilateral cleft lip and palate.

Figure 1: Establishing the existence of cleft lip and palate

Objective assessment of nasal air emission in speech

The See-Scape instrument is used for instrumental examination [15] of nasal air emission in speech. The examination is indirect, non-invasive and simple to conduct. In addition to the clear visual representation, the instrument gives the opportunity to objectively measure the nasal air emission of the patient during the speech. Measuring nasal air emission using the See-Scape instrument (Figure 2) begins by inserting the nasal tip in one of the patient's nostrils.

Figure 2: Measuring nasal air emission using the See-Scape instrument

The nasal tip is connected through a small flexible tube to a rigid plastic vertical tube, graded from 1 to 7, where 1 represents the lowest degree of nasal air emission, while 7 the highest. During the speech, if the patient releases air nasally, the foam piston in the rigid plastic vertical tube reacts instantly and rises.

The examination with See-Scape includes procedures at phoneme, word and sentence level. At the phoneme level, the respondent repeats isolated phonemes in words previously tested and established that they are nasalised (e.g. raka-maka, Viki-Miki). At word level, respondent repeats words that do not contain nasal sounds /M/, /N/ and /Nj/ (e.g. zhaba-kapa), while at sentence level, the respondent repeats short sentences not containing /M/, /N/ and /Nj/ (e.g. Kate kupi kaput; Tode vide dete). All these procedures indicate which phonemes, words and sentences raise the foam piston in the rigid plastic vertical tube, respectively. The highest degree obtained through the examination is considered as a final degree of nasal air emission.

Statistical analysis

The statistical analysis of the data obtained from the research was done in the statistical programs Statistica for Windows 7.0 and SPSS 17.0. The obtained data is represented in tables and figures. The categorical (attributive) variables are represented with absolute and relative numbers. For comparing the analysed variables between the experimental and control group, a non-parametric test (Fischer exact test) was used. The correlation between the results within the experimental group was analysed with the Spearman's Rank-Order Correlation coefficient. The statistical significance was defined at level $p < 0.05$.

Results

Results of the total sample

Twenty (50%) of the respondents with a cleft palate of different type had their cleft classified. According to the Veau classification, the largest number and percentage of those respondents belonged to Class III (Table 1), i.e., they had a complete unilateral cleft lip and palate – 9 (45%), followed by respondents of Class I, whose cleft only included the soft palate – 7 (35%), then 3 (15%) respondents of Class II, where cleft included the hard and soft palate and was limited to the secondary palate, and only one respondent belonging to Class IV, which included bilateral cleft lip and palate.

Table 1: Number of respondents according to the type of cleft

Veau classification of cleft lip and palate	n (%)
Class I. The cleft includes only the soft palate	7 (35)
Class II. The cleft includes the hard and soft palate and is limited to the secondary palate	3 (15)
Class III. The cleft is a completely unilateral cleft lip and palate	9 (45)
Class IV. The cleft is bilateral cleft lip and palate	1 (5)

The results from the experimental examination with the See-Scape instrument (Table 2) revealed that 16 (40%) of the respondents had no nasal air emission. In the group of respondents where the instrument registered nasal air emission, the most common level was 7, which is equivalent to the highest degree of nasal air emission, and this was the case with 10 (25%) of the respondents. Among the 24 respondents where nasal air emission was registered hypernasality was also noticed.

Table 2: Number of respondents according to the degree of nasal air emission

See-Scape The degree of nasal air emission		n (%)
No nasal air emission	0	16 (40)
Degree of nasal air emission	1	5 (12.5)
	2	3 (7.5)
	3	2 (5)
	4	1 (2.5)
	5	1 (2.5)
	6	2 (5)
	7	10 (25)

Comparative analysis of results from the experimental and control group

The results from the experimental examination with the See-Scape instrument (Table 3) revealed that all 16 respondents with no nasal air emission belonged to the control group. On the other hand, the presence of nasal air emission was established in 20 (100%) of the respondents from the experimental group and only 4 (20%) from the control group. Also, the statistical difference in the presence of nasal air emission between both of the examined groups was confirmed as significant for p = 0.0000002. In the experimental group, the most commonly measured degree of nasal air emission was 7 i.e. 10 (50%) respondents, while among 4 of the respondents with nasal air emission from the control group, 3 were with degree 1, while 1 respondent was with degree 2.

Table 3: Number of respondents according to the degree of nasal air emission

See-Scape The degree of nasal air emission	Group		*p*-value
	Experimental n (%)	Control n (%)	
No nasal air emission	0	16 (80)	no/has p = 0.0000002**
Presence of nasal air emission	20 (100)	4 (20)	
The degree of nasal air emission			
1	2 (10)	3 (15)	
2	2 (10)	1 (5)	
3	2 (10)	0	
4	1 (5)	0	
5	1 (5)	0	
6	2 (10)	0	
7	10 (50)	0	

p (Fisher exact test); ***p* < *0.01.*

Correlation between results within the experimental group

Table 4 shows the distribution of respondents from the experimental group with Class I, Class II, Class III and Class IV type of cleft established with the Veau classification, and concerning the degree of nasal air emission measured instrumentally with the See-Scape instrument. The highest degree of nasal air emission was measured in 2 of the 7 respondents with cleft only of the soft palate (Class I), 4 of the 9 respondents with cleft lip and palate (Class II), all 3 of the respondents with a completely unilateral cleft lip and palate (Class III), and the respondent with bilateral cleft lip and palate (Class IV).

Table 4: Distribution of respondents according to the type of cleft and degree of nasal air emission

See-Scape The degree of nasal air emission	Veau classification of cleft lip and palate			
	Class I n (%)	Class II n (%)	Class III n (%)	Class IV n (%)
1	1 (14.29)	1 (11.11)	0	0
2	1 (14.29)	1 (11.11)	0	0
3	1 (14.29)	1 (11.11)	0	0
4	1 (14.29)	0	0	0
5	1 (14.29)	0	0	0
6	0	2 (22.22)	0	0
7	2 (28.57)	4 (44.44)	3 (100)	1 (100)

Positive, statistically significant correlation was established between the results obtained with the Veau classification of cleft lip and palate and the degree of nasal air emission (R = 0.46; p = 0.04) (Figure 3), which points to the conclusion that the degree of nasal air emission rises with the increase of the severity of cleft, and vice versa.

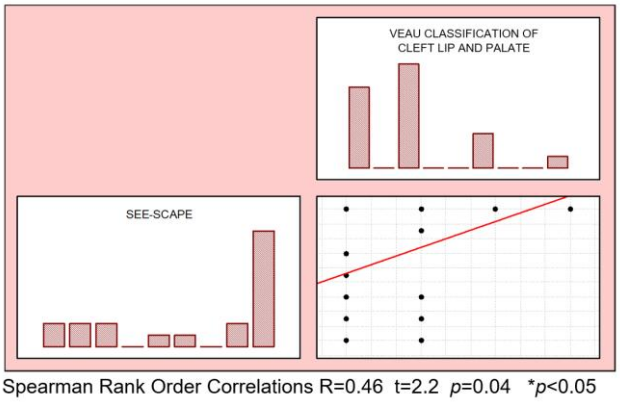

Figure 3: Correlation between the type of cleft and the degree of nasal air emission

Discussion

An anatomical and physiologically functional vocal apparatus plays an important role in correct articulation and speech. The relation of cleft palate or cleft lip and palate – nasal air emission – regular speech requires a separate analysis of each of them because, in such a way, unique information will be obtained about their condition and role in the speech of children with cleft palate or cleft lip and palate. Since it is primarily a matter of dealing with a population of the youngest age, it was important to consider the non-invasiveness and simplicity of the examinations, as well as a fast and precise diagnosis during implementation. These advantages and benefits were gained with the application of the perceptual examination method which determined the grade of severity of cleft palate, but also with the application of an instrumental non-invasive indirect method for determining of the nasal air emission in the speech of children with cleft palate or cleft lip and palate.

The results obtained from the examined variables in this research for the respondents with cleft palate or cleft lip and palate and irregular speech (experimental group) differed from those of the respondents with only irregular speech (control group), i.e., the values obtained for nasal air emission during speech are much higher for the experimental group. Specifically, our research results revealed statistically significant more common presence of nasal air emission in the respondents with cleft palate or cleft lip and palate (experimental group) than those with no cleft (control group).

Within the experimental group, our research results revealed that in the speech of respondents with cleft palate or cleft lip and palate the degree of nasal air emission rises with the increase of the severity of cleft and vice versa.

Some authors worldwide have conducted different research in this field, but each of them analyses and presents, with their approach and from their perspective, the importance of the type of cleft and nasal air emission in the speech of children with cleft palate or cleft lip and palate. Taking into consideration all the research that has been done so far, there is no study, or studies, in the international scientific literature identical to this research where the same methods and number of respondents are used so that a complete comparison of the results obtained could be made. Still, there was an opportunity to make limited comparisons and correlations with similar research by other authors, but only about certain defined variables. It has been proven that the results obtained in our research are, to a certain level, compatible with the results obtained in other research, but also different at times.

Kaewkumsan et al., [16], who examined the degree of hypernasality and formation of an oronasal fistula in 40 patients, aged between 5 and 6,9 years, with non-syndromic cleft palate with or without cleft lip, established that the patients with cleft Class IV and Class III (according to the Veau classification) had a higher total score for hypernasality than patients with Class I. Garcia-Vaquero et al., [17] conducted research about the hearing and speech of 121 patients aged above 6 who had already undergone intervention of the cleft palate. Among other things they also examined the relation between Veau classification and hypernasality. They established a direct association between hypernasality and the Veau grade of cleft palate ($p = 0.053$).

In our paper, the increasing severity of cleft is related to a higher degree of nasal air emission; while it must also be pointed out that hypernasality was noticed in all respondents where nasal air emission was registered. The See-Scape instrument used in our examination indicates nasal air emission during speech rather than nasality, but in that way, it also indicates towards lack of velopharyngeal competence, which is perceived as hypernasality [14]. Also, when it comes to nasal air emission and hypernasality during the speech, Dotevall et al., [18] have established a strong link between the results of velopharyngeal function and hypernasality and nasal air emission. They examined the correlation between perceptual assessment of speech variables related to velopharyngeal function and the behaviour of nasal air emission in the phase of velopharyngeal closure in the speech of children with or without cleft palate. According to them, the results for nasal air emission related to velopharyngeal closure during the speech were closely related to the perceptual results for velopharyngeal function and hypernasality.

Marrinan et al., [19] had researched 228 patients with cleft palate to determine the relative importance of the surgical technique, the year when it was conducted and the type of cleft on velopharyngeal function. According to them, it was commonly believed that the more severe the cleft was (according to the Veau classification), the weaker the speech results would be. However, according to Marrinan et al., [19] the patients with Veau Class II and IV had weaker speech results than those with Class I and III. They suggest that the predicted gradation of the severity of the cleft described with the Veau classification may not be clinically progressive, at least in respect to the speech results. Similar is the attitude of Timmons et al., [20], who studied a speech of patients after correction of the isolated cleft palate or cleft lip and palate. They point out to the existence of many studies in which focus is to make a correlation between speech results and the degree of anatomical disruption of the primary and the secondary palates (Veau classification). According to their study, some authors found that there was no correlation between the type of cleft and degree of speech disorders, while others that severe cleft entailed worse speech. Still, the results of Timmons et al. did not reveal a simple correlation between the Veau classification and speech. Lam et al., [4] imply that among the studies that examined speech results upon palate correction, certain researchers noticed negative or non-linear association with the severity of the cleft as defined by the Veau classification.

In their experiment, Coston et al., [21] assessed the surgical intervention of m.levator veli palatini based on the velopharyngeal competence acquired as a result of the intervention. An important finding which the results of Coston et al., the show is that the more severe the cleft is, the less likely the surgical intervention is to produce favourable results, i.e., have normal nasality and no nasal air emission [21].

Our study contains certain limitations that should be pointed out. First, future research should include a greater number of respondents so that the obtained results have greater relevance. The second limitation is that this research only established the existence and classification of cleft (cleft palate or cleft lip and palate) using the Veau classification, but no a

measurement of its width. Considering that the types of cleft from the same Veau class can have different width [22] which can affect the degree of nasal air emission during the speech, future research should explore this aspect as well.

Given that certain speech aspects are directly related to the condition of cleft palate or cleft lip and palate and nasal air emission establishing unique information about their condition and status is of great importance for correct diagnosis and rehabilitation of speech disorders in children with cleft palate or cleft lip and palate.

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The Pattern of Fasting and Post 75 G Glucose Loading of Glucagon-Like Peptide 1 Levels in Obese and Non-Obese Subjects

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Abstract

BACKGROUND: Prevalence of obesity increased sharply recently; it was associated with an increased prevalence of several cardiometabolic diseases. Reduced glucagon-like peptide 1 (GLP-1) secretion is observed among obese subjects in many studies, and it may mediate the failure of insulin secretion response to food intake.

AIM: To evaluate the pattern of fasting and post 75 g glucose loading of GLP-1 levels in obese and non-obese subjects.

METHODS: An experimental study on the pattern of GLP-1 levels in fasting state and response in post 75 g glucose loading in obese and non-obese subjects, was conducted. Sixteen obese and 16 non-obese subjects were enrolled in the study, with age- and sex-matching in both groups. GLP-1 levels were measured at fasting state (0), 15, 30, 60, and 120 minutes post-glucose loading.

RESULTS: The GLP-1 response to glucose loading were similar in obese and non-obese subjects, which increased from fasting state to post glucose loading and reaching the peak levels in 15 minutes, then declined until the end of observation. There was tendency that GLP-1 levels in fasting state and post glucose loading were lower in obese subjects compared to in non-obese subjects (in fasting state, 5.67 vs. 6.16 ng/mL, P = 0.338; in 15 minutes, 6.20 vs. 6.94 ng/mL, P = 0.239; in 30 minutes 6.20 vs. 6.90 ng/mL, P = 0.264; in 60 minutes, 5.77 vs. 6.12 ng/mL, P = 0.242), but the difference were not statistically significant, except in 120 minutes (5.24 vs. 6.67 ng/mL, P = 0.049; in obese and non-obese subjects, respectively). Similar finding was also seen in the pattern of response (delta) of GLP-1 from time-to-time observation among obese and non-obese subjects (0-15 minutes [0.52 vs. 0.8 ng/mL, P = 0.350], 0-30 minutes [0.53 vs. 0.74, P = 0.550], 0-60 minutes [0.11 vs. 0.31 ng/mL, P = 0.546], in 0-120 minute [-0.42 vs. 0.31, P = 0.006]).

CONCLUSIONS: The patterns of GLP-1 levels post glucose loading were similar in obese and non-obese subjects which increased from fasting state to post glucose loading, reaching the peak levels in 15 minutes and then declined until the end of observation, except in non-obese subjects where the GLP-1 levels were increased at 120 minutes. There was a tendency of GLP-1 levels in fasting state and post-glucose loading to be lower in obese subjects compared within non-obese subjects.

Introduction

Based on the World Health Organization report, the prevalence of obesity has doubled between 1980 and 2008. The highest prevalence of obesity is presented in the American region (62% have overweight, and 26% are obese), and the lowest prevalence is shown in Southeast Asia (14% are overweight, and 3% experience obesity). As many as 2.8 million people die each year from being overweight or obese [1]. Overweight and obesity trigger 44% of the incidence of diabetes, 23% of the incidence of ischemic heart disease and around 7-41% of cancer incidence [2]. The prevalence of obesity in many countries tends to be increased, including in Indonesia. Data from Basic Health Research in 2007 and 2013 reported by Ministry of Health Republic of Indonesia revealed that the prevalence of obesity in adults was increased from 13.9% to 19.7% in men and from 14.8% to 32.9% in women, and followed by increasing the prevalence of

diabetes from 5.7% to 6.9% [3], [4]. A survey on obesity and diabetes in Bali by Suastika et al., in 2011 showed that the prevalence of central obesity, impaired fasting glycemia and diabetes in adults were 35%, 13.1% and 5.9%, respectively [5].

Glucagon-like peptide-1 (GLP-1) is an incretin hormone produced by L cells in the small intestine and released in response to meal intake. Postprandial GLP-1 secretion in response to oral carbohydrate meal was reduced in obese subjects [6]. The ADDITION-PRO Study published by Faerch et al., in 2015 revealed that subjects with overweight and obesity had up to 20% reduced GLP-1 response compared with normal weight subjects. And, compared to individuals with normal glucose tolerance (NGT), women with prediabetes or type 2 diabetes mellitus (T2DM) had 25% lower GLP-1 response to an OGTT, and both in women and men with prediabetes or T2DM had 16-21% lower 120-minutes GLP-1 concentration [7]. A similar finding was seen in a study by Munoz et al., that subjects with normal weight had lower fasting GLP-1 levels significantly compared to subjects with overweight and obesity (8.27 *vs* 9.02 *vs* 8.86 pM, respectively) [8].

Impaired function of GLP-1 contributes to the impairment of insulin secretion in patients with type 2 diabetes mellitus (T2DM) [9]. A study on GLP-1 levels among normoglycemia and type 2 diabetes in Sanglah Hospital showed that fasting and 1-hour post 75 g glucose loading GLP-1 levels was lower in type 2 diabetes than in normoglycemic subjects [10].

Since there were in the variations data from several studies and there was no detailed data on the patterns of GLP-1 levels post glucose loading in obese and non-obese subjects in Indonesia, the study was conducted to evaluate the pattern of fasting and post 75 g glucose loading of GLP-1 levels in obese compared with non-obese subjects. Hopefully, the result of the study can be used to determine the peak response of GLP-1 levels post loading in the term for further study.

Methods

An experimental study on fasting and post 75 g glucose loading of GLP-1 levels among obese and non-obese subjects at Sanglah Hospital during April-June 2018 was carried out. Total of 32 subjects, 16 obese and 16 non-obese subjects was enrolled in the study and was matched for age and sex in both groups. Age of subjects was between 20-50 years (31.46 ± 4.81 years), and they had no diabetes. Subjects with obesity were confirmed if body mass index (BMI) ≥ 25 kg/m^2 and waist circumference (WC) ≥ 90 cm for men and ≥ 80 cm for women; and non-obese subjects were determined if BMI < 25 kg/m^2 and WC < 90 for men and < 80 for women.

Blood samples for determination of plasma GLP-1 levels were drawn in fasting state (0 minutes), 15, 30, 60, and 120 minutes post 75 g glucose loading. Subjects were fasting for at least 8-12 hours before performing the procedure; after drawing blood samples in fasting state, subjects drank 75 g anhydrous glucose dissolved in 250 ml water. The plasma human GLP-1 was measured by Elisa kit with a double antibody sandwich method produced by Yanaihara Institute Inc. (multispecies specificity), Cat. No. RSCYK160R.

The study was approved by the Ethical Committee of the Faculty of Medicine, Udayana University and Sanglah Hospital (No. 2145/UN.14.2/KEP/2017), and it was authorised by the Director of Sanglah Hospital (No. LB.02.01/IXIV.2.2.1/34463/2017). All subjects were given information regarding this study and signed the informed consent. This study was conducted by the Declaration of Helsinki.

Data were expressed descriptively in mean ± SD and analysed for normality by Shapiro-Wilk test. The difference of plasma GLP-1 levels pattern among obese and non-obese subjects was analyzed by multivariate analysis with the general linear model repeated measurement. In all statistical analyses, values of $P < 0.05$ were considered to indicate a significant difference between means.

Results

The experimental study enrolled 32 subjects consist of each 16 subjects with obesity and 16 subjects without obesity was conducted. The mean age was 31.56 years in obese subjects, and 31.37 years in non-obese subjects ($P = 0.914$), BMI was 31.10 kg/m2 in obese subjects and 21.71 kg/m2 in non-obese, and WC was 97.31 cm in obese subjects and 77.62 cm in non-obese subjects. Male to female ratios in obese was 8/8 and in non-obese subjects was 9/7 ($P = 0.723$) (Table 1).

Table 1: Characteristic of subjects

Variables	Obese subjects	Non-obese subjects	*P*
Sex (male/female ratio)	8/8	9/7	0.723
Age (years)	31.56 ± 4l.76	31.37 ± 5.03	0.914
Height (cm)	164.40 ± 9.89	163.25 ± 10.16	0.747
Weight (kg)	84.74 ± 15,94	58.08 ± 9.10	< 0.001
Body mass index (kg/m^2)	31.10 ± 2,91	21.71 ± 1.69	< 0.001
Waist circumference (cm)	97.31 ± 10.38	77.62 ± 6.92	< 0.001

The general patterns of GLP-1 levels post glucose loading were similar in obese and non-obese subjects, who increased from fasting state to post glucose loading and reaching the peak levels in 15 minutes then declined until the end of observation, except in non-obese subjects where the GLP-1 levels

were increased at 120 minutes although it never reached the peak levels.

Table 2: GLP-1 levels in fasting state and after 75 g glucose loading in obese and non-obese subjects

GLP-1 levels (ng/mL)	Obese subjects	Non-obese subjects	*P*
Fasting state (0 minutes)	5.67 ± 2.14	6.16 ± 2.85	0.338
15 minutes	6.20 ± 2.21	6.94 ± 3.21	0.239
30 minutes	6.20 ± 2.19	6.90 ± 2.90	0.264
60 minutes	5.77 ± 2.29	6.12 ± 2.47	0.242
120 minutes	5.24 ± 2.04	6.67 ± 2.87	0.049

There was tendency (indicated by absolute levels) that GLP-1 levels in fasting state and post glucose loading were lower in obese subjects compared to in non-obese subjects (in fasting state, 5.67 *vs.* 6.16 ng/mL, *P*=0.338; in 15 minutes, 6.20 *vs.* 6.94 ng/mL, *P* = 0.239; in 30 minutes 6.20 *vs.* 6.90 ng/mL, *P* = 0.264; in 60 minutes, 5.77 *vs.* 6.12 ng/mL, *P* = 0.242), but the difference were not statistically significant, except in 120 minutes (5.24 *vs.* 6.67 ng/mL, *P* = 0.049; in obese and non-obese subjects, respectively) (Table 2 and Figure 1).

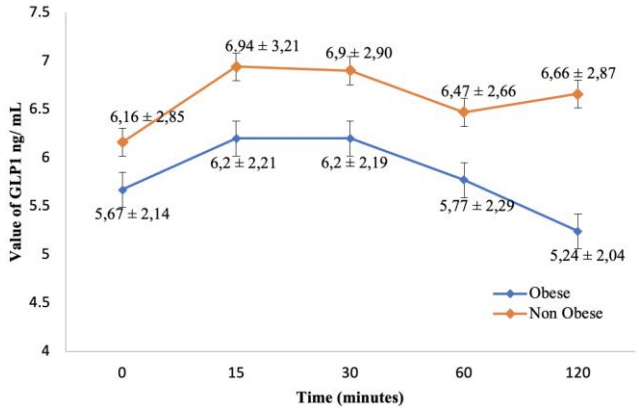

Figure 1: GLP-1 levels in fasting state and after 75 g glucose loading in obese and non-obese subjects

In this study we also compared the difference or delta (Δ) of GLP-1 levels between fasting state (0 minutes) and 15 minutes, 0 and 30 minutes, 0 and 60 minutes, and 0 and 120 minutes among obese and non-obese subjects. Similar like above finding, although obese subjects showed lower response of GLP-1 post glucose loading compared to non-obese subjects, actually there were no significant difference of ΔGLP-1 levels in 0-15 minutes (0.52 *vs.* 0.8 ng/mL, *P*=0.350), in 0-30 minutes (0.53 *vs.* 0.74, *P* = 0.550), and 0-60 minutes (0.11 *vs*. 0.31 ng/mL, *P* = 0.546), except in 0-120 minute (-0.42 *vs.* 0.31, *P* = 0.006) between obese and non-obese subjects (Table 3).

Table 3: Delta (Δ) GLP-1 levels after 75 g glucose loading in obese and non-obese subjects

Delta (Δ)GLP-1 (ng/mL)	Obese subjects	Non-obese subjects	*P*
ΔGLP-1 (0 – 15)	0.52 ± 0.84	0.80 ± 0.70	0.350
ΔGLP-1 (0 – 30)	0.53 ± 0.86	0.74 ± 1.12	0.550
ΔGLP-1 (0 – 60)	0.11 ± 0.97	0.31 ± 0.92	0.546
ΔGLP-1 (0 – 120)	-0.42 ± 0.56	0.31 ± 0.92	0.006

Discussion

The main objective of this study was to know the pattern of GLP-1 levels in fasting state and post-glucose loading. Our findings showed that the GLP-1 levels in fasting state and post-glucose loading were lower, but there was no significant difference, in obese compared to non-obese subjects, except at 120 minutes; and the pattern of GLP-1 levels in obese and non-obese subjects was very similar, except at 120 minutes. Peak GLP-1 levels in this study was noted in 15 minutes post-glucose loading both in obese and non-obese subjects. The response of GLP-1 measured by the delta of GLP-1 (0-15, 15-30, 30-60, 60-120 minutes) showed a similar figure with the absolute GLP-1 levels (above). In non-obese subjects, although there was increased of GLP-1 levels at 120 minutes, statistically was not different from the GLP-1 levels at 60 minutes.

The pattern is almost similar to other studies already conducted. Adam et al., in their study reported that peak levels of GLP-1 after ingestion of galactose/guar gam and standard breakfast was observed at 30 minutes and declined during observation at 60, 90, and 120 minutes. In their study there was no difference in GLP-1 levels at 0, 30, 60, 90, and 120 minutes in normal weight and obese subjects after ingestion galactose/guar gam and standard breakfast, except in 30 minutes after ingestion standard breakfast that GLP-1 levels at 30 minutes found higher in normal weight than those in obese subjects [11]. Report from Danish ADDITION-PRO study population on GLP-1 levels at 0 minutes, 30 minutes, and 120 minutes after glucose loading showed similar patterns of GLP-1 levels which increased sharply at 30 minutes from fasting state and declined at 120 minutes among 5 groups of subjects. Levels of GLP-1 at fasting state, 30 and 120 minutes were not different significantly among subjects with normal glycemic tolerance, impaired fasting glycemia (IFG), impaired glucose tolerance (IGT), IFG+IGT, and T2DM; except women with IFG+IGT and T2DM had lower GLP-1 levels at 120 minutes [7]. A study by Munoz et al. found that fasting GLP-1 in normal weight was lower than in overweight and in obese subjects, and higher fasting GLP-1 in overweight than obesity [8]. GLP-1 levels profile during OGTT in this study was similar to the observation by Manell et al., which the peak GLP-1 levels was noted at 15 minutes and declined until 120 minutes observation. Postprandial GLP-1 levels tended to be lower in adolescents with obesity, impaired glucose tolerance and T2DM compared to lean adolescents. In fasting state, GLP-1 levels in obese adolescents with normal glucose tolerance was higher compared with lean adolescents with normal glucose tolerance, adolescents with obesity and IGT and with obesity and T2DM. The finding was different in our study, and there are several studies where GLP-1 levels in fasting state among non-obese were higher than

those in obese subjects [12].

In this study, although fasting GLP-1 levels tend to be lower in obese compared to non-obese subjects, we observed no statistically significant difference. This result might be caused by the inclusion of subjects that were overweight (BMI between 23 to < 25 kg/m^2) in the non-obese group; which might influence the result.

There were several studies on GLP-1 response to glucose loading among the Asian population reported. A study from Malaysia in subjects with young-onset T2DM and healthy control showed that Asian young-onset T2DM showed similar GLP-1 response to oral glucose as a control but reduced incretin effects, beta cell functions, and insulin sensitivity. The peak GLP-1 levels were observed at 15 minutes in both groups [13]. Study on GLP-1 levels during 75 g glucose loading in a Japanese population with normal glucose tolerance showed that GLP-1 levels increased post glucose loading from fasting state and the peaked levels was observed at 60 minutes and declined until the end of observation at 180 minutes [14]. Another study in Japanese by Kozawa et al., on incretin secretion in obese and non-obese subjects with T2DM and non-diabetic revealed that the peaked GLP-1 levels was observed in 30 minutes and declined until 180 minutes observation. Among the 3 groups of subjects, the peak of GLP-1 levels was tended to be higher in obese diabetic, and the lowest in a non-obese diabetic. The two finding from Japanese studies is different from our study and other studies which the peaked GLP-1 levels were found at 15 minutes [15].

In general it can be concluded that pattern of GLP-1 levels is similar in subjects with lean normoglycemia, obesity, and glucose intolerance which increased post glucose loading and achieving the peak levels at around 15-30 minutes and declining thereafter until 120-180 minutes of observation; but normal or lean subjects have higher GLP-1 levels than those with obesity, prediabetes or T2DM. If we compare between our study and other studies, especially in Caucasians, increasing response of GLP-1 at 15 minutes post-glucose loading from the fasting state in our study was not as steep as other studies have observed. We do not know the certain reasons, but we assume that the blunted response of GLP-1 may cause T2DM earlier in the Indonesian population. We also could not explain the phenomenon of increased GLP-1 levels at 120 minutes in non-obese subjects in this study.

In conclusion, the patterns of GLP-1 levels post glucose loading were similar in obese and non-obese subjects, which increased from fasting state to post glucose loading and reaching the peak levels at 15 minutes and then declining until the end of observation, except in non-obese subjects where GLP-1 levels were increased in 120 minutes. There was a tendency of lower GLP-1 levels in fasting state and post-glucose loading in obese subjects compared to non-obese subjects.

Authors' Contributions

IBAN design the study and performed data analysis, interpreted the data, and drafted the manuscript. MRS and KS participated in the design of the study and helped revise the final manuscript. All authors read and approved the final manuscript.

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Clinical Science

Positive Correlation between Left Hemisphere Lesion and Erectile Dysfunction in Post-Stroke Patients

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Abstract

BACKGROUND: Stroke is a serious health condition and the leading cause of disability, including erectile dysfunction (ED). The mechanisms and factors that predict ED in stroke are not fully elucidated. Several studies have shown a relationship between the location of the lesion in stroke with the onset of ED. The left hemispheric lesion was believed to disrupt the parasympathetic nervous system which is responsible for the regulation of erection. Stroke attack in this region therefore can be the underlying reason for ED. However, there are still contradictory findings in this area, and very few studies attempted to look at this problem, particularly among Asian male.

AIM: We would like to evaluate the association between left hemisphere lesion after ischemic stroke and the subsequent risk of developing ED.

METHODS: The study was conducted in the neurology polyclinic of Sanglah and Wangaya Hospital from February to August 2013. Subjects are all post-stroke patients who visited the neurology polyclinic of Sanglah and Wangaya Hospital that met inclusion and exclusion criteria.

RESULTS: There is a significant positive correlation ($p < 0.05$) between left hemisphere lesions and ED in stroke patients with a correlation coefficient (r) = 0.361 which means weak correlation and $p = 0.032$.

CONCLUSION: Left hemisphere lesions positively correlated with ED in stroke patients. Further longitudinal research is needed to see whether left hemisphere lesion in post-stroke patients is a risk factor for ED.

Introduction

Stroke is a serious health condition in which late and inadequate treatment leads to death in a short amount of time. Stroke is also one of the leading causes of permanent disability such as paralysis, speech difficulty, and facial drooping, and this gives a morbid perception of stroke in society.

According to the World Health Organization (WHO) Multinational Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) Project in 1988, stroke is clinical syndrome with symptoms of focal or global disturbance of brain function, lasting 24 hours or longer, or resulting in death less than 24 hours, with no apparent cause other than of vascular origin [1]. Stroke is the third leading cause of death in the world with an incident of 5.5 million persons per year and is responsible for 50 million disability-adjusted life years (DALY) [2]. In the next 20 years, stroke is predicted to increase from its current position in the DALY table, from 7th to 4th [3].

Epidemiology studies in developed countries have shown that the number of deaths caused by stroke is proportional to the number of deaths caused by heart disease. A systematic review of literature published from 1980 to 2010 has shown the incidence of stroke in Southeast Asia varies from 123-145 per 100,000 persons with a prevalence of 45-471 per 100,000 [4]. Based on a community-based survey in 120 regions in Indonesia with a total of 4, 269, 629 samples, a prevalence of 1.7-2.2 per 100,000 [5].

Stroke increasingly causes more deaths and disability in Indonesia. From sporadic data collected from hospitals, there was a trend of increase in morbidity rate from stroke as the life expectancy increased and the lifestyle changed. According to the Indonesia Health Department in 2007, stroke is the

number one cause of death and disability in Indonesia.

Post-stroke disability frequently involves physical problem such as walking and speech difficulty but also causes a no-physical problem such as depression. This will eventually affect overall daily activities, including sexual activity. There is still very little resources that explored this issue [6], [7], regardless of frequent complaints of sexual problems in patients with chronic diseases such as cerebrovascular accident [8], [9]. Several literature reviews and studies have shown that sexual function is frequently disturbed after a stroke attack [6], [8], [10], [11], [12] This will affect the quality of life [7]. In one systematic review, sexual dysfunction was mentioned as one of five social consequences from stroke aside from work life, family relationship, financial issue, and social activity [13].

In Indonesia, there are very little reports regarding sexual dysfunction following a stroke attack. There are several possibilities as to the reason behind this: doctor's attention is mostly focused on life-saving procedures, treating the risk factors, and toward the physical disability resulting from the stroke attack. Stroke patients are mostly above 50 years old and are usually not paying much attention to their sexual life at this period. Patients mostly feel ashamed and self-conscious to bring up their sexual problem to their doctors as they may think that such a problem is a minor issue compared to the more life-threatening symptoms of stroke. The partners also usually take sexual dysfunction as "normal" after such a major attack such as stroke, so they don't feel the need to bring it up. Thus, post-stroke sexual dysfunction is rarely reported in Indonesia. In men, erectile dysfunction (ED) is the most common sexual problem found in post-stroke patients.

Although the relationship between stroke and ED has been widely studied, however, the mechanisms and factors that predict ED in stroke are few known. Several studies have shown a relationship between the locations of the lesion in stroke with the onset of ED. The regulation of erectile function is more influenced by the parasympathetic than the sympathetic nervous system [14]. Some studies have shown consistent results that the left hemisphere is predominantly regulating parasympathetic modulation, while the right hemisphere is dominant for sympathetic modulation [15], [16], [17]. Several studies have suggested that ED is more commonly obtained in stroke with right hemispheric lesions [18], [19]. Other studies have shown opposite results, namely in the left hemispheric lesions [6], [20] Jung et al., [10] stated lesions in the left basal ganglia and right cerebellum are associated with decreased sexual desire and ejaculation disorders. Sikiru et al., [20] wrote that DE is often found in stroke patients with right hemiplegia. However other study stated there is no significant relationship between the location of the lesion and the occurrence of ED in patients after stroke [21], [22], [23]. These contradictory results are what interested us in making a study in this field.

Also, comorbid factors that are thought to have important roles in the occurrence of ED in stroke patients are hypertension, diabetes mellitus (DM), and hypercholesterolemia [6], [11], [24], [25], [26], [27]. The risk factors are age, smoking and obesity [8], [22], [28]. Besides these physical factors, psychic factors also have a role in the occurrence of ED [6], [8], [10], [12], [29], [30], [31]. Post-stroke depression is the most common emotional disorder in post-stroke patients, and sexual dysfunction is also commonly found in patients who experience post-stroke depression [7], [32]. Left hemisphere stroke lesions are associated with depression [33], although other systematic reviews concluded that post-stroke depression was not significantly associated with the hemisphere location of the lesions [34].

This study aimed to evaluate the association between left hemisphere lesion after ischemic stroke and the subsequent risk of developing ED.

Methods

This research is an analytic observational study with a cross-sectional design. In the study subjects (post-stroke patients) analysis will be conducted to determine the relationship between the location of the lesion and the occurrence of ED. The study was conducted in the neurology polyclinic of Sanglah and Wangaya Hospital from February to August 2013. Subjects are all post-stroke patients who visited the neurology polyclinic of Sanglah and Wangaya Hospital that met inclusion and exclusion criteria.

Inclusion criteria include the following: Post-stroke patients minimum 6 months after stroke, men aged 40-59 years, married and living with his wife at the time of the study, Modified Rankin Scale (mRS) < 3. Exclusion criteria include the following: neurological disorders other than stroke, confirmed to have been diagnosed with ED before the stroke. Samples were taken by consecutive sampling.

Instruments used to collect data included data collection form and questionnaire. The data collection form was used to record basic characteristics such as age, type of stroke, location of lesion, hypertension, diabetes mellitus, smoking, obesity, and hypercholesterolemia, the result of the international index of erectile dysfunction-5 item (IIEF-5) questionnaire, modified Rankin scale (mRS), and Hamilton depression rating scale. IIEF-5 is a simple, reliable, and well-validated questionnaire to diagnose ED [35]. A score between 22-25 indicates no ED, 17-21 indicates mild ED, 12-16 indicates mild-to-

moderate ED, 8-11 indicates moderate ED, and 5-7 indicates severe ED [36]. The data was then analyzed with the SPSS version 20 for Mac. The correlation was analysed using a Lambda correlation test.

Results

In this study, a total of 74 post-stroke patients visited the Neurology Polyclinic of Sanglah and Wangaya during the period from February to August 2013. This study was an observational study with a cross-sectional design which aimed to determine the correlation between left hemisphere lesions with ED in stroke patients.

Characteristics of Subjects

There were 74 stroke patients who met the inclusion and exclusion criteria as a sample in this study. The mean age of stroke patients sampled in this study was 52.19 ± 4.37 with the youngest age of 42 years and the oldest 59 years. Characteristics of subjects including age group, type of stroke, the location of lesion, hypertension, diabetes, smoking, obesity, hypercholesterolemia, depression, erectile dysfunction, and degree of severity of ED are presented in Table 1.

Table 1: The characteristic of subjects

Characteristic	Frequency (n)	Percentage (%)
Age group		
40-49 years old	23	31.1
50-59 years old	51	68.9
Stroke type		
Hemorrhagic	20	27.0
Ischemic	54	73.0
Location of lesion		
Left hemisphere	35	47.3
Right hemisphere	39	52.7
Hypertension		
Yes	45	60.8
No	29	39.2
DM		
Yes	19	25.7
No	55	74.3
Smoking		
Yes	40	54.1
No	34	45.9
Obesity		
Yes	27	36.5
No	47	63.5
Hypercholesterolemia		
Yes	22	29.7
No	52	70.3
Depression		
Yes	39	52.7
No	35	47.3
Erectile Dysfunction*		
Yes	38	51.4
No	36	48.6
The degree of severity of ED*		
Severe	9	12.2
Moderate	11	14.9
Mild-Moderate	8	10.8
Mild	10	13.5
Normal	36	48.6

*According to IIEF-5 score.

The table found the highest age group in the 50-59 years group (n = 51; 68.9%). Based on the type of stroke, most of the samples experienced ischemic stroke with as many as 54 subjects (73.0%), while those with hemorrhagic stroke were 20 subjects (27.0%).

Based on the location of the lesion, more samples experienced lesions in the right hemisphere, with as many as 39 subjects (52.7%), compared to lesions in the left hemisphere (47.3%). Most of the samples experienced hypertension (n = 45; 60.8%). Only 19 subjects (25.7%) had diabetes. There were 40 subjects (54.1%) who smoked. Most of the samples were not obese (63.5%), and 52 subjects (70.3%) were not hypercholesterolemic. There were also 39 subjects (52.7%) who were depressed. Mean ± SD IIEF-5 score was 17.82 ± 6.56. From the sample, 38 subjects (51.4%) had erectile dysfunction, with the most being moderate (14.9%), followed by mild degrees (13.5%), severe (12.2%) and mild-moderate (10.8%).

Correlation between Left Hemisphere Lesions and ED in Post-stroke Patients

In stroke patients with lesions in the left hemisphere, 25 subjects (33.8%) had ED, and 10 subjects (13.5%) did not develop ED, whereas in stroke patients with lesions in the right hemisphere, 13 subjects (17.6%) had ED, and 26 subjects (35.1%) did not. The correlation between left hemisphere lesions and ED in stroke patients was analysed using the Lambda correlation test. The results of the significance analysis are presented in Table 2.

Table 2 shows that there is a significant positive correlation ($p < 0.05$) between left hemisphere lesions and ED in stroke patients with a correlation coefficient (r) = 0.361 which means weak correlation and $p = 0.032$.

Table 2: Correlation between left hemisphere lesion and erectile dysfunction (ED) in post-stroke patients

		ED		Total N (%)	r	p
		Yes N (%)	No N (%)			
Location of lesion	Left Hemisphere	25 (33.8)	10 (13.5)	35 (47.3)	0.361	0.032*
	Right Hemisphere	13 (17.6)	26 (35.1)	39 (52.7)		
Total		38 (51.4)	36 (48.6)	74 (100)		

*, significant ($p < 0.05$).

Discussion

Stroke is the third most common cause of death in the world, and one of the main causes of disability [8]. The relationship between stroke and ED has been widely studied, but the mechanisms and factors that predict ED in stroke have not been widely known. This study looked at the correlation between left hemisphere lesions and other variables with ED in

post-stroke patients.

In this study, 74 stroke patients who fulfilled the inclusion and exclusion criteria were sampled. The average age of post-stroke patients sampled was 52.19 ± 4.37 years with the youngest age of 42 years and the oldest 59 years. This study grouped the age into two: the 40-49 years age group and 50-59 years age group. This age grouping was done to reduce bias due to the hormonal status which was influenced by age. Derouet et al., [37] in their study stated that there were significant differences between free testosterone levels in men aged 40 years and over and 40 years and under. The incidence of ED increases after the age of 50 years [26]. The risk of ED also increases with age; it is reported that the risk increases up to 6 times in the age group 60-69 compared to the age group 40-49 [38].

Toglia et al., [39] reported that in 67 stroke patients, 40.3% came with left hemisphere lesions and 59.7% with right hemisphere lesions. This finding is slightly different what was reported by Sikiru et al., [20] Wherein out of 105 stroke patients, 55 people (52.4%) had lesions in the left hemisphere, and 50 people (47.6%) had lesions in the right hemisphere. It was reported that the difference in the distribution of hemispheric lesions was not significant [40]. Also, the accuracy of determining the location of lesions is also determined by the availability of diagnostic tools such as CT-scan or MRI.

In this study, as many as 45 subjects (60.8%) had hypertension. In men with hypertension, impaired erectile function is not due to increased blood pressure itself, but because of arterial stenosis. Vascular failure to close during erection (venous-occlusion dysfunction) can cause ED [29]. Post-stroke patients suffering from DM in this study were 19 people (25.7%). These results are not much different from those reported by Icks et al., [41], who found 25.6% of male stroke patients aged 45-64 years with DM. The research used data taken from insurance in Germany between 2005 and 2007. DM has been well known as a risk factor for ED. Phé and Rouprêt [42] stated that increasing HbA1c levels and hyperglycemia in men with DM type 2 could reduce NO activity and reduce endothelial relaxation factors, resulting in increased risk for ED. A HbA1c level above 8.1% increases the incidence of ED threefold. Smoking is also closely related to the incidence of ED. Austoni et al., [43] reported an association between ED and smoking, defined by a smoking history of 10 cigarettes or more per day. Smoking can induce vasoconstriction and penile venous leak due to contractile effects on the cavernous smooth muscle.

The relationship between obesity, hypercholesterolemia, and stroke has been widely investigated about primary prevention [44], [45]. In this study 27 subjects (36.5%) were obese, and 22 subjects (29.7%) had hypercholesterolemia. Bener et al. [8] obtained the prevalence of obesity in stroke and ED patients by 42.1% compared to non-ED patients at 28.4% (p = 0,0005). High-Density lipoprotein (HDL) cholesterol and the ratio of total/HDL cholesterol were found to be significant predictors of ED. Furthermore, post-stroke depression is common. In this study, 39 subjects (52.7%) were depressed. This statement is by Bhogal et al., [33] who reported the prevalence of post-stroke depression by 20-50%. Gaete and Bogousslavsky [32] also reported the prevalence of depression in the first four weeks after a stroke at 17-52%. The relationship between ED and depression has been investigated and shown significant results [6], [8], [10], [12], [29], [30, [31]. Post-stroke depression is the most common emotional disorder found in post-stroke patients [7], [32].

This study found a weak correlation between left hemisphere lesions and ED in post-stroke patients. However it was statistically significant (Table 2). The correlation is positive which means the post-stroke patient who has left hemisphere lesions are more likely to develop ED. These results are supported by the research of various studies. Kimura et al., [6] reported from 67 male samples in his research, the proportion of the sexual function disorder occurred higher in post-stroke patients with left hemisphere lesions versus right (40.3% vs 19.4%; p = 0.013). Sikiru et al., [20] studied the correlation of hemiplegia and ED in stroke patients. There were 105 stroke patients with left hemiplegia and 55 stroke patients with right hemiplegia, then assessed with the IIEF-5 questionnaire. Erectile function was found to decrease significantly in both patients with left and right hemiplegia. The mechanism for the relationship of left hemisphere lesions to ED is not known with certainty. But some studies that try to link hemispheres with the autonomic nervous system can indirectly support this statement. Regulation of erectile function is more influenced by the parasympathetic nervous system than by the sympathetic [14]. Avnon et al., [17] stated that brain function about the autonomic nervous system is asymmetrical, where the dominant left hemisphere regulates parasympathetic modulation, whereas the right hemisphere brain is dominant for sympathy. This statement is also by other studies [15], [16]. Braun et al., [46] revealed patients with left hemisphere lesions (especially in the temporal lobe) tend to experience hyposexual activity, whereas patients with right hemisphere lesions tend to experience hypersexual drive.

Of note, Winder et al., [47] had performed lesion mapping using voxel-wise analysis and found that ischemic stroke was related to erectile dysfunction if the lesion was found in the right occipital-parietal cortex and thalamus which are responsible for visual and somatosensory input integration, as well as lesions in the left insular and parietal-temporal region, areas responsible for generating and mapping visceral arousal state. A lesion affecting insula due to another insult (i.e., multiple sclerosis) was also associated with ED [48].

Different results were reported by other researchers, where ED was more frequently found in post-stroke patients with right hemisphere lesions [19], [49]. This difference can occur because of this study excluded depression from the study sample, whereas the number of depression in stroke patients was almost one third and Bhogal et al., [33] reported about 70% of stroke patients with depression had lesions in the left hemisphere. So, when the studies excluded depression, a direct proportion of stroke patients with left hemisphere lesions consequently would decrease and could, therefore, affect the results of the study. In this study, the proportion of ED patients who suffered from depression was 35.1%. When connected between depressed patients and location of lesions, this study obtained the proportion of stroke patients who experienced lesions in the left hemisphere and suffered from ED as 33.8% compared to 18.9% of those who experienced lesions in the right hemisphere. This show the tendency for stroke patients with lesions in the left hemisphere to experience depression.

Furthermore, the same study also confirmed a relationship between left hemisphere lesions and the occurrence of depression in post-stroke patients. One of the inclusion criteria in this study was using a sample of right-handed patients, but not differentiated further whether the right-handedness was absolute or not. Absolute certainty was known from the history where the patient always used his right hand and leg, and there was no history of left-handedness in his family. The dominant hemisphere in absolute left-handed people is the left hemisphere. The relative right-handed person was defined by the history of a left-handed person in the family. The right-handed group did not have a cerebral ambivalence. Likewise, in the left-handed group, the patient could use both of his left and right hand and legs, even though usually better with the left side.

In conjunction with erectile function, the role of the autonomic nervous system is not solely determined by the hemispheric sympathetic and parasympathetic activity of the brain, but it also involves the somatomotor, endocrine, and autonomic system itself. These systems are represented by overlapping areas of the brain. Three important components of the central autonomic control pathway include the solitary nucleus who receive visceral sensory information, the hypothalamus which is the centre neural control most important for controlling endocrine and visceral functions, and the nucleus ventrolateral rostral reticular (nRVL) which is the motor nucleus that regulates the autonomic nervous system. The hypothalamus is part of the limbic system which has two-way connections between the cortical and subcortical areas complexes. Assessing depression is of more value in this study than in the research done by Jung et al., [10], although this study also has a weakness because it does not check hormonal levels in post-stroke patients.

In conclusion, based on the results of the research above, the conclusion can be drawn as following: left hemisphere lesions positively correlated with ED in stroke patients. Further longitudinal research is needed to see whether left hemisphere lesion in post-stroke patients is a risk factor for ED.

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Age Characteristics and Concomitant Diseases in Patients with Angioedema

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Abstract

BACKGROUND: Angioneurotic oedema (AE) is an unpredictable and dangerous disease directly threatening the patient's life due to a sudden onset of upper respiratory tract obstruction. The disease is associated with various causes and triggering factors, but little is known about the conditions that accompany AE.

AIM: The study aims to determine the age-specificities and the spectrum of concomitant diseases in patients with AE.

MATERIAL AND METHODS: The subjects of observation were 88 patients (53 women and 35 men) with angioneurotic oedema who underwent diagnostics and treatment in the Department of Occupational Diseases and Clinical Allergology of University hospital "Saint George"-Plovdiv.

RESULTS: The highest level of disease prevalence was found in the age group over 50 years, both in males (45.71%) and females (54.72%). We found that the most often concomitant diseases in our patients with AE are cardiovascular (33%). On second place are the patients with other accompanying conditions outside of the target groups (27.3%). Patients with AE and autoimmune thyroiditis were 14.8%, and those with AE and skeletal-muscle disorders-10.2%. Given the role of hereditary factors in this disease, the profession of the patients is considered insignificant.

CONCLUSION: Angioedema occurs in all age groups, but half of the cases are in people over 50 years of age. The most common concomitant diseases in angioedema are cardiovascular diseases.

Introduction

Angioneurotic oedema (AE) is an unpredictable and dangerous disease directly threatening the patient's life due to a sudden onset of upper respiratory tract obstruction. Hereditary angioedema (HAE) is a rare form of AE. The disease is associated with various causes and triggering factors, but little is known about the conditions that accompany AE [1], [2], [3], [4].

The study aims to determine the age-specificities and the spectrum of concomitant diseases in patients with AE.

Material and Methods

The subjects of observation were 88 patients (53 women and 35 men, 1.5:1) with angioneurotic oedema who underwent diagnostics and treatment in the Department of Occupational Diseases and Clinical Allergology. We accepted as a technical unit of observation the Department of Occupational Diseases and Allergology at the University General Hospital for Active Treatment "Saint George"-Plovdiv, and as a logical unit-every patient hospitalised in the ward. The study is complete and covers all the patients hospitalised in the ward.

The study is retrospectively longitudinal, with AE patients being monitored throughout 4 years (from 01.01.2013 to 31.12.2016).

Signs of observation are divided into two groups:

• factorial - age, sex, place of residence, region, social group, occupation;

• resultant - leading diagnosis, diagnosis at the point of discharge, outcome of the disease during hospitalization, consecutive hospitalizations, etiology, other accompanying diseases, family history, occupational nature of the disease, determined diagnosis, how many attacks have been documented, criteria for acceptance of the diagnosis, localization and dynamics of swelling, treatment and outcome, laboratory tests.

Two basic methods of medical sociology are used to collect the primary statistical information - questionnaire and documentary.

The questionnaire contains 56 open, semi-open and closed questions, grouped into 5 sections:

1. Socio-demographic characteristics of the patient

2. Hospital treatment

3. Aetiology

4. Clinical diagnosis

5. Laboratory diagnostics

In the documentary method, the main document was the history of the disease from the archive of the primary documentation. The necessary information from the document was imported into a database. The individual survey was conducted on the day of the patient's discharge from the clinic. A basic element of the documentary sociological method was the study of the history of the disease, the registered stage epicrises, outpatient consultations, clinical and paraclinical data.

Statistical processing of information

The collected primary information was translated, coded and entered into a computer database. A primary grouping was precisely performed. On this basis, by combining the factorial and resultant signs, a second group was established to address the specific needs of the study. Statistical data processing utilised abundant methods of medical statistics.

- Variation analysis-to handle quantifiable signs. The normality of the distribution was determined by the λ criterion of Kolmogorov-Smirnov. For comparison of the mean values, u-criterion for normal distribution was used. Existing differences were considered statistically significant and confirming H1 when they exceeded the critical value of Δ for λ = 0.05.

- Alternative analysis-for processing qualitatively measurable signs. Depending on the type of signs, the classic method and Fischer's transformation were applied. For relative shares corresponding to the requirements of the classical methodology, the Van der Waerden criterion was used.

- Correlation analysis-to reveal a cause and effect relationship between some of the factorial and resultant signs. Regression analysis could be used to quantify the relationship.

- The nonparametric analysis-in need of hypothesis verification, for distributions different from the normal Gauss-Laplace, and analysis of complex composite tables.

- Dispersion analysis-the Tukey's method was used to compare more than two averages.

- Analysis of dynamic changes.

- Graphical analysis-to illustrate the observed processes and phenomena and to illustrate the existing regularities.

Data were processed by SPSS (SPSS Inc., IBM SPSS Statistics) 20.0 and Microsoft Office 2010 statistical analysis programs.

Results and discussion

During the study, a total of 2,198 patients were admitted to the department. Due to incomplete data, 87 (3.8%) patients were excluded from the study. Only 8 out of 88 were suspected for hereditary angioedema due to family history (most often the father had episodes of AE): 3 underwent treatment in the department, and 5 were observed ambulatory.

Age characteristics and sex of AE patients

The total number of patients with AE for the studied period was 88. Table 1 shows the distribution of patients by sex and age. The highest level of disease prevalence was found in the age group over 50 years, both in males (45.71%) and females (54.72%).

No statistically significant difference in disease prevalence levels between the two sexes was found. Women with AE slightly prevailed-53 (60.23%) then men-35 (39.77%). The nonparametric analysis confirmed that there is no significant difference between patients with AE treated in the ward by sex. The same-sex dependency was also observed in individual age groups ($\chi 2 = 5.702$, $P = 0.127$) (Table 1).

Table 1: Distribution of patients with AE by age and sex

Age group	Male			Female			Total	
	Number	%	Sp	Number	%	Sp	Number	%
Up to 30 year old	7	20.00	6.40	4	7.55	4.15	11	12.50
30-40 year old	9	25.71	7.39	9	16.98	5.16	18	20.45
40-50 year old	3	8.57	4.74	11	20.75	5.58	14	15.91
Above 50 year old	16	45.71	8.42	29	54.72	6.84	45	51.14
Total	35	100.00	-	53	100.00	-	88	100.00

Concomitant angioedema diseases

The published literature on the relationship of AE to other concomitant diseases and syndromes is scarce. There is no clear answer to the question whether some non-allergic diseases and combinations of these are more common in AE patients or there are other factors involved that determine the onset and progression of the disease besides the harmful habits. The study results concerning the most often found concomitant diseases in our AE patients are listed in Table 2.

Table 2: Concomitant diseases in patients with AE

Disease	Data obtained on the patient's anamnesis						Total	
	No			Yes				
	Number	%	Sp	Number	%	Sp	Number	%
Autoimmune thyroiditis	75	85.2	3.78	13	14.8	3.78	88	100
Diabetes mellitus	84	95.5	2.22	4	4.5	2.22	88	100
Neoplasm	87	98.9	1.13	1	1.1	1.13	88	100
Hematological diseases	85	96.6	1.93	3	3.4	1.93	88	100
Parasitoses	87	98.9	1.13	1	1.1	1.13	88	100
Systemic diseases of the connective tissue	87	98.9	1.13	1	1.14	1.13	88	100
Diseases of the musculoskeletal system	79	89.8	3.23	9	10.2	3.23	88	100
Cardiovascular diseases	59	67.0	5.01	29	33.0	5.01	88	100
Respiratory system diseases	84	95.5	2.22	4	4.5	2.22	88	100
Cerebrovascular disease	85	96.6	1.93	3	3.4	1.93	88	100
Diseases of the peripheral nervous system	85	96.6	1.93	3	3.4	1.93	88	100
Kidney disease	86	97.7	1.59	2	2.3	1.59	88	100
Other	64	72.7	4.75	24	27.3	4.75	88	100

In the observed cases, besides the type of concomitant diseases, we also studied their number in each patient. We found that the most often concomitant diseases in our patients with AE were the cardiovascular (33%). On second place were the patients with other accompanying conditions outside of the target groups (27.3%). Patients with AE and autoimmune thyroiditis were 14.8%. The patients with AE and skeletal-muscle disorders were 10.2% among the most often: myalgia, myositis and tendovaginitis. Only one of the patients was with the systemic disease of the connective tissue (Lupus erythematosus), 2 with chronic myeloid leukaemia and 1 with polycythaemia vera (hematologic diseases).

Our data concerning the association of AE with other allergic conditions are shown in Figure 1. The results confirm the statement that urticaria is often found in patients with AE.

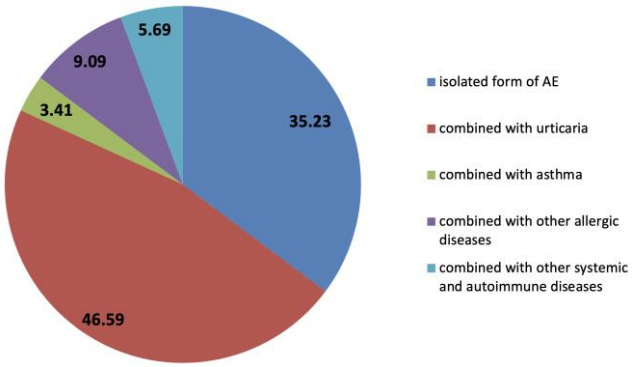

Figure 1: Association of AE with other allergic conditions in percentage (%)

Regarding the presence of concomitant diseases in one AE patient, in one third (30.68%) of the patients, we didn't prove any concomitant disease whereas in the other two thirds (69.32%) we found at least one up to five concomitant conditions (Figure 2).

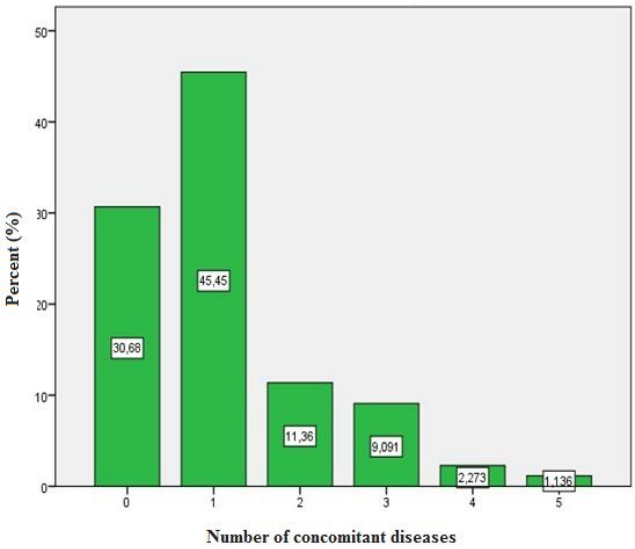

Figure 2: Number of concomitant diseases of one patient with AE

By the indicator "number of concomitant diseases" in one AE patient we found that the majority of patients with AE had only one accompanying disease (45.45%). Almost 1/3 of the studied patients have only C1 inhibitor deficiency.

Professional aetiology of angioneurotic oedema

In the majority of our AE patients (95.4%), the profession was excluded as a factor causing the onset of the disease. We consider that workplace risk factors may in some cases play the role of triggers leading to the clinical manifestation of swelling in AE patients, although such risk is objectively explored in one of our patients, and in other three is in the process of proving (Figure 3).

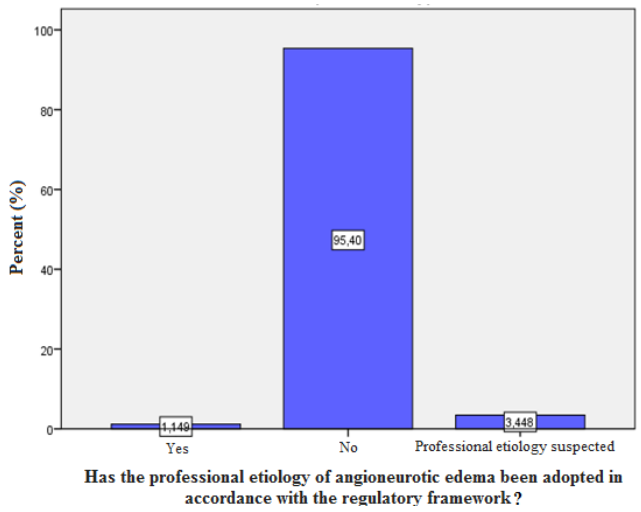

Figure 3: Professional aetiology of angioneurotic oedema

Discussion

Several literature sources discuss the association of AE with urticaria and other immune and autoimmune diseases [5], [6], [7], [8], [9], [10].

Our results correlate with the results published by Brickman CM et al., in two studies in 1986 regarding the frequency of autoimmune disorders in patients with AE (12%) [11], [12]. In 2012, Habal F. & Huang V reported a case of AE associated with Crohn's disease [13].

Half of the patients with AE have at least one concomitant disease (45.45%). The most common concomitant diseases in patients with AE are the cardiovascular (33%), followed by autoimmune thyroiditis (14.8%), musculoskeletal disorders (10.2%) and diabetes mellitus (4.5%). Given the role of hereditary factors in this disease, the profession of the patients is considered insignificant.

In conclusion, angioedema occurs in all age groups, but half of the cases are in people over 50 years of age. The most common concomitant diseases in angioedema are cardiovascular diseases, autoimmune thyroiditis and musculoskeletal system diseases.

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Salivary Secretor Status of Blood Group Antigens in Patients with Head and Neck Cancer

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Abstract

BACKGROUND: Head and neck cancers include malignancies of the scalp and neck skin, nasal cavity, paranasal sinuses, oral cavity, salivary glands, pharynx and larynx. The term ABO secretor refers to people who secrete blood group antigens in their body fluids such as saliva, sweat, tears, semen, and serum. Non-secretors refer to those who do not secrete their blood group antigens in their body fluids. The lack of blood type antigens in body discharge increases the susceptibility to certain types of diseases and infection.

AIM: Our study aimed to investigate the relationship between the secretion of blood groups in the saliva of patients with head and neck cancers.

MATERIAL AND METHODS: This case-control study was performed on 110 people (57 patients with head and neck cancer who were referred to Imam Khomeini Hospital, Tehran and 53 cancer-free controls). Five ml of non-stimulated saliva were collected by the spitting method. By agglutination or lack of agglutination in the test tubes, we determined the patient's secretor or non-secretor condition.

RESULTS: In terms of secretor status, 52.7% of all samples were secretors. In the case group, 19 out of 57 cases (33.3%) were secretors, and 38 were non-secretors (66.7%). In the control group, 39 out of 53 cases (73.6%) were secretors, and 14 cases were non-secretors (26.4%). There was a significant difference in the percentage of non-secretors between the two groups ($p = 0.00$).

CONCLUSION: People with non-secretor status may be more prone to develop head and neck cancer. The presence of these antigens in saliva may have a protective effect.

Introduction

Head and neck cancers include malignancies of the scalp and neck skin, nasal cavity, paranasal sinuses, oral cavity, salivary glands, pharynx and larynx [1], considered the sixth most common cancer in the world, accounting for 10% of malignancies worldwide and 3% of malignancies in the United States [2], [3], [4], [5], [6].

Tobacco and alcohol are two major risk factors for head and neck cancers, especially squamous cell carcinoma and the probability increases with higher intake [7], [8]. Also, researches have shown the association of other agents, such as human papilloma virus and blood group antigens with cancer development [9], [10], [11], [12].

The term ABO secretor refers to people who secrete blood group antigens in their body fluids such as saliva, sweat, tears, semen, and serum, and non-secretors refer to those who do not secrete their blood group antigens in their body fluids [13], [14]. The secretor gene (fucosyltransferase 2) is inherited in the autosomal dominant pattern: Se is the dominant form, and she is the recessive form. Therefore, Se Se or Se we are secretors, and se we are non-secretors [15]. According to studies, the lack of blood type antigens in body discharge is considered a limitation, as it increases the susceptibility to certain types of diseases, including peptic and duodenal ulcers,

periodontal disease and candidiasis [16], [17], [18], [19], [20], [21], [22].

The protective mechanism of the blood group antigens against a variety of infections is unclear, but it seems that it interferes on the bond between the microorganism and epithelial cells, because many bacteria show a reciprocal response to blood group antigens. Also, in non-secretors, lower IgA levels were reported in both serum and saliva. Hence, it is likely that specific immune responses at mucosal surfaces of non-secretors are weaker than secretors. Lower levels of IgG have also been observed in these individuals [13].

Studies on the relationship between secretor status and diseases such as pre-malignant and malignant oral lesions have shown different results. Cerovic et al., (2008) and Lamey et al., (1994) found no significant relationship between secretor status and oral cancer [23], [24]. While Rai et al., (2015) showed significantly more pre-malignant lesions in non-secretors [25]. In Hallikeri et al. 's study (2015), oral mucosal fibrosis, a pre-malignant lesion, was more common in non-secretors [26]. As Jamil et al., reported (2005) it was shown that lack of secretion of blood group antigens in saliva could be considered as a risk factor for oral cancer [27].

Our study aimed to investigate the relationship between the secretion of blood groups in the saliva of patients with head and neck cancers.

Material and Methods

This case-control study was performed on 57 patients with head and neck cancer who were referred to Imam Khomeini Hospital in Tehran (Cancer Institute), and their diagnosis was determined by histopathologic examination. Also, 53 patients' accompanies and hospital staffs were considered as the control group. Patients who were suffered from severe dry mouth for any reason, such as radiotherapy and those who were unable to give their saliva sample due to extensive oral surgery were excluded from the study.

First, the information checklist was completed based on patients' medical records, and then 5 ml of non-stimulated saliva were collected in a 15 ml falcon tube with a plastic cap between 9 am, and 12 am, while the patient was in sitting position, and then the tubes were placed in a boiling water bath for 10 minutes to denature the salivary enzymes. After that, the tubes were centrifuged for 10 minutes at 1700 rpm and the supernatant layer was removed and transferred to 2 ml micro tubes, kept in a freezer at -70°C until complete collection of the specimens.

According to Cerovic et al. 's study (2008), secretor status was detected using the Inhibition Agglutination Test (Wiener Test) [23].

For each case, four test tubes were prepared, and "A" or "B" anti-sera were added as presented in table 1. Test tubes 3 and 4 were considered as control groups. We shook all the tubes at room temperature. After one hour, the readings were ready. By agglutination or lack of agglutination in the tubes, we determined their secretor or non-secretor state.

Table 1: Content of test tubes

	Content
Test tube 1	One drop of saliva + one drop of an anti-B+ suspension of RBC B type
Test tube 2	One drop of saliva + one drop of an anti-A+ suspension of RBC A type
Test tube 3	One drop of *physiological* saline +one drop of an anti-B+ suspension of RBC B type
Test tube 4	One drop of *physiological* saline + one drop of an anti-B+ suspension of RBC B type

In tubes 3 and 4, control tubes, agglutination occurred, and the results in tubes 1 and 2 were interpreted as follows:

- Lack of agglutination in tube 1 showed neutralising anti-B with salivary antigen, which meant that antigen B was present in saliva.

- Lack of agglutination in tube 2 showed neutralising anti-A with salivary antigen, which meant that antigen A was present in the saliva.

- Agglutination in tube 1 showed the reaction between Anti-B and B red blood cells. As a result, antigen B was not present in saliva.

- Agglutination in tube 2 showed the reaction between anti-A and "A" red blood cells. As a result, antigen A was not present in saliva.

So, no agglutination in tube 1 and agglutination in tube 2 meant: B secretor; agglutination in tube 1 and no agglutination in tube 2 meant: A secretor; lack of agglutination in both tubes 1 and 2 meant: AB secretor; agglutination in both tubes 1 and 2 meant: non-secretor. The secretor status was considered as a nominal variable, and the difference between the patients' group, compared to the healthy control group was assessed using the Chi-square test. The significance level was considered less than 0.05.

In this study, there was no interference in the patients' treatment process, and the condition was only described. However, to ensure patients' awareness and obtain their consent, the consent form was signed by all individuals and confirmed by the Ethics Committee with the code IR.SBMU.RIDS.REC.1396.479.

Results

In this case-control study, a total of 110 people including 57 patients (25 female and 32 male) with a mean age of 58.69 ± 14.84 years with head and

neck cancer and 53 controls (26 women and 27 men) with a mean age of 49.13 ± 11.13 years participated. All patients in the case groupware admitted to the surgery department and Cancer Surgery Clinic of Imam Khomeini Cancer Institute.

In terms of smoking and alcohol in the case group, 17 out of 57 smoked (29.8%) and 2 consumed alcohol (3.5%); these two people were alcoholics and smokers at the same time.

There were 59 lesions in 57 patients, of which 39 cases were squamous cell carcinoma (SCC), 10 cases were basal cell carcinoma (BCC), 3 cases were sarcoid cell carcinoma, 3 cases were mucoepidermoid carcinoma (MEC), 2 cases were osteosarcoma, 1 was pleomorphic adenoma, and 1 was Acinic-cell carcinoma (ACC). Meanwhile, one patient had two lesions in the tongue and buccal area, and another in mandible and tongue simultaneously. The total number of lesions was 59 in 57 patients.

The most frequent lesion site was tongue with 13 cases; other sites involvement were as follow: mandibular bone 9 cases, buccal mucosa 8 cases, facial skin 8 cases, parotid 5 cases, larynx 4 cases, lip3 cases, maxillary bone 3 cases, nasal mucosa 3 cases, maxillary sinus 2 cases and one case had oral floor involvement.

The most common lesions were as amass (54.23%), and the rest (45.77%) were ulcerative. (Type of cancer, site, and shape are given in Table 2.)

Table 2: Features of malignancies in head and neck cancers

Variable	Description	Number	Percentage
Cancer type	Squamous cell carcinoma	39	66.1%
	Basal Cell Carcinoma	10	16.9%
	Spindle cell sarcoma	3	5.1%
	Mucoepidermoid carcinoma	3	5.1%
	Osteosarcoma	2	3.4%
	Pleomorphic adenoma	1	1.7%
	Adenoid cystic carcinoma	1	1.7%
	Total	59	100%
Anatomic location	Tongue	13	22..3%
	Mandible	9	15.3%
	Buccal mucosa	8	13.5%
	Skin	8	13.5%
	Parotid	5	8.4%
	Larynx	4	6.7%
	Lip	3	5.1%
	Maxilla	3	5.1%
	Nose	3	5.1%
	Sinus	2	3.3%
	The floor of the mouth	1	1.7%
	Total	59	100%
Clinical manifestation of the Lesions	Mass	32	54.23%
	Ulcer	27	45.77%
	total	59	100%

In terms of lesions' size, among the total of 59 lesions, the smallest reported lesion was 5 mm, and the largest was 70 mm. The mean size was reported to be 21.19 ± 16.13 mm.

Lymph node involvement was positive in 18 of 57 patients (31.6%). In the case group, 38 of 57 patients were new cases of cancer (66.7%), and 19 cases were diagnosed to have recurrent cancer (33.3%).

In terms of secretor status, 52.7% of all samples were secretors. In the case group, 19 out of 57 cases (33.3%) were secretors, and 38 were non-secretors (66.7%). In the control group, 39 out of 53 cases (73.6%) were secretors, and 14 cases were non-secretors (26.4%) (Figure 1).

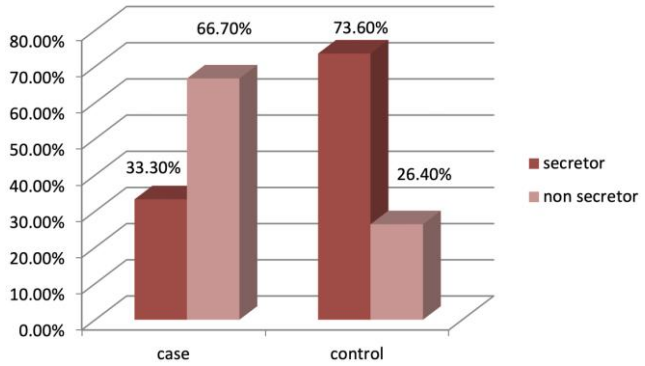

Figure 1: Secretory status in case and control groups

There was a significant difference in the percentage of non-secretors between the two groups (p = 0.00). In general, according to Wiener anti-agglutination test, 19 out of 57 patients were a secretor, among whom 12 were "A" secretor, 6 were "B" secretor, and one was "AB" secretor.

As mentioned above, the most common type of cancer among the case group was head and neck squamous cell carcinoma (HNSCC), 39 lesions (37 patients) among 59 lesions (66.1%). Therefore, the secretor status in patients with this type of cancer was analysed separately. In this group, 32.4% of subjects were secretors, and 67.6% were non-secretors. The difference between non-secretors in HNSCC and the control group was statistically significant (Table 2).

Discussion

Blood group antigens can be present in physiological fluids such as saliva. The hypothesis that non-secretors are more likely to develop infections, malignant and pre-malignant oral diseases, chronic periodontitis and dental caries, led us to assess the secretor status of patients with head and neck malignancies [28], [29], [30].

Fucosyltransferase 2 is an enzyme synthesizes by the secretor gene. This enzyme adds fucose to the sugar moiety of glycolipids and glycoproteins. By Fucosyltransferase 2 on precursor oligosaccharides chain (type A) H is formed in secretions. By adding fucose to H, fucosyltransferase 3 can lead to the production of Le/b. Thus, all people whose phenotype consists of Le/b are secretors. Fucosylated oligosaccharides which exist at the cell surface participating in severe biological processes such as cell differentiation, cell movement and

adhesion. Saliva and other body secretions of ABH secretors contain considerable amounts of carbohydrates compared to non-secretors. This can lead to interference with previously mentioned functions [15].

In this study, 52.7% of the total population were secretors (33.3% of the case and 73.6% of the control group) with statistically significant difference ($p < 0.001$). This means that people without blood group antigens in the saliva may be at increased risk for head and neck malignancies. Some previous studies have suggested a lack of secretion of blood group antigens in saliva as a risk factor for the development of oral epithelial dysplasia and malignant tumours [26].

It has been reported by Jamil et al., (2016) that 56% of the case group with oral cancer and 78% of the control group were secretors and there was a statistically significant relationship between secretor status and oral cancer ($p < 0.01$) [20]. The result of this study was consistent with the present study. While in studies by Cerovic et al., (2008) and Lamey et al., (1998), assessment of blood group antigens in patients with oral cancer showed no significant difference between the two groups in terms of secretor status ($P > 0.05$) [23], [24].

In Garrett's study (1971), there was no difference between the patients with benign and malignant salivary gland tumours and the control group in terms of secretor status [30].

Rai et al., (2015) evaluated the secretor status of the blood group antigens in oral premalignant lesions (leukoplakia, oral lichen planus, and subcutaneous fibrosis). In the case group, 87% of the subjects and in the control group only 16% were non-secretor, and there was a significant relationship between secretor status and pre-malignant oral lesions ($p = 0.00$) [25]. These results are consistent with the present study. The results of Vidas and Temmer's study (1999) showed no significant difference in saliva secretor status of patients with premalignant oral lesions (lichen planus, leukoplakia, etc.) ($p = 0.05$) meanwhile a higher intensity of these lesions was found in the non-secretor group ($p = 0.01$) that indicates the progression of pre-malignant oral lesions in this group of patients [31].

In Campi et al. 's study (2007) there was no significant relationship between oral lesions (malignant and premalignant) and secretor status ($p = 0.119$) [29].

Regarding oral submucosal fibrosis (OSMF) as a pre-malignant lesion, the study by Hallikeri et al., (2015) divided the samples into three groups. In the end, the results indicated that all members of the first group (tobacco users, with submucosal fibrosis) were non-secretors, in the second group (tobacco users, without oral lesions) 84% were non-secretors, and in the third group (healthy control subjects) 15% were non-secretors. These results showed a statistically significant difference between the first and third groups, indicating the role of secretor status in the development of oral submucosal fibrosis [26].

In the study of Shahidi Dadars et al., (2016), most patients with lichen planus were non-secretors (74%), while in Bakhtiari et al. 's study (2016) on patients with lichen planus 84% of the cases and 80% of the control group were secretors, and there was no statistically significant difference between the patient and control groups in terms of secretor status (32) ($P = 0.73$).

Another study by Lamey et al., (1991) showed that the percentage of non-secretors in the case group (68%) was significantly higher than the control group (38%) [21].

Lack of blood antigens in the saliva facilitates the long-term bonding of *Candida* to the mucosa. In potentially malignant diseases with *Candida* infection superimposition, nitrosamines produced by *Candida* can -directly or with other carcinogenic substances- activate specific proto-oncogenes and induce the development of malignant lesions [26].

Also, in non-secretors, lower IgA levels were reported in both serum and saliva. Therefore, it is likely that specific immune responses are weaker at mucosal surfaces of non-secretors, in comparison with secretors. Lower levels of IgG have also been observed in these individuals [13].

The study of Shahidi Dadras et al., (2016) on the secretor status of patients with oral lichen planus supported the hypothesis that the blood gene antigens on cell surfaces played a role in protecting mucosal surfaces from external pathogens [32].

In conclusion, the significant difference between secretor and non-secretor status in cancerous patients indicates that people with non-secretor status may be more prone to develop head and neck cancer. The presence of these antigens in saliva may have a protective effect.

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Allele Frequency of *SLC22A1* Met420del Metformin Main Transporter Encoding Gene among Javanese-Indonesian Population

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Abstract

BACKGROUND: Genetic variation in the genes that encode metformin transporters has been proven to cause pharmacokinetic variability and various glycemic response to metformin. Organic Cation Transporter (OCT) 1 protein encoded by the *SLC22A1* gene is primarily responsible for the process of metformin influx to the hepatocytes as the target of antihyperglycemic action as well as metformin elimination through the renal. This study aimed to determine the allele frequency distribution of the *SLC22A1* Met420del gene in OCT1 among the Javanese population, the largest ethnic group in Indonesia with T2DM.

METHODS: The research involved 100 adult patients from 9 healthcare facilities in Yogyakarta Province. The PCR-RFLP method was employed as a genotype analysis to detect polymorphism using 5'-AGGTTCACGGACTCTGTGCT-3' forward primer and 5'-AAGCTGGAGTGTGCGATCT-3' reverse primer.

RESULTS: No AA variant (wild type) type was found in the *SLC22A1* Met420del gene, and only 4% of the subjects had Aa heterozygote type. The allele frequencies of A and a were 2.0% and 98.0% in all subjects, respectively.

CONCLUSION: The allele frequencies in the Javanese-Indonesian population were almost the same as those in the studies involving Japanese, Chinese-Han, and Asian-American populations. This study recommends further research on the correlation between the influence of methionine deletion at codon 420 on the variability of pharmacokinetic profiles and the glycemic response to metformin as well as the incidence of gastrointestinal intolerance due to metformin administration.

Introduction

The prevalence of diabetes mellitus in Indonesia continues to increase, reaching 2.1% compared to the last 6 years [1]. Meanwhile, the International Diabetic Federation estimates that DM prevalence in Indonesia will reach 14.1 million in 2035 [2]. Therefore, good management of glycemic control is required to prevent as well as reduce morbidity and mortality due to diabetes mellitus [3].

From 2013 to 2017, metformin remained in the list of Indonesia's National Formulary as one of the oral antidiabetic drugs available up to primary healthcare facilities. The ability of metformin to reduce HbA1c levels in the range of 1.0-2.0% and the low hypoglycemic effects are among its advantages over other oral antidiabetic drugs. However, the glycemic response to metformin use is varied as 35 to 40% of patients have yet to reach the target of fasting blood glucose levels [4]. Variability in patients' response to antidiabetic drugs can result from genome variations that lead to variations in disposition and response to antidiabetic drugs including metformin [5].

Our previous study found a variety in the minimum as well as maximum metformin steady-state concentrations, reaching > 100-fold and 15-fold respectively, in 82 T2DM patients who received metformin at the similar dosage (1000 mg/day) [6]. As

a drug with renal excretion as the primary route of elimination, metformin has > 0.6 rGC (genetic component), indicating that variations in steady-state concentration can result from the involvement of genetic factors during the renal clearance of metformin [7].

As a hydrophilic base existing at physiological pH as a cationic species (> 99.9%), the effectiveness of metformin pharmacokinetics depends on the function of the transporters involved [8]. One of the major transporters known to play an important role in metformin pharmacokinetics to date is Organic Cation Transporter 1 (OCT1), a protein mainly expressed in liver sinusoidal cells, renal basolateral membrane [9], and apical membrane of tubule cells [10], which transports metformin to hepatocytes as the target of its antihyperglycemic action as well as plays a part in the elimination and reabsorption in the renal tubules. Variations in *SLC22A1* gene have led to changes in the function of OCT1 protein, which results in varied plasma concentrations of metformin and decreased amount of metformin in the receptors, making the therapeutic response to metformin decline. Such genetic variations can take the form of methionine deletion at codon 420 located in the ninth transmembrane domain of *SLC22A1*, which is the highest functional variant in the gene. Several studies have found that *SLC22A1* gene variants cause variability in both steady-state concentrations of metformin and glycemic response [11], [12], [13], [14]. Also, recent research showed that genetic variations in the gene are related to the level of gastrointestinal intolerance due to the use of metformin [15], [16].

This research is a part of pharmacogenetic studies of metformin use among the Indonesian population suffering from T2DM. Analysis of genetic variants in the target gene that encodes metformin transporters is important to provide information on the profile of genetic variation in Indonesian population which can then be further researched on the implications for the use of metformin as a first-line antidiabetic drug for T2DM and its safety for the gastrointestinal tract. Therefore, this study aims to determine the allele frequency distribution of *SLC22A1* Met420del gene encoding OCT1 among the Javanese population, the largest ethnic group in Indonesia with T2DM.

Methods

Recruitment of Subjects

T2DM patients were recruited from 9 existing healthcare facilities in Yogyakarta Special Province categorised as Javanese based on their three previous generations from Javanese ethnic. Informed consent was obtained from each patient who was willing to be involved in the study. The research has obtained ethical clearance from the Ethics Commission of the Faculty of Medicine of Gadjah Mada University.

Genotype Analysis of SLC22A1 Met420del in OCT1

Genotyping at *SLC22A1* Met420del was carried out using PCR followed by Restriction Fragment Length Polymorphism (RFLP). The PCR primer design used the forward primer 5'-AGGTTCACGGACTCTGTGCT-3' and the reverse primer 5'-AAGCTGGAGTGTGCGATCT-3'. The PCR conditions for amplification consisted of initial denaturation at 93°C for 3 minutes followed by 35 denaturation cycles at 93°C for 45 seconds, annealing at 58°C for 35 seconds, and extension at 72°C for 35 seconds as well as a final extension at 72°C for 5 minutes. The amplification products (600bp) were then analysed in 1% agarose gel for 30 minutes at 100 Volt followed by restriction digestion using *BspHI*, incubated for ± 12 hours at 37°C. *BspHI* enzyme cut T-CATGA sequence at the 197th base of DNA template. AA genotype was recognised and digested by the enzyme. PCR products with the T-CATTT sequence would not be recognised by the *BspHI* enzyme, leaving the product undigested. The resulted products were then analysed in 1% agarose gel and quantified using floor safe. Digestion of amplification products resulted in 600 bp fragments for AA (wild type) genotype as well as 403 bp and 197 bp fragments for aa (mutant) genotype, and 600 bp, 403 bp, and 197 bp for heterozygotes (Aa).

The results were presented in percentage using the Hardy-Weinberg principle. Referring to the previous research, the A allele (wild type) showed a GAT base deletion on DNA target sequences, and an allele (mutant) indicated GAT base insertion in DNA sequences [13].

$$\text{A allele} = \frac{(\text{AA genotype} \times 2) + \text{Aa genotype}}{2 \times \text{the number of samples}}$$

$$\text{a allele} = \frac{(\text{aa genotype} \times 2) + \text{Aa genotype}}{2 \times \text{the number of samples}}$$

Results

A total of 100 Javanese-Indonesian patients with T2DM were involved for the genotype analysis of the *SLC22A1* Met420del gene in OCT1. Characteristics of the research subjects are described in Table 1.

The patients involved in this study were mainly female (69%) with an average age and BMI of 52.88 ± 8 years old and 25.47 ± 4.5 kg/m^2, respectively.

Table 1: Characteristics of subjects for the genotype analysis of the *SLC22A1* Met420del gene in OCT1

Patient Characteristic	Male n	Female n	p-value
Age (years)			0.22
< 50	7	24	
≥ 50	24	45	
BMI (kg/m^2)			0.54
< 30	28	58	
≥ 30	3	11	
SLC22A1 Met420del genotype			*
AA	0	0	
Aa	0	4	
aa	31	65	

Presented only in descriptive statistics

Table 1 shows no differences in patient factors of both age and BMI between male and female patients with T2DM (P > 0.05). Also, there was no type of AA variant (wild type) found in the *SLC22A1* Met420del gene, and only 4% of the subjects had the Aa variant. The electrophoretic display of the results of the enzyme digestion for detecting polymorphism in *SLC22A1* Met420del is shown in Figure 1.

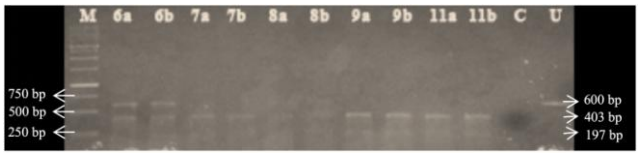

Figure 1: Result of analysis of the cutting region in BspHI restriction enzyme of Met420del polymorphism in SLC22A1 gene; aa homozygous/mutant (lane 7a-11b: 403bp and 197bp), Aa heterozygous variants (lane 6a and 6b: 600bp, 403bp, and 197bp). Note: lane M = marker/ladder 1 kbp; lane 6a and 6b = samples of heterozygote type in; lane 7a-11b = samples of mutant type; lane C = negative control; lane U = undigested sample

Also, to examine the allele frequencies in *SLC22A1* for both male and female patient groups, a descriptive analysis was employed with the results presented in Table 2.

Table 2: Allele frequency in the SLC22A1 gene and SLC47A1 gene according to gender

Allele Variant	Male (%)	Female (%)
A allele	0	2.90
a allele	100	97.10

Table 2 shows the highest proportion of allele in OCT1-Met420del of this study, namely mutant allele a (> 95%) in both male and female patients, and even in the male patient group, 100% of them had typed a mutant allele.

Discussions

No AA variant type was found in the *SLC22A1* Met420del gene in this study. Such non-existence of AA type was similar to the results of studies that involved 116 patients of Japanese, Chinese-Han, and Asian-American populations [17], [18], [19]. Meanwhile, among African-American and European-American populations, each with 200 research subjects, the frequencies of the mutant allele was found to be 2.9% and 18.5%, respectively [19]. In contrast, in studies involving 117 Iranian T2DM patients [20], 232 healthy Caucasian subjects [21], and 103 healthy Caucasian subjects in another study [10], as well as 246 T2DM patients in Latvian population [22] and 361 Danish patients [12], the frequency of wild-type genotype (AA) was higher than that of mutant genetic variant (aa) and heterozygote (Aa). Although infrequently conducted, pharmacogenomics studies that involve Indonesian population tend to find genetic profiles that are similar to those of Southeast Asian populations [23] and other Asian populations such as Chinese population [24] when compared to the genetic profiles of other populations. Different frequencies of Met420del genetic variants in OCT1 were also found in this study. This has certainly reinforced the importance of genetic profiles as a consideration in personal drug selection, effective dosage for a population/human race that is rarely involved in research into the safety and efficacy of novel drugs, such as in Indonesia.

Despite being performed only on experimental animal models, there were no differences in the expression of OCT1 in renal cells based on gender [25], [26]. Therefore, the difference in sex-type proportions in a pharmacogenomics study involving OCT1 transporters can be ignored, or no matching technique is needed in the data analysis for this patient-factor.

Several studies have been conducted to analyse the association of genetic variation in the *SLC22A1* gene with its effects, such as the variability of expression, disposition, and therapeutic response of a drug. A study of liver tissue samples from subjects of the Caucasian population identified the genetic variation as a critical factor of OCT1 hepatic expression [27]. This could lead to changes in the function of OCT1 as a protein transporter for several drugs that have liver as the action target, such as metformin. Expert studies of OCT1 distribution showed that such protein is found in the stomach, small intestine, kidney, and skeletal muscles in human, and is mainly expressed through the liver [28], [29]. Although early studies reported that OCT1 is found in the basolateral membrane [30], some other studies reported that the apical surface of intestinal epithelial cells also becomes the location of OCT1 [31], [32], [33]. To date, studies of the reduced function of OCT1 transporter in the intestine has been more associated with the level of gastrointestinal intolerance of metformin use because of the possibly higher effect on increasing local concentrations of metformin in the intestine (lumen and enterocytes)

when compared to the level of metformin transported to the blood [15]. Thus, genetic variation in SLC22A1 as an OCT1 encoding gene affects not only the absorption of metformin but also the function of OCT1 involved during the distribution to hepatocytes as the primary action target of such antidiabetic drug as well as during the reabsorption in the renal tubules. This analysis has been justified in several studies that found the effects of polymorphism on the pharmacokinetic and pharmacodynamic variability of metformin.

Methionine deletion at codon 420 (Met420del) located in the ninth transmembrane region of the *SLC22A1* gene, the polymorphism target of this research, has been the most commonly studied functional variant. In contrast to the majority of other functional *SLC22A1* variants that are population specific, the Met420 deletion can be found in some populations in different regions in the world [34], [35], [36], [37]. Such polymorphism causes a decrease in the activity of metformin transporter, leading to a reduced antihyperglycemic response [38]. This is also proven by a study of 20 healthy subjects receiving metformin as much as 1850 mg/day that indicates the presence of polymorphism, one of which is Met420del, causing the variant allele group to have higher AUC of plasma metformin concentration but lower volume of distribution in oral administration compared to the wild-type group [39]. Therefore, the metformin concentration transported to the hepatocytes as its action target is reduced, resulting in a decreased antihyperglycemic response [24]. Also, a study of 108 Iranian patients newly diagnosed T2DM and using metformin for 12 weeks also showed that the Met420del variant causes lower FBG reduction compared to the wild-type group [20]. Other studies also found a variation in the scores of metformin clearance in the kidney and the metformin uptake to the liver which will eventually affect blood glucose levels followed by an effect on the appropriate dose to administer to T2DM patients [37], [40]. In contrast, the research involving 1531 patients in GoDART study revealed that 420del does not affect A1C reduction in T2DM patients receiving metformin [41]. These contradictory differences require further research using more improved methods.

It is widely acknowledged that the effect of 420del polymorphism in *SLC22A1* on the expression of OCT1 in the apical membrane of renal tubule cells can reduce re-absorption in the renal tubule, leading to a decrease in the plasma metformin concentration, including its steady-state concentrations [42]. A significant reduction in the minimum steady-state concentration of metformin also occurs in patients with a heterozygous deletion of rs72552763 (Met420del) when compared to the wild-type group (P 0.06) [12].

As previously studied, OCT1 is also found in the basolateral membrane of intestinal cells, so polymorphism in OCT1 causes a decrease in the amount of metformin absorbed into the systemic circulation and increased its concentration in enterocytes. This has been believed to contribute to the occurrence of metformin intolerance [15]. A prospective study involving 92 newly diagnosed T2DM patients who received metformin found that Met420del variant in the group of patients with R61C (rs12208357) variant has twice higher OR to experience gastrointestinal side effects [43] and even 4 times higher OR in the group of patients who have 2 alleles of OCT1 functional variant including Met420del [44], but different types of polymorphism are found in another study involving 246 T2DM patients [22].

Also, although contradictory findings remain to appear, particularly related to the effect of Met420del polymorphism in *SLC22A1* gene on the glycemic response to metformin and gastrointestinal intolerance, the high frequency of mutant alleles in Javanese-Indonesian population requires further research. This is in line with the minimum involvement of the Indonesian population in the development of new drugs. Additionally, OCT1 is also an important transporter for several other drugs. Therefore, further studies of pharmacokinetic variability and therapeutic response to the use of other drugs that also require OCT1 in their pharmacokinetics, such as oxaliplatin, sorafenib, and lamivudine, are recommended [27], [45].

In conclusion, the results of allele frequency study on OCT1 involving the Javanese-Indonesian population is a novelty in the initial study of pharmacogenetics of metformin use which has never been conducted. The frequency of an allele in *SLC22A1* Met420del among the Javanese population in Indonesia is reasonably high (> 95%). Therefore, further studies are suggested to investigate the effect of genetic variation of these polymorphisms on the pharmacokinetic profile and glycemic response to metformin in Indonesian patients with T2DM.

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Case Report

Primary Seminoma Localized in Mediastinum: Case Report

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Abstract

BACKGROUND: Primary mediastinal seminomas most commonly occur in young men, and they are localised in the anterior mediastinum.

CASE PRESENTATION: The presented study is a case report of a 34-year-old man suffering from a mediastinal tumour in size of 19 cm, with pleural and pericardial effusion. The patient complains of cough, difficulty breathing, weight loss, and pronounced tiredness. CT of lungs and biopsy of the mediastinal change was performed. The histopathological analysis was in favour of a primary mediastinal seminoma. The patient initially had pronouncedly increased levels of LDH and β-hCG tumour marker. Pericardiocentesis was realised due to threatening tamponade of the heart, followed by 4 cycles of chemotherapy by BEP protocol. Following 2 cycles of chemotherapy, normalisation of LDH and β-HCG levels and significantly improved the clinical condition in the patient was found. Upon completion of 4 chemotherapy cycles by BEP protocol, the patient performed an FDG-PET scan with partial response and reduced dimension of a primary tumour in the mediastinum. Radiotherapy of residual tumour mass up to a total dose of 40Gy in 20 fractions was realised. Control FDG-PET scan had a finding of complete response to a tumour and absence of FDG uptake. The last follow-up examination was performed in October 2018, and the patient was disease-free for 54 months.

CONCLUSION: Multimodality treatment approach of chemotherapy followed by radiation consolidation ensured long-term survival in primary advanced mediastinal seminoma.

Introduction

Primary extragonadal germ cell tumours occur rarely, and they include 1-5% of all germ cell malignancies [1]. The most common extragonadal localisations are mediastinum and retroperitoneum. Primary mediastinal germ cell tumours are rare lesions accounting for only 10-15% of mediastinal masses. Most of these tumours are benign lesions; however, up to one-third of them could be malignant, and seminoma is the most common histologic subtype. The primary mediastinal seminoma is a rare tumour. In general terms, mediastinal seminomas are bulky tumours, and they tend to infiltrate into adjacent structures early in the growth process. Pure seminoma is sensitive to chemotherapy and radiotherapy, and the prognosis is good [2]. These tumours typically occur in men from the second to the fourth decade of life and could be present as asymptomatic, incidentally discovered lesions in 20-30% of patients.

Case Presentation

A 34-year-old patient was diagnosed with chest pain lasting for 2-3 months. Due to pain progression, the occurrence of pronounced fatigue, cough and body weight loss (more than 15 kg), X-ray of lungs was performed with a finding of mediastinal tumour mass, and consequently, CT of lungs was performed by intravenous application of contrast. CT finding of lungs was in favour of a large expansive process in the mediastinum, with irregular form and lobular contours and dimensions of 190 x 100 x 175 mm.

The substrate compressed and displaced the vascular structures in the mediastinum, primarily the aortic arch and its arterial branches toward the dorsal region. Pulmonary arteries were also suppressed. V.cava superior was suppressed as well towards the lateral region to the right, while its lumen was partially reduced. Pleural effusion was present bilaterally, more pronounced to the left side. Enlarged lymph nodes

were detected along the substrate in the proximal segment of the mediastinum. Enlarged lymph nodes were also detected bilaterally to the neck; the largest one was located to the right at the thyroid gland level, behind the right jugular vein (Figure 1).

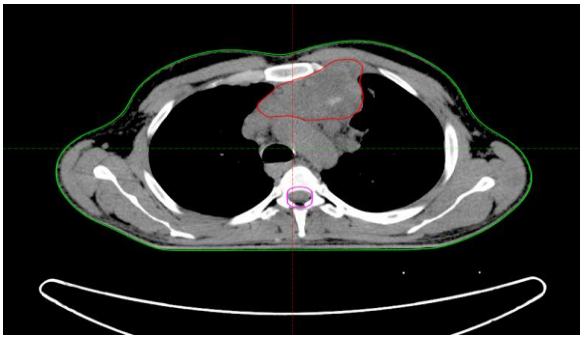

Figure 1: Computed tomography chest showing primary mediastinal seminoma with the involvement of major vessels

Fine needle biopsy and core biopsy were performed on three occasions, whereby the results were obtained in two laboratories; one of the results had undefined histology, and it was in favour of lymphoma or a tumour of neural origin, while the histopathological finding by the second laboratory was in favour of a thymoma. A third opinion indicated a finding in favour of an extragonadal seminoma. Biochemical analyses were performed resulting in pronouncedly increased levels of LDH 1482 U/L (normal range of 0-225), alkaline phosphatase level of 511 U/L (normal range of 40-129). Blood tests for germ cell tumour markers AFP were realised as well, with a normal level of 1.5 kiU/L and β-hCG with a pronouncedly increased level of 420.5 mlU/ml (normal range of 0-2.6).

Considering the increased β-hCG level in correlation with the histopathological finding for extragonadal seminoma, examination of testicles was performed to detect an occult primary testicular tumour. Normal ultrasound finding was obtained. Before the treatment initiation due to worsening of the general condition and occurrence of pronounced fatigue and suffocating, the patient performed an ultrasound examination of the heart with a finding of pericardial effusion and threatening tamponade of the heart. Pericardiocentesis was performed, and 500 ml of haemorrhagic pericardial effusion were evacuated. The patient started chemotherapy by BEP protocol, and 4 cycles of chemotherapy were realised. Following 4 cycles of chemotherapy by BEP protocol, the patient normalised the levels of LDH and alkaline phosphatase, and β-hCG level as well; clinical condition of the patient was significantly improved and following the chemotherapy he did not have symptoms of the disease.

After 4 weeks of the last chemotherapy cycle, the patient performed FDG-PET-CT scan; the result was in favour of partial response and presence of a pathologic metabolically active tumour substrate in the mediastinum. Radiotherapy for the residual tumour mass using IMRT technique was realised; a total dose of 40 Gy was achieved in the course of 20 fractions. The treatment was performed on LINAC by using X-ray of 6 MV energy (Figure 2). The patient had no side effects related to the treatment during its realisation.

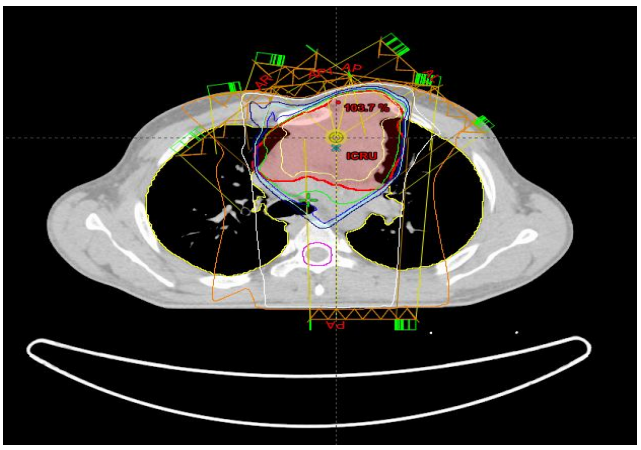

Figure 2: Radiotherapy for the residual tumour mass is realised using IMRT technique; a total dose of 40 Gy

Control FDG-PET-CT scan was performed 6 weeks after the radiation therapy, and a finding of complete response to the treatment and absence of pathologic metabolic active tumour mass in the mediastinum was obtained (Figure 3).

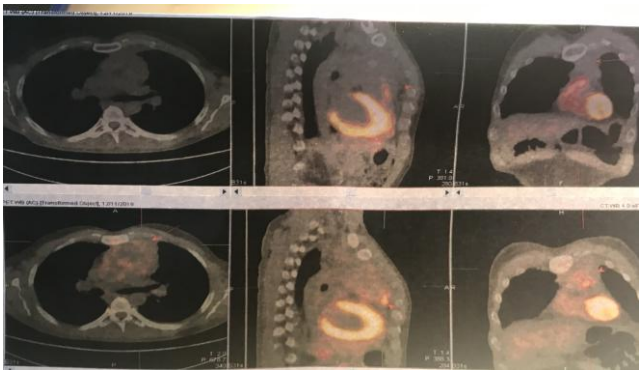

Figure 3: Comparative images from PET-CT before and after radiotherapy (upper part of a picture is PET-CT showing results after radiotherapy)

The patient continued the regular follow-up examinations, and he was disease-free for 54 months since the initial diagnosis. The last follow-up examination performed in October 2018, had a normal finding and the patient was disease-free.

Discussion

Extragonadal germ cell tumours (GCTs) typically are located along the median line of the body,

including the mediastinal and retroperitoneal spaces. Mediastinal seminomas usually affect the anterior mediastinum involving the thymus; more rarely the tumour could be presented in the middle and the posterior mediastinum. In some cases, large tumours could expand from the anterior to the medium mediastinum and lead to difficulty in distinguishing the primary tumour localisation [3].

These extragonadal tumours are considered to develop from germ cell precursors trapped during the embryonic migration and survived on ectopic localisation. Approximately 90% of primary malignant mediastinal germ cell tumours develop in mail in the third decade of life. Metastatic disease occurs in less than half the cases; in case of occurrence, it is usually spread to adjacent lymph node groups in the neck, the mediastinum, or the abdomen. Hematogenous metastases are unusual but can be detected in the lungs, bones, liver, spleen, thyroid gland and brain.

Primary mediastinal seminomas are manifested by non-specific symptoms. Symptoms are closely related to tumour localisation and dimensions. The most commonly reported symptoms include chest pain, dyspnea, cough, and weight loss [4]. The prognosis for pure seminomas in the mediastinum is excellent, with a 5-year survival rate in more than 90% using appropriate therapy.

When a tumour mass in the mediastinum develops, it could be a metastatic or primary benign or malignant mediastinal tumour. Most common differentially diagnostic cases could be lymphoma, a tumour of neurogenic origin such as schwannoma; in case of the anterior mediastinum it could be a thymoma; if there are packages of lymph nodes, they could be metastatic deposits of primary lung carcinoma, or an extragonadal mediastinal seminoma or a non-seminoma tumour [5].

Considering the difficult radiology distinction of these tumours, histopathological analysis of the biopsy material is necessary for definitive diagnosis. Another complicated step in the diagnosis of these tumours is the localisation, hardly accessible for biopsy. A biopsy can be done within the mediastinoscopy, or it could be CT-guided biopsy as in the case of our patient. There is significant morphological overlapping when it comes to embryonic carcinoma or seminoma, poorly differentiated carcinomas (such as pulmonary or thymus carcinoma, and metastatic carcinomas as well). Immunohistochemical analysis by a pathologist is of particular importance to specify the diagnosis by individual elimination of these entities. During the diagnosis of a germ cell tumour, a sample of blood may be collected to test for levels of serum tumour markers. High levels of any one of three tumour markers, called alpha-fetoprotein (AFP), beta human chorionic gonadotropin (β-hCG), and lactate dehydrogenase (LDH), may indicate a germ cell tumour. High AFP levels can also help identify the type of germ cell tumour, by showing whether it is a pure seminoma or mixed with non-seminoma, since AFP is not made by seminomas. However,

β-hCG and LDH can be higher if a man has a seminoma, non-seminoma, or mixed tumour. The diagnosis in our case was based on the histopathological finding, as well as the biochemical analysis in favour of pronouncedly increased LDH and β-HCG levels. Monitoring of serum tumour markers is significantly important for the diagnosis and prognostic evaluation of patients with mediastinal seminoma.

According to the 2010 American Society of Clinical Oncology clinical guidelines, in rare male patients presenting with testicular, retroperitoneal, or anterior mediastinal primary tumor and whose disease burden has resulted in an urgent need to start treatment, substantially elevated serum AFP and/or β-hCG may be considered sufficient for the diagnosis of GCT [6].

Patients at an early stage of mediastinal seminoma could be cured by surgical treatment and postoperative radiotherapy. Patients suffering from an advanced disease should be treated by chemotherapy and radiotherapy to achieve a good treatment result [7]. Seminoma is a highly radiosensitive tumour among mediastinal tumours, and therefore radiotherapy is considered an important treatment that increases the long-term survival rate of patients by 60-80% [8]. Clinical trials have demonstrated excellent results when multimodality chemotherapy is combined with radiation for large, localised mediastinal seminomas or extensive residual disease [9].

In conclusion, multimodality treatment approach of chemotherapy followed by radiation consolidation ensured long-term survival in primary advanced mediastinal seminoma.

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Case Report

"Transforming the Beast to A Beauty"- Fifteen Years into the Making - Case Report of Congenital Neurofibromatosis

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Keywords: Neurofibromatosis; Recklinghausen; Madelung's disease

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Abstract

BACKGROUND: In 1882, the German pathologist Friedrich Daniel von Recklinghausen described a series of patients with a combination of cutaneous lesions and tumours of the peripheral and central nervous system. Succeeding this paper, all of the patients with similar symptoms were given the diagnosis "von Recklinghausen disease". In the 20th century, a distinction was made between Neurofibromatosis type 1 (NF1) and Neurofibromatosis type 2 (NF2) with the help of molecular testing.

CASE REPORT: We are presenting the results from multiple surgical esthetic and reconstructive surgical procedures performed on a female patient with severe congenital neurofibromatosis during 15 years (2000-2015). The external appearance of our patient was not reflected in the general public's beauty standards. Convinced that she was unusual and unaccepted by the society, she gathered all of the strength and became our patient at 15 years of age.

CONCLUSION: Transforming the patient's life in the next fifteen years improved her overall health and her life quality.

Introduction

In 1768, Akenside was the first one to publish a scientifically-based description of a disease that later has been described as Neurofibromatosis type 1 [1]. At that time, he had acknowledged that the monsters of scholars, such as Pare and Aldrovandi, suffered from a disorder of the nerves [2]. The neurofibromas of NF1 were first described in details by Smith in 1849 [2]. In 1882, the German pathologist Friedrich Daniel von Recklinghausen described a series of patients with a combination of cutaneous lesions and tumours of the peripheral and central nervous system [1]. Succeeding this paper, all of the patients with similar symptoms were given the diagnosis "von Recklinghausen disease".

In the 20th century, a distinction was made between Neurofibromatosis type 1 (NF1) and Neurofibromatosis type 2 (NF2) with the help of molecular testing. NF1 evolved from the von Recklinghausen disease, a name which now is only an antiquated synonym for the disease [3]. It has been estimated that in 1 out of every 2500 to 3500 individuals are affected, and only one half are results of the inherited (familial) disorder. The remaining half is considered to be sporadic (de-novo) mutations [4]. The classic manifestation of café-au-lait macules, axillary or inguinal freckling, iris hamartomas (Lisch nodules) and neurofibromas. These findings usually prompt further clinical examination, sometimes requiring genetic testing (in questionable cases) for the mutation of the NF1 gene on chromosome 17q11.2. The consensus for diagnosis of NF1 was developed by the NIH (National Institute of Health) at the National Institutes of Health Consensus Development Conference in 1987, later updated in

1997, which required having two or more of the clinical features shown in Table 1 [5], [6], [7].

Table 1: Clinical features of the patient

NIH diagnostic criteria for neurofibromatosis type 1
6+ café-au-lait macules (prepubertal individuals: bigger than 5 mm and postpubertal individuals: bigger than 15 mm)
Axillary or inguinal freckling
Neurofibromas (2 or more) or one Plexiform neurofibroma
Optic glioma
Iris hamartoma (Lisch nodules, 2 or more)
Bone lesions (sphenoid dysplasia, medullary narrowing or cortical thickening of long bone cortex with or without pseudoarthrosis)
First degree relative with NF1

Ethical Approval: Our patient has been informed of potential publication, has given her consent, and all steps to ensure patient confidentiality have been taken.

Case Presentation

The female patient was only 15 years of age when first presented to our hospital. The first impression was remarkable for her innumerable skin neurofibromas and skeletal abnormalities. Born in 1985 in a small mountain village in the eastern part of Republic of Macedonia, she spends almost her entire life hiding from the public due to her external appearance. Living in an isolated area of a third world country additionally limited her to access appropriate medical care.

The patient had neurofibromas involving almost all anatomical regions of the body, most prominent on the face, head, thorax, hands, feet and the inguinal area. The neurofibromas were ranging in size (from 0.5 to 10 cm in greatest dimension) and in addition to their location were incapacitating her daily living. The patient was not able to walk or use her hands. In addition to the neurofibromas, the patient had multiple café-au-lait macules and cervical ankyloses.

The MRI of the head, kyphosis of the spine and ankyloses of the thoracolumbar part, are combined with the different size of round and fairly well-circumscribed nodules. Also, there is neurofibroma in the subcutaneous soft tissue in the forehead, nasal area, intraoral region, upper and lower extremities, sternum, spine and gluteal regions. Serial blood laboratory tests were performed and were within normal limits. The genetic evaluation was not performed for definitive in addition to the clinical diagnosis since it was compliant with the NIH NF1 diagnostic criteria (see Table1). However, maybe one of the most debilitating aspects of the patient's life was her emotional status. Her mental status and intellect were appropriate for her age, and she was attending school until graduation from high school. The everyday stress due to her presence and the constant unpleasant remarks from her peers were leaving scars in her soul much bigger and unbearable if compared to any surgical scars.

After multiple surgical procedures, the results come slowly. She understood and accepted our plan saying "I am a beast, please help me" with despair but and not very hopeful for the future.

The first surgery was performed with general anaesthesia on June 28th 2000, with concentration to the lesions on her face, especially bulky nose and her head (Figure 1).

Figure 1: A) Pre-operative presentation (June 28, 2000); B) Pre-operative presentation (June 28, 2000); C) Initial surgery, removal of facial lesion (June 28, 2000)

The second surgery was done the following year in local anaesthesia, since the patient was already experienced and familiar with what to expect. The patient continued to return almost annually for the removal of the lesions.

On March 30th 2011, we came to the point when her psychological and overall health was improving, and we could concentrate more on the smaller lesions on her body which will improve her quality of life even more.

a) b)

Figure 2: March 30, 2011

Our focus was aimed at restoring her fine motor movement by removing the digital lesions (Figure 2a) and multiple lesions on her bilateral feet, including the lesions affecting the toes (Figure 2b).

After this surgery, the fine motor movements, and use of her hands in general drastically improved. On December 7th 2012, multiple large and diffuse subcutaneous lesions were removed from her anterior thorax region (Figure 3a). The last time we saw the patient was on December 10th 2015. She was functioning on her own and much more independent

than the time we first met her (Figure 3b).

a) b)

Figure 3: December 7, 2012

The histopathological evaluation of the multiple lesions throughout the years was consistent, showing spindle-cells shaped cells with a scant amount of cytoplasm separated by collagen fibres and myxoid material (Figure 4).

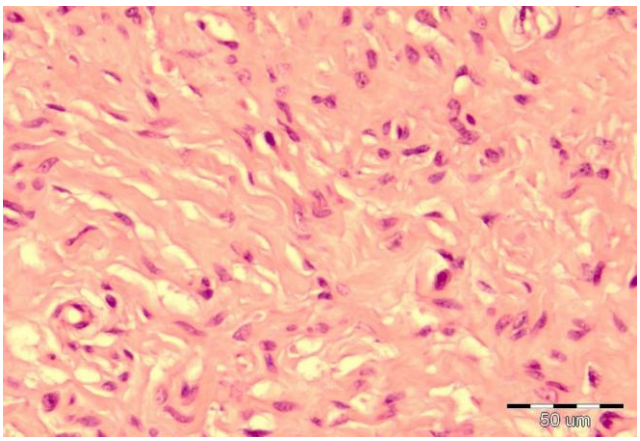


Figure 4: Spindles cells in the fibrous stroma (H&E, 200x)

Immunohistochemical staining with S-100 highlighted the fraction of the cells (Figure 5). Some of the lesions were small (less than 2.0 cm), and others was larger, diffuse and involving nerves (Figure 6). No apparent malignant transformation was identified [8].

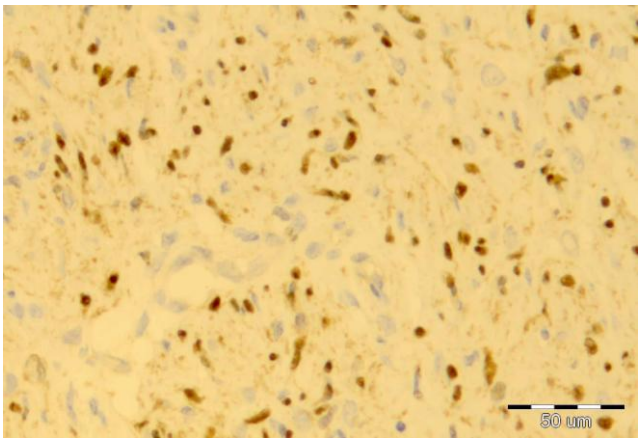
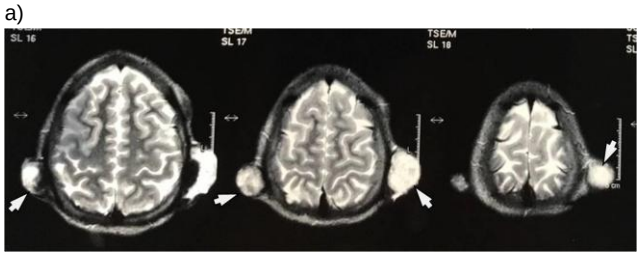


Figure 5: S-100 highlights the Schwann cells (S-100, 200x)

Discussion

Neurofibromatosis type 1 is a genetic disorder that predominantly affected our patient's look [2]. The shame of her physical disabilities attributed to the delay in her family seeking medical help for the multiple deformities [4]. The progression of fibromatoses nodules affected nerves and combined with her spinal ankyloses had limited her mobility, making her dependent on her mother.

a)



b) c)

Figure 6: a) MRI of the head; b) MRI of the body; c) MRI of the spine

The solution for the inability to walk the family had overcome by carrying the patient rather than seeking a permanent medical solution. None of the siblings or close relatives is known to have NF-1, which is not an uncommon finding. Her surgical treatments have abruptly stopped in 2015, after the death of her mother who was her principal caregiver and support throughout her life.

The initial operation involved removing the huge neurofibromas from the head and face region. Within the subsequent fifteen years (2000 – 2015) an additional ten surgical procedure was performed in various regions of her body, all of them in local anaesthesia. Her wounds were healing fairly quickly. In each of the wounds, only one drain was left for only one day. We performed to used stitches Vicryl 4-0. No complications were observed, including infections and keloid formations. After the removal of numerous neurofibromas, we release some peripheral nerves structures, and she has achieved complete restoration of movement in her upper extremities to the point that she can perform delicate tasks with her hands. Our last communication was 16.09.2018 when she gave us the consent form to publish her pictures.

In conclusion, the goal of any esthetic intervention in congenital disorder illness is to re-establish near “normal” contour. Immediate results are evident on the patient’s psychological wellbeing. The elimination of unusual appearance contributed and brought out our patient’s pleasant appeal to those that surround her.

The known risk of malignant transformation of the plexiform and intraneural neurofibromas currently has been estimated to be up to 5-10%. Aside from the risk of malignant transformation and deceptive physical deformity, psychological trauma, such as depression and anxiety, almost inevitably accompany physical deformities. Often the suffering does not end with only the patient but continues with the family thus burdening a wider circle of people.

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Case Report

Subcutaneous Dirofilariasis in Female Pubic Region - Case Report

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Keywords: Human dirofilariasis; Subcutaneous dirofilariasis; Dirofilaria repens; Zoonoses; Croatia

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Abstract

BACKGROUND: Number of reported cases of human dirofilariasis in the last couple of decades has been increasing. Dogs are the main reservoir hosts, while various mosquito species represent/serve as vectors. Human infections are caused by two different parasites: Dirofilaria immitis and Dirofilaria repens. Dirofilaria repens is currently considered to be one of the most rapidly-spreading human and animal parasite species in Europe. Clinical features are characterised by subcutaneous or ocular lesions which can persist for months without any symptoms. The disease is considered to be endemic in Mediterranean countries, central Asia, Israel, and Sri Lanka with increased incidence in Central and East Europe. Apart from the local population, tourists are also susceptible to this disease. Export of domestic animals can transfer parasites between different countries. This disease is rare in Croatia.

CASE PRESENTATION: We are presenting a case of a 58-year-old female patient with dirofilariasis of the genital region caused by Dirofilaria repens, confirmed with PCR and sequencing.

CONCLUSION: From the clinician point of view, subcutaneous dirofilariasis should be considered as a differential diagnosis in cases of long-lasting subcutaneous swellings.

Introduction

Members within the genus Dirofilaria, namely D. repens and D. immitis are zoonotic parasites transmitted by the different mosquito species. The number of reports on human dirofilariasis in a couple of last decades has been increasing across Europe and is considered to be one of the most extensively spreading human and animal parasite in Europe. Dogs are the most important reservoirs for infection with this species. Mosquitoes of the genus Aedes, Anopheles and Culex, serve as vectors of both parasites [1], [2], [3], [4]. Vector-borne diseases are usually transmitted to human by mosquitoes [1], [5]. Human infections caused mostly by D. repens are still overlooked, and usually represent unexpected finding, most frequently manifested as skin or subconjunctival infiltrates. Dirofilaria immitis infections are clinically characterised by solitary or multiple lung lesions which must be diagnostically evaluated for differential diagnosis as tumours; primary or metastatic [1], [3]. Clinical manifestations are characterised by subcutaneous or ocular lesions that can persist for months without any symptoms [1], [3], [6]. Subcutaneous nodules can occur in any part of the body but are most frequently detected on the face, neck, eyelid, chest, back, and scrotum [1], [3], [6]. Rarely, the parasite could be located/present in the buccal mucosa, liver, spermatic cord and abdomen [6], [7]. The disease is prevalent in tropic climates, with a tendency of spreading to moderate climate regions [2]. In Europe, the disease is considered to be endemic in Mediterranean countries with increasing incidence in Italy, France, Greece, and Spain [1], [3], [4], [5], [6]. Except in the Mediterranean region, the

number of reported cases has been increasing in Central Europe [6], [8], [9]. Hungarian National Centre for Epidemiology recorded 13 cases of dirofilariasis from 1879 to 2000 and 88 cases from 2001 to 2013 [6], [10], [11]. In Croatia, the disease is still considered to be rare with sporadic occurrence [1], [3].

In the following paper, we present the case of 58-year-old woman with a subcutaneous node in the genital area caused by infection with D. repens.

Case Report

A 58-year-old female patient has been admitted to the gynaecology clinic in Zagreb, Croatia due to the presence of hard (firm), painless nodule in the pubis region, without a history of any serious disease. She did not travel in the last few months from her place of residence. Patient lives in a rural area in Zagreb County with a dog and other domestic animals near a forest, without springs of natural water nearby.

After the inspection and gynaecological examination, an enlarged lymph node was suspected. The ultrasonographic finding of the nodule appeared normal. For the next two weeks, “nodule” was followed up and since there were no visible changes, excision was proposed. During the excision, a seven-centimetre filarial worm was found and removed together with surrounding subcutaneous tissue. Histopathology revealed a worm-like parasite and based on morphological characteristics, D. repens infection was suspected (Figure 1).

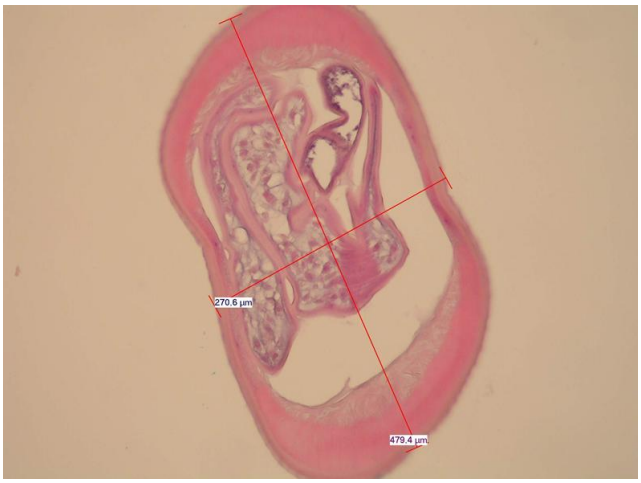

Figure 1: Histology of the worm suggested the diagnosis of Dirofilaria repens

The worm was surrounded by fibrous tissue infiltrated with lymphocytes and plasma cells associated with extensive eosinophil infiltration (Figure 2).

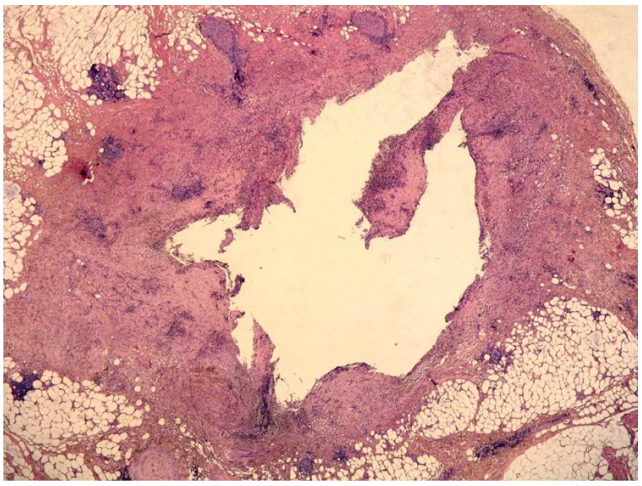

Figure 2: Low power magnification of the tissue that surrounded the warm

DNA was done using commercial Blood and Tissue Kit (QIAGEN) system according to manufacturer's instructions. Molecular identification of the parasite was performed using DIDR-F1 (5' – AGT GCG AAT TGC AGA CGC ATT GAG-3') and DIDR-R1 (5' – AGC GGG TAA TCA CGA CTG AGT TGA-3') primers. Amplification was carried out according to the method developed by Rinshiw and coauthors [12]. Primers were used to amplify Internal transcribed spacer region 2 (ITS2) for D. immitis (542 bp), D. reconditum (578 bp) and D. repens (484 bp). The amplification products were analysed by capillary electrophoresis (QIAxcel System®, QIAGEN) and purified (ExoSAP-IT®, USB Corp., Cleveland, United States). The sequencing was performed by Macrogen Europe in both directions. The resulting sequences were assembled using the SeqMan program and edited with EditSeq (DNA Star). Obtained Sequence was compared to sequences in the GenBank database using BLAST. Blood with EDTA was collected from the patient and analysed with modified Knott’s method to exclude the possible presence of microfilariae. A search against the GenBank database using the BLAST algorithm revealed that the sequence with the highest similarity (99%) with the isolate from the dog (GenBank Accession No. AY693808). The patient was followed for the next two years, and no additional symptoms were noted.

Discussion

Dirofilaria is parasitic nematode of dogs that can infect humans accidentally via vectors. The first case described in the literature is by Babes in Hungary who described the parasite in 1879 [6].

D. repens is zoonotic nematode able to cause dirofilariasis in humans and is considered to be an

emerging zoonosis. Some parasitic diseases are still neglected in “industrialised” world since they are considered to be tropical. Due to global changes including warming, animal trading and travelling, together with a change in vector ecology; evidence for their emerging are increasing. In the current paper, we are reporting an additional case of subcutaneous dirofilariasis in a female patient with a nodule in the pubic region, living in Central Croatia.

Subcutaneous dirofilariosis was suspected based on morphological characteristics of the worm after the parasite has been surgically removed. Worms belonging to the genus Dirofilaria are identified by their thick laminated cuticle, broad lateral ends and large muscle cells [1], [2]. D. repens was confirmed with sequencing which represents the first human sequence from Croatia. Exact identification of species may be possible only after studying the fully matured worm. Microfilaremia is absent in the case of human infection with D. repens as was the case in the current study [2]. Dirofilaria repens parasite enters the lymphatic system and forms local subcutaneous or subconjunctival lesions. Subcutaneous nodules are up to 1 cm in diameter, and the treatment is surgical removal similar to the presented case [1], [6].

In Croatia, similar to other European countries, number of reported cases has been increasing, however, the exact number of human infections with D. repens remains unknown [13], [14], [15], [16], [17], [18], [19], [20], [21], [22]. As of today, there is no obligation to report human dirofilariasis in Croatia. Global warming allows an increase in the mosquito population as well as the introduction of new species with the capacity to transmit both of Dirofilaria species [23]. The same part of increased incidence of the disease is due to better diagnostical modalities [1], [2], [6], [8].

In conclusion, to have accurate data on the incidence of the human dirofilariasis, all individual cases should be analysed with molecular methods for species confirmation. Better reporting system could be a helpful tool in providing answers on prevalence, distribution, and an actual increase in the number of occurrences, rather than an increase in some published cases.

From the clinician point of view, subcutaneous dirofilariasis should be considered as differential diagnosis in cases of long-lasting subcutaneous swellings.

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Case Report

Pulmonary Embolism Masquerading as Severe Pneumonia: A Case Report

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Keywords: De novo pulmonary embolism; Deep vein thrombosis; Severe pneumonia; Computed tomography of pulmonary angiography

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Abstract

BACKGROUND: De novo pulmonary embolism (DNPE) is a term used when pulmonary embolism (PE) occur in the absence of deep vein thrombosis (DVT). Most DNPE cases occur in a patient who had a recent injury to the chest.

CASE PRESENTATION: However, here we report a case of DNPE with a slightly different presentation where there is no preceding trauma and has symptoms that mimic severe pneumonia. He presented with high fever, dyspnoea and pleuritic chest pain. Despite on 10 L of oxygen supplementation via high flow mask and already given bolus intravenous antibiotic, the patient still tachypnoeic and was persistently in type I respiratory failure. His chest X-ray showed consolidative changes. Upon further investigation revealed no evidence of DVT on Doppler ultrasound and normal D-dimer level. Due to the high index of suspicion by the attending physician, PE was suspected and later confirmed with computed tomography pulmonary angiography scan. He was successfully treated with anticoagulation therapy. The objective of this case report is to share the difficult experience of diagnosing PE when the presentation highly atypical and mimics severe pneumonia.

CONCLUSION: And with such a masquerading presentation, one can easily miss the diagnosis. To the best of our knowledge, there are very few similar cases reported.

Introduction

Pulmonary embolism (PE) is a serious medical condition that occurs when a clot usually from the deep vein thrombosis (DVT) dislodged and occluded the pulmonary circulation. It is clinically manifested as shortness of breath, pleuritic chest pain, coughing, orthopnoea, haemoptysis and much other less common presentation [1]. If the PE is massive, the patient can even present with circulatory collapse. When an established PE occurs without any evidence of DVT, the condition is deemed to be called de novo pulmonary embolism (DNPE). A recent study showed that DNPE is not uncommon, and it is associated with a patient who has a history of recent trauma or accident, especially to the chest. However, here we present a patient who developed PE but has no history of recent trauma. Subsequent investigations noted that the patient has no evidence of DVT and any intracardiac clots or vegetations from echocardiography. Therefore, it is deemed suitable for this patient to be labelled as DNPE and will be the centre of the discussion of this case report.

Case Report

A 70-year old man with underlying hypertension, diabetes mellitus, dyslipidaemia and ischemic heart disease who had undergone multiple percutaneous coronary interventions between the

year 2009 to 2012, was referred from another tertiary hospital after being admitted there for eight days for degenerative spinal disease and prolapse intervertebral disc involving the L3/L4, L4/L5 and L5/S1. He has no history of recent trauma, fall or accident before that admission. In that hospital, he was managed conservatively with only physiotherapy and analgesia. The patient went back home for a few days before current admission, and he was partially bed bound where he spent most of his time in bed and did not ambulate much due to the severe back pain. Upon arrival at the emergency department, he was febrile with a temperature of 38.7°C and breathless with the oxygen saturation of 90% on air. His pulse rate was 114 beats per minute, irregularly irregular rhythm and has good volume. His blood pressure was 130/80 mmHg unsupported.

Further history from the family member noted that the patient has been having a fever and feeling unwell for the past 2 days. On examination, the patient was obese and tachypnoeic. There was reduced breath sound, and coarse crackles heard over the right lower zone. Examination of the cardiovascular system was unremarkable; his abdomen was mildly distended but otherwise soft, not tender and no palpable organomegaly. The muscle power of his lower limbs was reduced to 2/5 from the hip downward. Electrocardiogram (ECG) showed atrial fibrillation with normal ventricular response Q-wave over the inferior lead and no acute ischemic changes. Preliminary blood investigations were done (as shown in Table 1).

Table 1: Initial blood investigation taken upon arrival to the emergency department showed marked leucocytosis and mild renal impairment. Otherwise, no elevated liver enzyme, coagulation profile was normal, no elevated cardiac enzyme, and the D-dimer was not raised

TEST	VALUE	NORMAL RANGE
Haemoglobin	11.8 g/dL	12 – 18 g/dL
Haematocrit	37.1%	35 – 48%
White cell count	27.1 x 10^9/L	4.0 – 11.0 x 10^9/L
Platelet	415 x 10^9/L	150 – 400 x 10^9/L
Urea	15.7 mmol/L	1.7 – 8.0 mmol/L
Creatinine	103.9 umol/L	60 – 120 umol/L
Sodium	133 mmol/L	135 – 150 mmol/L
Potassium	4.3 mmol/L	3.5 – 5.0 mmol/L
ALT	32 u/L	5 – 35 u/L
ALP	38 u/L	30 – 100 u/L
PT	14.2 second	11.8 -14.5 second
aPTT	44.3 second	30.0 - 44.5 second
INR	1.09	< 1.1
D-dimer	0.35 mg/L	< 0.5 mg/L

ALT = Alanine aminotransferase ALP = Alkaline phosphatase; PT = Prothrombin time; aPTT = Activated partial thromboplastin time; INR = International Normalised Ratio.

The initial impression was severe community-acquired pneumonia given the respiratory symptoms associated with high fever and leucocytosis. Intravenous (IV) Ceftriaxone 2 gm once daily was started. However, despite on 10 L oxygen supply via a high flow mask, the patient was still tachypnoeic and oxygen saturation still lingering over the 90%. Repeated arterial blood gas still showed type 1 respiratory failure without any improvement. Chest X-ray showed mild consolidation over the right lower zone (as shown in Figure 1), which coincide with clinical finding of coarse crackles on that area.

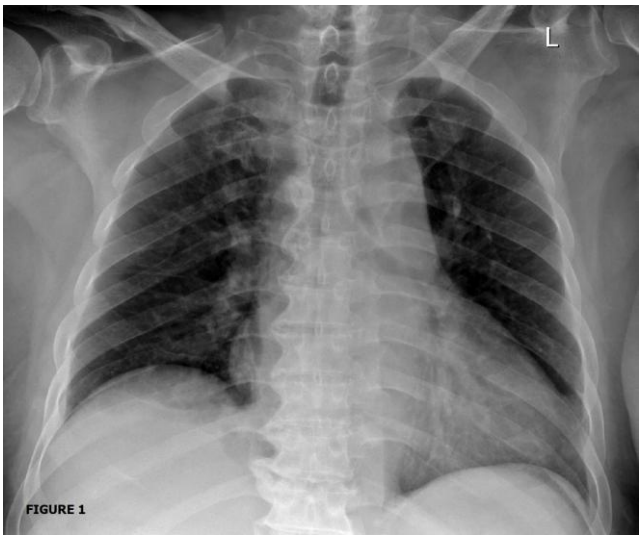

Figure 1: Chest X-ray is taken a few hours after arrival showed minimal consolidation over the right lower zone

Given the atypical presentation of pneumonia and high suspicion of pulmonary embolism, the respiratory physician was referred to review the patient. Although Well's score was only 1.5 which was low, given the risk of partial immobilisation for a few days before referral and also having persistent type 1 respiratory failure despite on oxygen supplementation, he was subjected to Computed Tomographic Pulmonary Angiography scan. The scan showed impaired filling over the secondary branch of the descending right pulmonary artery (as shown in Figure 2a and 2b), in keeping with the diagnosis of pulmonary embolism. There was also right-sided pleural effusion and collapsed consolidation over the ipsilateral posterobasal segment of the lower lobe.

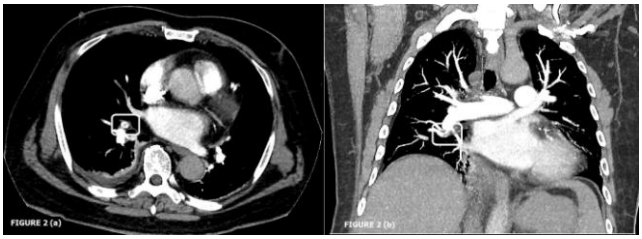

Figure 2: a) Computed Tomography of Pulmonary Angiography (CTPA) Axial view of the thorax showing pulmonary embolus effecting the secondary branch of descending right pulmonary artery. The partial central filling defect surrounded by contrast material produces polo mint sign, in keeping with acute embolus; b) CTPA Coronal view of the thorax shows filling defect of the right basal trunk with no extension into the right lower lobe segmental branch

He was immediately started on subcutaneous Fondaparinux 10 mg once daily and then overlapped with oral warfarin a few days after his condition stabilised. After he achieved his target INR of between 2 to 3, the subcutaneous Fondaparinux was subsequently discontinued. The oral warfarin was planned to continue for 3 months. The patient has shown great recovery and able to maintain good

oxygenation with spontaneous respiration on air. His antibiotic was changed to IV tazobactam 0.5 mg and piperacillin 4mg three times daily given recent hospital admission and was continued for a week. Upon further investigation, Duplex Doppler ultrasonography of the lower limb was done and showed no evidence of deep veins thrombosis. Echocardiograph was also done and show good left ventricular ejection fraction, no abnormal motility of the ventricular wall, no dilated chambers, and no vegetations or clots visible.

Discussion

Pulmonary embolism (PE) is a life-threatening condition that occurs when a dislodged blood clots usually from the deep vein of lower limb occluding the pulmonary vessel. It is clinically manifested as shortness of breath, pleuritic chest pain, coughing, orthopnoea, painful swelling over the calf or thigh, wheezing, haemoptysis and less commonly heart arrhythmia. It can also present with a syncopal attack or more devastatingly as circulatory collapse [1]. The best modality or so-called “gold standard” investigation to diagnose PE is pulmonary angiogram. However, due to the adverse effect and the limited availability of the modality, the best alternative will be Computed Tomography Pulmonary Angiography (CTPA). It has high sensitivity and specificity to diagnose PE which will show filling defect upon contrast enhancement in the pulmonary artery or any of its branches [2]. If CTPA is contraindicated (e.g. patient with a history of contrast allergy, hypotensive, has advanced heart failure, unable to lie flat or morbidly obese and unable to perform CT scan, etc), or when the CTPA finding is inconclusive, and further testing is required, ventilation-perfusion (V/Q) scanning is recommended. V/Q scan has slightly lower sensitivity, and specificity compared to CTPA and will show impaired perfusion with normal ventilation in PE [3]. Other modality to diagnose PE includes echocardiography and also measuring the level of D-dimer in the body. Echocardiography is usually done over the bedside especially in condition when other preferable modalities are not available or when the patient condition is too unstable to transfer, and it will show a nonspecific right ventricle (RV) strain in PE, such as enlarged RV size with reduced functionality, abnormal septal wall movement, and tricuspid regurgitation. Sometimes, RV thrombi can be visualised in echocardiography and usually signify a poor prognosis. The d-dimer level is usually measured when all other modalities are unavailable or in a condition when a patient with high risk but has normal CTPA finding. However, normal D-dimer level does not rule out pulmonary embolism [4], as we see in our patient where the D-dimer level was not elevated.

The management algorithm for PE begins by assessing the severity of the condition [5]. In a hemodynamically unstable patient (systolic blood pressure below 90 mmHg or a drop of more than 40 mmHg from baseline), thrombolysis may be warranted [6]. And if thrombolysis failed or contraindicated, embolectomy sometimes performed. In a hemodynamically stable patient, on the other hand, anticoagulation medication will be started as soon as possible and continued for a certain duration depending on whether the PE is provoked or not. And if the patient developed another episode of PE while on anticoagulation therapy, a longer period of treatment or maybe lifelong maybe indicated. If there is any contraindication for anticoagulation therapy, inferior vena cava (IVC) filter can be inserted in the source of emboli is from deep vein thrombosis (DVT). And once the patient is suitable for anticoagulation medication, it should be started immediately, and the IVC filter should be removed [7].

It is strongly believed that most PE patients have underlying DVT and that the occluding clots in the pulmonary vasculature are dislodged from DVT. Some even claim that PE and DVT are the clinical manifestations of the same entity, which is called venous thromboembolic disease [8]. Until recently, Van Gent JM et al., [2014] reported that PE could occur without DVT especially in a patient who had recent trauma. He also suggested that DNPE most likely occur in response to injury or inflammation [9]. However, there was a slightly different presentation in this case report, whereby our patient who was obese and has a short history of recent partial immobilisation due to severe back pain secondary to degenerative spinal disease and intervertebral prolapse disc, developed PE. Upon further investigation noted that there was no evidence of DVT nor any intracardiac clots or vegetation. And unlike what has been reported by Van Gent JM et al., our patient has no preceding history of trauma. Another peculiar thing about this case was the initial presentation of the patient which was rather not typical and can be easily missed, especially to those who work in the setting with no CTPA or V/Q scan modality.

In conclusion, this case report served only to share the diagnostic challenge in treating a patient with pulmonary embolism (PE) who presented with a clinical manifestation that mimics severe pneumonia. It is by-all-mean not to recommend the reader to treat all patient with a similar presentation as PE, but to keep PE as one of the possible causes in mind whenever treating a patient with a similar presentation.

Consent

Written informed consent was obtained from the patient to publish this case report.

Acknowledgement

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Case Report

Hidradenitis Suppurativa (Hurley I/II): Serial Excisions with Primary Wound Closure Under Local Anesthesia As Most Adequate Treatment Approach!

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Keywords: Hidradenitis suppurativa; Acne inversa; Treatment; Serial excisions; Local anaesthesia; Recurrence

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Abstract

BACKGROUND: Acne inversa as a chronic inflammatory disorder can be divided into three stages according to Hurley's classification. It affects the axillary and anogenital region predominantly, and its chronic course of development is associated with a major negative impact on quality of life, especially in young patients. We discuss the different types of treatment in patients with acne inversa and the benefits of two-stage surgical treatment by serial excisions with primary wound closure under local anaesthesia.

CASE REPORT: We present a 28-year-old man with hidradenitis suppurativa stage I in the right axillary region and also in the pubic area. The patient is an active smoker. The patient was treated with Rifampicin 2x 300mg / day without any particular effect and preoperatively, systemic therapy with Clindamycin 4x 600mg / day was performed, combined with daily dressings with jodasept ointment for 7 days. The patient was treated through two surgical sessions under local anaesthesia with elliptical excision of the lesions located in the right axillary and the pubic area. Both of the two surgical defects were initially closed with single interrupted sutures. Histological examination of both lesions revealed the presence of suppurative folliculitis.

CONCLUSION: The literature describes various methods for treating acne inversa which include both systemic and local approaches. However, it is considered that drug therapy achieves only a temporary improvement in patients with hidradenitis suppurativa. For this reason, the surgical treatment of acne inversa is indicated as the only curative treatment, especially for recurrent lesions and serial excisions under local anaesthesia, followed by primary wound closure is a valuable treatment for patients with mild to moderate HS (Hurly stage I & II).

Introduction

Acne inversa (AI) (Hidradenitis suppurativa (HS)) is a chronic inflammatory disorder of the apocrine glands, which usually affects the axillary and anogenital region [1]. The main problem with this disease is the chronic course of development with a major negative impact on quality of life and significant co-morbidity in the cases of severe acne inversa (secondary lymphedema, hypertension, diabetes mellitus type II, obesity, metabolic syndrome and others) [1].

According to Hurley's classification, hidradenitis suppurativa can be divided into three clinical stages: Stage I: abscess formation, single or multiple without sinus tracts and scarring; Stage II: recurrent abscesses with sinus tracts and scarring; single or multiple widely separated lesions; Stage III: diffuse or almost diffuse involvement or multiple interconnected tracts and abscesses [2)].

Case Report

We present a 28-year-old man in good overall condition. The patient is an active smoker for 14 years (1 box per day). For several years, an acne inversa has been diagnosed. The patient was hospitalized for the surgical treatment of hidradenitis suppurativa within two surgical sessions. During the dermatological examination in the right axillary region and the pubic area the presence of erythema nodules filled with purulent content, clinically defined as hidradenitis suppurativa stage I, was established (Figure 1a, 1b, and 2a). In the right axillary region,

post-inflammatory hyperpigmentations and a scar of surgical intervention performed in the past were observed (Figure 1a, and 1b).

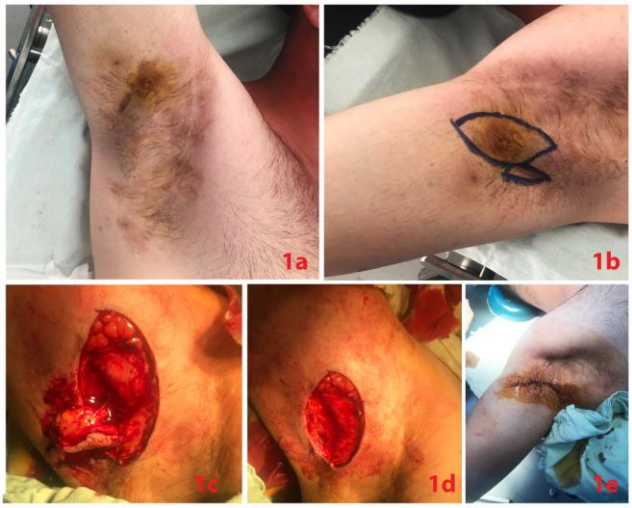

Figure 1: a) Clinical view of acne inversa: erythema nodules in the right axillary region; b) Preoperative finding: outlining the surgical margins; d) Intraoperative view: elliptical excision of the lesion in the right axillary region; e) Postoperative finding: surgical defect closed by single interrupted sutures

Post-inflammatory hyperpigmentations were also observed in the pubic area (Figure 2a). The patient was treated with Rifampicin 2 x 300 mg/day without any particular effect. Preoperatively, systemic therapy with Clindamycin 4 x 600 mg/day was performed, and dressings with jodasept ointment were applied topically for 7 days. In the first operative session, an elliptical excision was performed under local anaesthesia of the lesion located in the right axillary region (Figure 1b, and 1d). The surgical defect was primarily closed with single interrupted sutures (Figure 1e). A sterile bandage with jodasept ointment was made. The subsequent histological examination revealed the presence of suppurative folliculitis. In the second stage, an elliptic excision of erythema nodule in the pubic area was performed again under local anaesthesia with lidocaine (Figure 2b, and 2c). The occurred surgical defect was initially closed with single interrupted sutures (Figure 2d). Histological data was again about hidradenitis suppurativa. A smooth postoperative period was observed without complications and perfect cosmetic results.

Discussion

Treatment of acne inversa includes several basic options-topical options, systemic options and surgical methods, including laser therapy [1], [3]. About drug therapy, long-term antibiotics (clindamycin, rifampicin) or acitretin monotherapy, antiandrogens, tumour necrosis-α inhibitors (TNFαI) may be used, which may lead to improvement in milder cases of AI, but they are not curative [1], [3], [4].

Figure 2: a) Clinical view of acne inversa: erythema nodules in the pubic area; b) and c) Intraoperative view: elliptical excision of the lesion in the pubic area; d) Postoperative finding: surgical defect closed by single interrupted sutures

It is believed that treatment with antibiotics or anti-inflammatory drugs such as prednisone or TNF-alpha-inhibitors usually achieves only temporary improvement and patients with advanced AI treated by combinations of antibiotics need up to 12 months before some of them achieve a temporary remission [1], [4]. As HS has a follicular pathogenesis Lasers and intense pulsed light (IPL) treatment have been found useful by reducing the numbers of hairs in areas with HS [5]. However, according to some authors, as we think, surgery should be introduced earlier in the management of acne inversa [6]. Furthermore, according to the literature, surgical intervention is considered as the only curative treatment for recurrent lesions, and for patients with mild to moderate HS (Hurly stage I & II), local excision followed by primary closure is a valuable treatment with low morbidity and a high patient satisfaction rate [4]. The risk of long-term signs of recurrence, most often near the surgical field, should be noted in surgical excisions under local anaesthesia (mainly wide excisions for Hurley Stages II to III) [7].

In conclusion, based on the results of our patient, we support the thesis that, for mild to moderate cases of hidradenitis suppurativa, serial excisions under local anaesthesia should be considered as first-line therapy.

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Case Report

Successful Endovascular Treatment of a Giant Hepatic Artery Aneurysm with Dual Layer Stents Placement as Flow-Diverting Option: Case Report

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Keywords: Visceral artery aneurysms; hepatic artery aneurysm (HAA); stent; case report

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Abstract

BACKGROUND: Visceral artery aneurysms are rare conditions. The incidence of hepatic artery aneurysm (HAA) accounts for approximately 20% of all splanchnic aneurysms. HAA can become a life-threatening situation because there is a great risk of rupture when it grows more than 2 cm in diameter.

CASE PRESENTATION: In this article, we describe a case of a 54-year-old female patient with large hepatic artery aneurysm which was incidentally founded. From symptoms, she had abdominal discomfort slightly worsening after meals, frequent pain in mesogastrium and reported some unexplained weight loss in the last few months. The lesion was detected by CT examination of the abdomen and pelvis.

CONCLUSION: The aneurysm was successfully treated at our department with the endovascular approach, by covering the aneurysmal sac with two stents, one closed cell and one double-layer micromesh new generation stent as a flow diverting option.

Introduction

Visceral artery aneurysms (VAAs) and pseudoaneurysms (VAPAs) are rare lesions among other vascular diseases with an incidence from 0.01-2%, although the real percentage is not known because of their asymptomatic nature [1]. The incidence of hepatic artery aneurysm (HAA) is a relatively rare condition, accounting for approximately 20% of all splanchnic aneurysms. Within all splanchnic aneurysms, HAAs are not uncommon, being on the second place just after splenic aneurysms [2], [3].

True aneurysms and pseudoaneurysms of the hepatic artery can become life-threatening situation because of their increased risk of rupture [4], [5]. In the majority of the cases patients remain asymptomatic. Thus HAAs are often detected incidentally by routine computed tomography (CT), magnetic resonance (MR) or abdominal ultrasonography (US) examinations. When the diameter of the aneurysm increases, patients tend to present with nonspecific symptoms such as upper abdominal pain, back pain or unexplained discomfort. Rupture of HAA can cause abdominal bleeding, jaundice, hypotensive shock, biliary tract bleeding and sometimes gastroduodenal bleeding. The mortality rate at the time of rupture is reported to be as high as 20%, so if an HAA is detected incidentally an early treatment is desirable. Before the era of endovascular techniques, open surgical aneurysmectomy with or without reconstruction was the conventional treatment for HAA [4]. In recent years, with the advances of new tools and embolisation materials, endovascular treatment, a minimally invasive and safe method has been applied by interventional radiologists to treat HAAs, giving satisfactory results [4], [5].

In this article, we present a case of a giant hepatic artery aneurysm that was successfully treated with 2 stents, one closed cell and one double-layer micromesh stent as a flow diverting option.

Case Presentation

During routine abdominal US examination due to unexplained abdominal discomfort and loss of weight a hypervascular mass in the upper abdomen was found. The lesion was located at the hilum of the liver, between the left and right lobes described as a possible pancreatic mass in the region of the head of the pancreas with consecutive stenosis of the main trunk of portal vein at this level.

Figure 1: Computed tomography CT findings. A) and B) Enhanced axial abdominal CT scans show large, irregular pseudoaneurysm of common hepatic artery with partially thrombosed wall and compression of the portal vein (arrows); C) 3-dimensional VR angiography shows the aneurysm with wide neck arising from vertically oriented CHA

The patient had neither history of previous abdominal trauma nor a family history of the aneurysmal disease. The only fact that she gave was a severe peritoneal infection after childbirth more than 20 years ago. Standard multiphase multidetector computed tomography- MDCT of the upper abdomen was performed, and large aneurysm of the common hepatic artery was detected. Common hepatic artery was with an extremely vertical course and proper hepatic artery, and gastroduodenal arteries were patent.

The aneurysm was approximately 70 mm in its largest diameter with the very big active portion of 50 mm, mural thrombosis and ill-defined borders which can be a radiological sign of pseudoaneurysm. Also, the neck of the aneurysm was very wide.

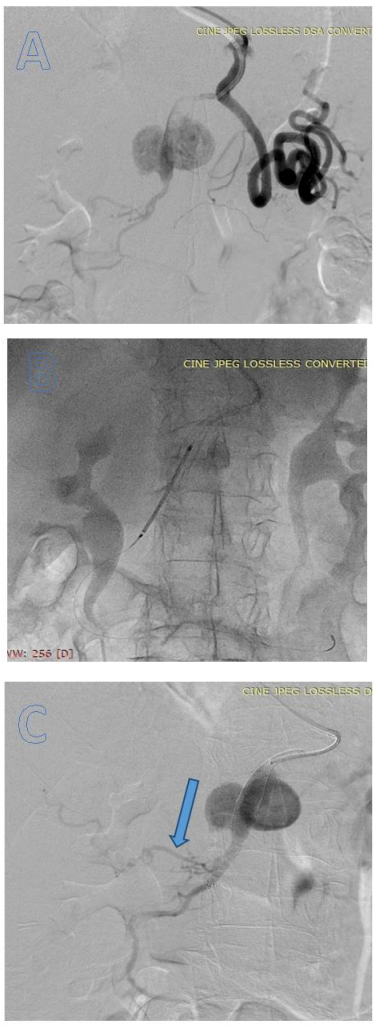

Figure 2: Angiographic findings and treatment. A) Celiac trunk arteriography in 30" left anterior oblique projection shows the aneurysm at the distal site of common hepatic artery; B) Guidewire placed deeply in GDA and 2 carotid stents placed along the neck with complete overstenting; C) Poststenting angiography shows patency of both stents and reduced flow in the aneurysmal sac, patent proper hepatic artery with distal origin(arrow)

There was compression of the main trunk of the portal vein with consecutive stenosis (Figure 1 A, B, C). Our treatment strategy was to go with an

endovascular approach first with stent placement as a flow diverting option because coiling alone seemed to be impossible due it's the irregular wide neck.

Because of the vertical origin of the celiac trunk (Figure 1, C), we decided that a brachial approach was more suitable than femoral in this case. A 90 cm long, MP shaped, 6F guiding sheath (Destination, Terumo) was placed in the celiac trunk through left brachial approach. Manipulation with catheters through a brachial approach was not very easy in this particular case because of high tortuosity of the thoracoabdominal aorta. Dynamic angiography of the celiac trunk was performed which showed the common hepatic artery aneurysm with very turbulent flow in the sac (Figure 2 A).

Proper hepatic artery origin was unusually distal, and both left and right hepatic arteries were gracile. The gastroduodenal artery was with a normal calliper. The wide aneurysm neck was successfully crossed with a guidewire (260 cm Advantage, Terumo, 0.014") and then the wire was placed deeply into the gastroduodenal artery. After that followed placement of 2 closed cell stents across the sac (7 x 40 mm Wallstent, Boston Scientific and 7 x 30 mm, Roadsaver, Terumo), both primarily carotid stents (Figure 2 B, C).

The final angiography showed good stent position and an immediate decrease of flow in the aneurismal sac which led us to think that overstating the aneurysm could be a definite treatment option. All branches of the hepatic and gastroduodenal artery were preserved.

At this point, we decided to stop the procedure and follow the patient after 1-2 months with CT angiography. Hemostasis was achieved by manual compression.

Standard dual antiplatelet therapy was administered; post-stenting, with 100 mg Aspirin and 75 mg Clopidogrel per day for 3 months. The patient was discharged from the hospital the next day without any early complications and with normal laboratory liver function tests. We scheduled her for an abdominal CT angiography after one month. The control CT scan which was performed almost 40 days after the procedure showed complete thrombosis of the aneurysm with patent common hepatic artery and both stents. Good flow in the left and right hepatic arteries and GDA (Figure 3, A and B).

Patient symptoms decreased dramatically since the procedure.

After 3 months we performed DSA of the visceral arteries via right femoral approach as a final invasive control study. 5F SIM 2 catheter was used to cannulate the celiac trunk and angiography performed.

Angiography confirmed the same findings as last CT scan with excellent perfusion in the hepatic artery, complete thrombosis and shrinkage of the aneurysm without signs of residual filling (Figure 3, C).

Figure 3: CT and angiographic findings post-treatment. A) and B) 3D VR angiography and axial contrast-enhanced CT 40 days post-treatment both shows good position of stents and patent CHA, complete thrombosis of the aneurysm; C) DSA three months after procedure performed via right femoral approach SIM 2 catheter used to cannulate celiac trunk ,clearly confirms stents patency,no residual aneurysmal sac filling and good flow in hepatic and splenic arteries

Discussion

The incidence of hepatic artery aneurysm (HAA) is rare, accounting for approximately 20% of all splanchnic aneurysms, being in second place after splenic artery aneurysms [6], [7]. Aetiology of HAAs varies and includes abdominal trauma, infection, atherosclerosis, pancreatitis, necrotising vasculitis and other [1]. HAAs are generally asymptomatic and usually found incidentally

by CT or ultrasonography examinations. Most common symptoms are abdominal pain, obstructive jaundice, intra-abdominal or gastrointestinal haemorrhage.

HAA can become a life-threatening situation when in ruptures with mortality rates according to some reports > 25%. Treatment for HAAs is always needed and recommended when the diameter surpasses 2 cm. High mortality rates up to 50%, made the surgical repair no more preferred treatment, but the endovascular one. In this case, we chose brachial approach due to the vertical origin of the celiac trunk, and we placed two stents (Wallstent and Roadsaver) over the aneurysmal sac to simulate a covered stent and to divert the blood flow, a technique which is already established in the treatment of cerebral aneurysms. Of course, there are dedicated stents-flow diverters for this purpose in the neurovascular field. The Roadsaver stent is characterised by an external self-expanding nitinol layer and an internal micromesh layer, and Wallstent is a traditionally closed cell stent.

Reviewing the literature, we found that Akkan et al. in 2018 [8] reported the use of double-layer micromesh stent (Roadsaver, Terumo) for the treatment of 5 aneurysms (common carotid, vertebral, mesenteric and renal artery) and achieved successful embolisation in all cases only by the stent. Akasaka J. et al. 2018 [9], reported treatment of hepatic artery aneurysm with balloon occlusion technique and coils. In 2013 Sugihara et al. reported a case of proper hepatic artery embolisation with balloon-assisted occlusion of the left, right and proper hepatic artery without liver dysfunction [10]. Stent-assisted coiling, a technique that is already established in the treatment of intracranial aneurysms can also be performed for visceral aneurysms sometimes. There is no similar case in the literature like this particular one with the use of non-covered stents for treatment of HAA. Due to an immediate decrease in flow within the aneurysmal sac, we decided not to proceed with coiling in our case.

After the procedure, there were no signs of liver dysfunction or infarction. The patient was placed on dual antiplatelet therapy with 100 mg Aspirin and 75 mg of Clopidogrel per day for 3 months. Forty days after intervention CT angiography showed complete thrombosis of the aneurysmal sac with both stents completely patent and normal flow in hepatic and gastroduodenal arteries. DSA performed 3 months after embolisation confirmed successful treatment. In conclusion, we can say that embolisation with dual-layer stents alone or together with other closed cell design stent, used as “flow diverters” can be used in the treatment of HAA in selected cases. Of course, this is an off- label use, but potentially it can be an option for some cases. The hypothesis of preserving side branches that arise from the aneurysm or close to it may be an additional potential advantage of dual layer stents over traditional stent-grafts. Of course, this is a case report only and further studies with larger cohorts are needed in future with longer follows of patients.

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A Promising Probiotic Irrigant: An *In Vitro* Study

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Abstract

AIM: The present study aimed to investigate the inhibitory effect of *Lactobacillus rhamnosus* (B-445) as a probiotics irrigant on the growth of *Enterococcus faecalis*.

METHODS: Forty-two extracted single human canal anterior teeth were prepared with rotary instrumentation and sterilised. Teeth were divided into 3 groups according to the type of irrigant, N = 14. Three experimental groups were inoculated with *E. faecalis* and cultured for 21 days before use; Group 1 was 2.5% NaOCl (positive control), Group 2 was saline (negative control), Group 3 was the experimental probiotic irrigant. Paper point sampling of the canals of each group was obtained before irrigation (S1), immediately after irrigation (S2) and after 24 hours (post irrigation samples) (S3) to determine remaining colony forming units for *E. faecalis*. Also, Colony counts for *L. rhamnosus* in Group 3 after immediate irrigation, as well as 24 hours post irrigation, was performed to determine the survival profile of these bacteria in infected root canal with *E. faecalis.*

RESULTS: The NaOCl irrigant group had the lowest mean value of (log 10 CFU/mL) of *E. faecalis* after immediate irrigation and after 24 hrs post irrigation followed by the probiotic group, while the highest mean value was the saline group ($P \leq 0.001$). The survival profile for *L. rhamnosus* in Group 3 after immediate irrigation and post-irrigation were slightly higher than for *E. faecalis* ($P \leq 0.001$).

CONCLUSION: *Lactobacillus rhamnosus* which revealed a potential inhibitory effect on the growth of *Enterococcus faecalis*, could be used as a new natural, safe probiotic irrigant agent.

Introduction

In Endodontics, success depends on chemomechanical removal of bacteria, bacterial endotoxin and debris from the root canal system (Sjögren et al., [1] 1997; Katebzadeh et al., [2] 2000). *Enterococcus faecalis* (*E. faecalis*) is an anaerobic, facultative microorganism that is highly resistant to conventional chemomechanical preparation and usually found in failing root canal cases [3]. *E. faecalis* is a part of the human flora and appears to be highly resistant to the medicaments used in treatment [4]. Also, *E. faecalis* can form a surface attached microbial community known as a biofilm. This allows it to be protected from host defences as well as systemic treatment.

Different methods of combatting *E. faecalis* were explored through the use of different irrigants [5]. Sodium hypochlorite has remained a popular root canal irrigant because of its ability to dissolve necrotic tissue and organic remnants and its antimicrobial activity. On the other hand, sodium hypochlorite has certain adverse effects such as ineffectiveness against some microorganisms when used at low concentration, corrosion of endodontic instruments and lack of differentiation between necrotic and vital tissues when in contact with apical and periapical tissues [6]. “Out of the box” treatments were evaluated for use against *E. faecalis* such as the use of passion fruit juice as an endodontic irrigant as well as the use of Photomedicine and laser surgery [7]. In endodontics because of the cytotoxic reactions of irrigants used and their inability to eliminate bacteria from dentinal tubules, the trend of recent medicine attends to use biologic medication.

Probiotics have been shown to promote health in the intestines and the oral cavity through the consumption of certain yoghurts and lozenges. The mechanisms of action of these probiotics include the productions of bacteriocin-like inhibitory substances and the altering of the local pH, competing for nutrients, forming physical barriers and stimulating the immune response [8]. The World Health Organization recognises probiotics as a significant avenue of health preservation if current antibiotics become useless due to the development of bacterial resistance. The purpose of this study was shifting the established paradigm of endodontic treatment from eliminating all bacteria from the canal system to focusing on the elimination of the problematic bacteria through introducing probiotics.

Therefore, this study aimed to explore the probiotics as new irrigant agent that would probably have an inhibitory effect against the growth of Enterococcus faecalis.

Material and Methods

Forty-two freshly extracted single-rooted human teeth with fully formed apices were collected for this study. All teeth were examined under a stereomicroscope (Technical, Germany) to exclude teeth with any existing defects or cracks. Teeth were thoroughly washed under running water and immersed in 5.25% Sodium Hypochlorite (Clorox, Egyptian company for household products, Egypt) for 30 minutes to remove soft tissues on the root surface. Teeth were gently cleaned with ultrasonic scaler to remove any remaining soft tissues or calculus. The teeth were stored in saline solution until instrumentation. Before canal instrumentation, the crown of each tooth was sectioned at cementoenamel junction using a sectional disc (Toolouip, Germany) mounted in a low-speed handpiece (NSK, Japan) under water coolant. This was done for ease of manipulation and to standardise the tooth length at 15 mm.

A size 15 k-type file (Mani, Japan) was used to assure patency of the canal and the apical foramen. The working length was established by subtracting 1 mm from the length obtained when the file tip just appeared at the apical foramen. Cleaning and shaping were performed by using rotary Ni-Ti Protaper system that was driven by an endodontic handpiece of 1:16 gear reduction and powered by X-Smart electric motor (Speed 400 rpm and torque setting 5 Ncm, Dentsply, Japan). All canals were enlarged to the same file size F5. During instrumentation, irrigation was performed with 3 ml of 5.25% sodium hypochlorite after each file. After completion of the instrumentation, the smear layer was removed with 3 ml 17% ethylene-diamine-tetra-acetic acid solution (EDTA-Q.A-Dent, Egypt) by using a disposable plastic syringe. This was followed by irrigation with distilled water. Teeth were then autoclaved at 121°C for 30 minutes. After that, the apical foramen of each tooth was sealed by using Putty silicone for impression, and the root surface was sealed with varnish after which the samples were handled under strict aseptic measures.

Lactobacillus rhamnosus (*L. rhamnosus* B-445) was selected as an example of probiotic species and provided in lyophilised form by the Northern Regional Research Laboratory, Illinois, USA. Bacterial activation was carried out through inoculation of *L. rhamnosus* in De Man, Rogosa, Sharpe (MRS) broth medium (Oxide, Basingstoke, Uk) followed by anaerobic incubation (BBL Gas Pak, Becton Dickinson, Cockeysville, MD, USA) at 37°C for 48 hrs. The experimental probiotic irrigant was formulated through inoculation 5 ml of *L. rhamnosus* ($2x10^8$ CFU/ml) in 10 ml of sterile distal water.

A Total of Forty-two samples were randomly assigned to three main groups according to the type of irrigant was applied (N = 14); Group1 was 2.5% NaOCl (positive control), Group2 was saline (negative control), Group3 was the experimental probiotic irrigant.

The three groups were infected by using a 24-hours pure culture suspension of *Enterococcus faecalis* (*E. faecalis*; ATCC 19434) in Brain heart infusion broth medium (BHI broth medium; 53286 Sigma-Aldrich, USA). In each previously sterilised samples 1 x 10^{-8} CFU/mL suspension of *E. faecalis* (determined by serial dilution and plating) was inoculated in root canals and incubated at 37°C and 95% humidity for 21 days. The BHI broth was removed from the canal by gentle aspiration and renewed every 48 hours.

At the end of 21 days infection with *E. faecalis*, sterile paper point pre-irrigation sample was taken from each specimen. The sterile paper points were placed in the canals to the apical foramen and were moved circumferentially along the walls of the canal. Each paper point was left for 1 minute in the canal to collect the pre-irrigation samples from the canals of all teeth. Paper points were placed in airtight sterile vials containing 5 ml of nutrient broth for transportation to the microbiological laboratory for culturing procedure. After that, each group was irrigated immediately with 5 mL of its specific irrigant, and all irrigants were delivered by using a side-vented 30-gauge needle (Max-I-Probe; Dentsply). The immediate paper point irrigation sample was obtained from each root canal of each tested groups. Again the experiment was repeated after 24 hours irrigation of root canals (post irrigation samples) and storage in an incubator at 37°C.

In colony counting method, each pre-irrigation, immediate, post irrigation paper points samples were placed in airtight sterile vials containing 5 ml of saline and vortexed at the highest speed for 3

consecutive intervals of 15 seconds each. This was followed by serial dilutions 1:10, 1:100, 1:1000 and 1:10000. A volume of 50 µl was taken from each dilution using the automatic micropipette and was plated on BHI agar in triplicate. All plates were incubated at 37°C and 95% humidity for 24 hours, after which the colonies for *E. faecalis* on BHI agar plates were enumerated, and the most countable plates from pre-irrigation, immediate, post irrigation paper point's samples of each group were selected. If any sample was too numerous to count, it was diluted, plated, and counted.

Colony counts for *E. faecalis* and *L. rhamnosus* in Group 3 after immediate irrigation as well as 24 hours post irrigation was performed to determine the survival profile of these bacteria. Colony count method was carried out as mentioned above through serial dilutions followed by a volume of 50 µl was taken from each dilution and plated on BHI agar in triplicate. Also, the duplicated paper point samples were plated on MRS agar in triplicate. The experiment was repeated after 24hrs post irrigation. All plates were incubated at 37°C and 95% humidity for 24 hours, after which both the colonies for *E. faecalis* on BHI agar plates and *L. rhamnosus* on MRS agar plates were enumerated and the most countable plates for each microorganism were selected.

Data presented as means and standard deviation (SD) values. One-way repeated measures ANOVA used to compare between the follow-up within each Irrigant solution. One Way ANOVA used to compare different irrigant solutions within each time.

For the Survival profile of microorganisms (Log10 CFU/mL); independent t-test used to compare between different type of bacteria, Dependent t-test used to compare between follow-up periods for each irrigant. The significance level was set at P ≤ 0.05 and was performed with IBM® SPSS® (SPSS Inc., IBM Corporation, NY, USA) Statistics Version 24 for Windows.

Results

Regarding the colony counting method, the results showed that there was a statistically significant difference in the mean (log10 CFU/mL) of *E. faecalis* between the three irrigants groups immediately and after 24 hrs post irrigation (P ≤ 0.001). While there was no statistically significant difference in the mean (Log10 CFU/mL) of *E. faecalis* between tested groups pre-irrigation (P = 0.001).

Table 1: Mean and SD for the Log10 CFU of (*E. faecalis*) for different irrigants

		Groups						p-value
		Saline		NaOCl		Probiotic		
		Mean	SD	Mean	SD	Mean	SD	
Log10 CFU (E. faecalis)	Pre-irrigation	7.04^{Ab}	0.03	7.20^{Aa}	0.07	7.09^{Ab}	0.09	0.001*
	Immediately	4.95^{Ba}	0.11	0.00^{Cc}	0.00	4.40^{Bb}	0.10	≤ 0.001*
	After 24 hours	6.89^{Aa}	0.32	2.20^{Bc}	2.01	4.04^{Bb}	0.16	≤ 0.001*
p-value		≤ 0.001*		≤ 0.001*		≤ 0.001*		

Means with same Capital Superscript within each Column are not significantly different at p=0.05; Means with same Small Superscript within each Row are not significantly different at p = 0.05; NS = Non-significant; * = Significant.

The previous results revealed that the NaOCl irrigant group had the lowest mean value of (log10 CFU/mL) of *E. faecalis* after immediate irrigation (0.00) and after 24 hrs post irrigation (2.20 ± 2.01) followed by the probiotic group with mean value (4.95 ± 0.11) for immediate irrigation and (4.40 ± 0.10) for post irrigation, while the highest mean value immediately and after 24 hrs post irrigation was the saline group (4.95 ± 0.11, 6.89 ± 0.32) respectively (Table 1, Figure 1).

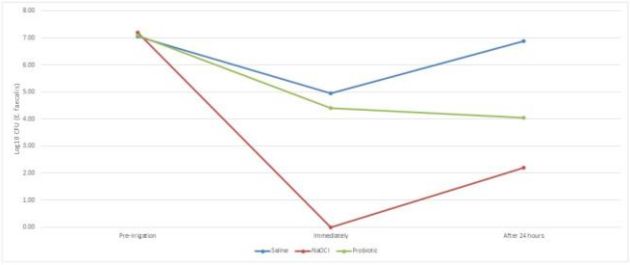

Figure 1: Line chart showing the mean Log10 CFU (E. faecalis) for follow-up periods for each group

Concerning the survival profile for both bacterial growth *E. faecalis* and *L. rhamnosus* in Group 3, the results showed that the mean colony count (Log10 CFU/mL) for *L. rhamnosus* after immediate irrigation and post-irrigation (6.41 ± 0.06, 4.77 ± 0.44) was slightly higher than for *E. faecalis* (4.40 ± 0.10, 4.04 ± 0.16 respectively) (Table 2, Figure 2).

Table 2: Mean and SD for the Survival profile of microorganisms (Log10 CFU/mL)

		Bacterial				p-value
		E. faecalis		Lactobacillus Rhamnosus (L.rhamnosus)		
		Mean	SD	Mean	SD	
Survival profile of microorganisms (Log10 CFU/mL)	Immediately	4.40	0.10	6.41	0.06	≤ 0.001*
	After 24 hours	4.04	0.16	4.77	0.44	≤ 0.001*
p-value		≤ 0.001*		≤ 0.001*		

*NS = Non-significant; * = Significant.*

Discussion

As *Enterococcus faecalis* (*E. faecalis*) became more and more commonly isolated in failing root canal cases, it became the focus of interest in the medical and dental communities. Hence, *E. faecalis*

was selected in our due to its ability to form a surface attached microbial community known as a biofilm. This allows it to be protected from host defences as well as systemic treatment [9], [10]. Additionally, It is the most common bacterial species found in persistent infections due to its relatively small cell diameter [11], [12]. The present study was designed to prepare a standardised tooth length of 15 mm, and the root canals were enlarged to F5 Protaper at the working length as this could attribute to create an adequate environment for bacterial growth.

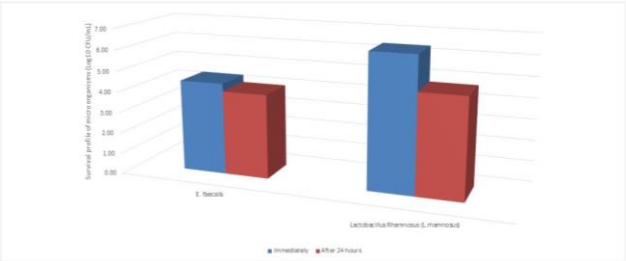

Figure 2: Histogram showing the mean Survival profile of microorganisms (Log10 CFU/mL)

Based on previous studies, the counting of CFUs for bacterial growth that was expressed in log CFU/mL could be used in the present study to evaluate the effects of the tested irrigants on *E. faecalis* growth [13], [14], [15], [16]. Several endodontic irrigants have been used for decontamination, but the most common was sodium hypochlorite (NaOCL) for its antimicrobial activity [17], [18], [19] and its ability of dissolution of organic tissues [20], [21]. However, due to its high toxicity [22], new irrigants were introduced. Therefore, this study was trying to explore a potential new way of treating endodontic infections which would probably be as effective or more and at the same time less irritating to the tissues than sodium hypochlorite. The use of probiotics has not yet been evaluated for use against *E. faecalis*. So, the present study was proposed to test known probiotics as an experimental irrigant in the presence of the established post-endodontic treatment disease bacteria *E. faecalis* and observe what the effect of the probiotics would have against *E. faecalis.*

Regarding colony counting method, the results showed that *E. faecalis* colonies statistically significant decrease from (7.09 log CFU/mL) pre-irrigation to (4.04 log CFU/mL) after 24 hrs post irrigation with probiotic irrigant. The latter result was supported by [23] who documented that killing of pathogens through probiotic could be due to one of the mechanisms of probiotic action such as; the production of Bacteriocin-like inhibitory substance (BLIS) and acids/peroxides along with altering the local environments pH. However, (BLIS) is used to describe bacterial products that have inhibitory effects.

For the results that concerning the survival profile for both bacterial growth *E. faecalis* and *L. rhamnosus* in Group 3, it revealed that the mean colony count for *L. rhamnosus* after immediate irrigation and post-irrigation was statistically significantly higher than for *E.faecalis* ($P \leq 0.001$). A more sophisticated method of observing the effects of the probiotic agents against *E. faecalis* could be conducted. The method of “Deferred Antagonism” which was demonstrated by [24] could explain the latter result that been used to evaluate the antibacterial properties of the normal flora of the nasopharynx. This deferred antagonism test which previously mentioned could be used to evaluate our hypothesis presented in a more controlled fashion as probiotic might compete for *E. faecalis* on nutrition and adhesion site which contribute to decreasing the survival profile of *E. faecalis* as found in the latter result of the present study. *E. faecalis* appears to be highly resistant to the medicaments used in the treatment and is one of the few microorganisms shown in vitro to be resistant to calcium hydroxide, due to its proton pump [4]. It is also able to survive as a single organism without the support of other bacteria. The fact that *E. faecalis* is not normally present or is present in very low numbers in untreated root canal cases implies that it can enter the canal, survive the antibacterial treatment and then persist after obturation [25]. Therefore, this deferred antagonism test which previously mentioned could be used to evaluate our hypothesis presented in a more controlled fashion as probiotic might compete for *E. faecalis* on nutrition and adhesion site which contribute to decreasing the survival profile of *E. faecalis* as found in the latter result of the present study.

In conclusion, within the limitation of this study; *Lactobacillus rhamnosus* which revealed a potential inhibitory effect on the growth of *Enterococcus faecalis*, could be used as a new natural, safe probiotic irrigant agent. Further studies using other known probiotics should be considered.

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Effects of Different Parameters of Diode Laser on Acceleration of Orthodontic Tooth Movement and Its Effect on Relapse: An Experimental Animal Study

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Abstract

AIM: Recent studies have demonstrated that low-level laser therapy (LLLT) can accelerate orthodontic tooth movement. However, there is still controversy about the optimum parameters that can cause acceleration. The present study was designed to examine two different doses of LLLT in the acceleration of orthodontic tooth movement and their effect on relapse.

METHODS: An orthodontic appliance was designed to induce tooth movement on lower incisors of rabbits. The in-al-as diode laser was used to radiate different groups of rabbits according to a specific protocol. The amount of tooth movement was measured for 21 days, and then the orthodontic appliance was removed, and the relapse was measured till day 28.

RESULTS: The amount of tooth movement was significantly greater in the group with irradiation of low dose in comparison with the high dose group and the control group. The relapse was greatest in the group of low dose irradiation and least in the control group.

CONCLUSION: The findings suggest that LLLT with a low dose accelerate the orthodontic tooth movement while LLLT with a high dose was not able to have the same effect. LLLT had a reverse effect on relapse tendency; an increase in the relapse tendency was seen with low dose irradiation.

Introduction

The principle of orthodontics is to apply light pressure on the tooth for a long time, so that bone remodelling occurs and the tooth starts to move [1]. So, the orthodontic tooth movement normally takes a long time. This is usually non-desirable from the patient`s point of view and may also have adverse side-effects as the development of white spot lesions and root resorption. Moreover, the prolonged time of retention after the orthodontic treatment is another concern for the patient.

Previous methods for stimulation of Orthodontic tooth movement (OTM) have been investigated such as drug injections, electric stimulation and ultrasound application. One promising way to enhance tooth movements is the use of low-level laser therapy (LLLT). Many researches support the claim of increased osteoblastic activity following LLLT in vivo [2], [3], [4], [5] and in vitro [6], [7], [8], [9], [10], [11], [12], [13].

Other researchers suggest that bone resorption is the rate-limiting step in tooth movement. So, any procedure which potentiates osteoclastic activity can accelerate the rate of orthodontic tooth

movement. Recent studies have indicated enhanced osteoclastic activity after low-level laser therapy in vivo and in vitro [14].

Low-level laser therapy (LLLT) has shown to affect many biological processes in the body and has many beneficial effects including effects on fibroblasts, chondral proliferation, collagen synthesis, nerve regeneration.

However, there is a great controversy in the literature concerning the effective parameters of (LLLT) that produce an acceleration of tooth movement [14] and there are many recommendations for further studies concerning the effective doses. Thus, the primary aim of the present study is to investigate the effect of different parameters of (LLLT) on orthodontic tooth movement.

A long period of retention is necessary to prevent early relapse. Although the reason for early relapse is not fully clear, bone regeneration after orthodontic treatment may affect the post-treatment relapse. It would be beneficial therefore to accelerate bone formation to prevent relapse to abbreviate the retention period [15]. This study will also investigate the effect of (LLLT) on the relapse of orthodontically moved teeth.

Material and Methods

The material and methods of this study were designed according to the ARRIVE guidelines for reporting In Vivo Experiments for animal research. All the checklist items were accurately followed.

Ethical statement

The ethical committee of the National Research Centre in Egypt has approved this research according to the protocol submitted in November 2015, and the approval had the reference number 15083.

Number of experimental and control groups

Forty-five rabbits were randomly divided into three groups according to the treatment modality: Group I, non- irradiation control group (n = 15); Group II, High dose irradiation group (n = 15); Group III, Low dose irradiation group (n = 15). The orthodontic appliance is fixed on the right and left lower incisors of each rabbit so the number of incisors in each group (n = 30).

Timeline of the experiment

The appliance was activated immediately before its fixation on the lower incisors of each rabbit, and a photograph (Fuji film, Finepix, 4X, 8.2 megapixels, Macro mode) is taken before the fixation of the appliance and immediately after the fixation. A scale is added to the photo so that the distance between the incisors can be measured accurately using computer software.

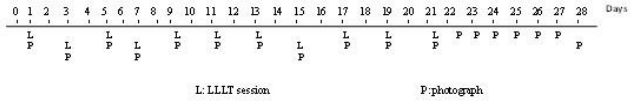

Figure 1: The timeline diagram

Then each rabbit was photographed at days 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 and the rabbits of group II and III undergone the laser sessions according to the specific protocol of each group at the same days while group I didn't receive any laser.

On day 21 all the appliances were removed, and the teeth were allowed to relapse, and they were photographed every day until day 28 which is the last day of the experiment.

The Orthodontic appliance

An orthodontic appliance was constructed for each rabbit which consisted of:

1. Two bands formed of stainless steel band material with 0.18-inch width and 0.005-inch thickness.

2. Omega loop constructed of stainless steel round orthodontic wire (0.012 inches) welded to the bands.

The bands were custom made on a lower incisor extracted from a rabbit so that all the bands had the same size. All the omega loops were standardised in size and shape and were welded at the middle of each band. (Height of omega loop is 0.4 mm approximately) (Figure 2).

Figure 2: The orthodontic appliance

The omega loops were placed on a grid and activated so that the range of movement was about

3mm. And the force was calibrated with a Stress and Tension gauge (1 pcs/set, Lotus Global Co., Ltd.) to 30 g so that it would be 15 g on each incisor [16], [17], [18]. The orthodontic appliance was activated once just before fixing it in the rabbit's mouth with no further activation during the experiment.

Animal sedation

Intramuscular injection of a mixture of Xylazine 20 mg\kg and Ketamine 50 mg\kg was used to anaesthetize each rabbit.

a) b) c)

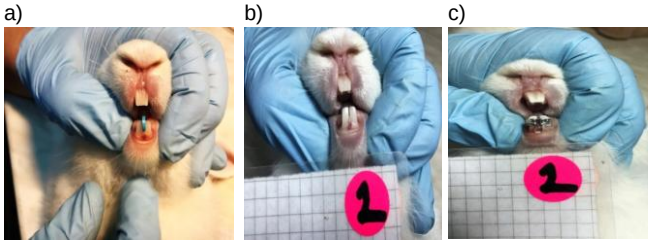

Figure 3: a) Separator between incisors to open space for appliance; b) After separation; c) Orthodontic appliance fixed

After anaesthetising each rabbit, the orthodontic appliance was fixed on the lower incisors of each rabbit using glass ionomer cement (Medicem; Promedica; Dental Material GmbH; Domagkstr.31; 24537 NeumÜnster; Germany) (Figures 3a, 3b and 3c).

Soft Laser application:

Group I (non-irradiation): Did not receive any laser throughout the experiment

Group II (High dose): Received laser with a high dose every other day from day 1 till day 21

Group III (Low dose): Received laser with a low dose every other day from day 1 till day 21

Figure 4: Laser machine Biolase Epic X

The soft laser was applied using Biolase (Epic X) (Figure 6) with active medium InGaAs (indium gallium arsenide) Semi-conductor diode using a probe with spot size area 0.002 cm2. The laser was applied distal to the distal incisal edge of the lower incisor, parallel to the long axis of the tooth and in contact with the distal periodontal pocket of the lower incisor [18] (Figure 4).

Figure 5: LLLT application distal to the right and left incisors

The laser irradiation was done on the lower right, and lower left incisor of each rabbit.

1. Wavelength: 940 ± 10 nm.

2. Power density: Group II (High dose): 250 W/cm^2; Group III (low dose): 250 W/cm^2.

3. Energy density: Group II (High dose): 5000 J/cm^2; Group III (Low dose): 2500 J/cm^2

4. Joules/session: Group II (high dose): 10 J; Group III (low dose): 5 J.

Total energy does: **Group II:** At day 3: 20 J; At day 7: 40 J; At day 15: 80 J; At day 21: 110 J; **Group III:** At day 3: 10 J; At day 7: 20 JAt day 15: 40 J; At day 21: 55 J.

5. Continuous wave

Experimental animals

The study included 45 white New Zealand rabbits. The rabbits used were males about 14 weeks and the average weight of 2 kg ± 100 gm. They were provided by the Animal House of the National Research Centre.

The orthodontic appliance was fixed on the lower incisors of each rabbit.

Housing and husbandry

All animals were housed in a 12-h light/dark environment at a constant temperature of 23 C and fed a standard pellet diet with tap water and libitum. The rabbits were kept in individualised cages, and

special caretakers, as well as the veterinarian specialist of the animal house, is monitoring the animals around the clock, in regular shifts.

Sample size determination

Based on the assumption of normal distribution and equal variances of the amount of movement variable, assumed standard deviation of 1.2 and means equal 9.25 and 7.84 for the null and alternative hypothesis, Using ANOVA test, the required sample size is 12 rabbits in each group for a confidence level 95%, at alpha = 0.05 and power = 0.80[10]. To consider for drop- out, 15 rabbits per group was advised. So the total number of rabbits in the three groups was 45 rabbits.

Allocating animals to different groups

The animals were randomly divided using the online computer program Random sequence generator; random.org, into three groups. Groups I (non-irradiation control group), Group II (High dose), and Group.

(Low dose) Served as experimental groups containing 15 rabbits each. This randomisation was performed by a researcher who didn`t participate in the rest of the study.

In the first group, the orthodontic appliance was fixed on lower incisors for orthodontic tooth movement. In the second and third groups, the orthodontic appliance was fixed on the lower incisors, and laser application was made with different doses.

Blinding

Blinding of the operator was not possible due to the nature of the experiment, but blinding was done with the assessor doing the postoperative assessment.

Assessment of the rate of tooth movement

Each rabbit was photographed using a digital camera (Fuji film, Finepix, 4X, 8.2 megapixel, Macro mode) and a scale (Figure 6a), before the fixation of the appliance, immediately after fixation and at days 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 22, 23, 24, 25, 26, 27 and 28.

Figure 6: a) Photo was taken using a digital camera and a scale; Image converted into actual scale image; c) Lines showing the exact measurement of the space

The photographs were assessed using computer software (Corel Draw X6, Corel photopaint X6, Autocad) to exactly measure the distance moved by each tooth on these days (Figures 6b and 6c).

Statistical methods

Statistical analysis was done between groups comparing different variables using ANOVA test SPSS version 20. A p-value < 0.05 was accepted as statistically significant.

Results

Forty-five white New Zealand rabbits were included in this study. The study period lasted for twenty-eight days. During this period, the animals received veterinary care and were housed under the same environmental conditions. The animals lost some of their body weight during the first week then weight regain started during the second week and continued until the end of the experiment.

The rate of tooth movement

Days from zero to 3

The mean distances during the first 3 days were: (0.58 ± 0.39 mm, 0.62 ± 0.35 mm, 0.51 ± 0.28) for groups I, II, and III respectively.

The rate of tooth movement was highest in group II (high dose) than group I (no laser) then group III (low dose). Statistically, there was no significant difference (p > 0.05) between the three groups during the first 3 days (Figure 7).

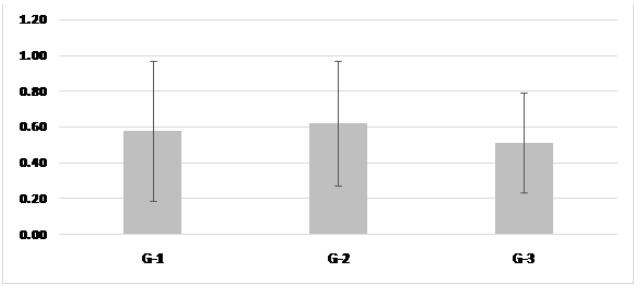

Figure 7: Mean (SD) of the distance from 0 to 3 days

Days from 3 to 7

The mean distances from day 3 to 7 were: (1.06 ± 0 mm, 1.02 ± 0.03 mm, 1.11 ± 0.11) for groups I, II and III respectively.

The rate of tooth movement was highest in group III then the group I than group II. Statistically, there was no significant difference (p>0.05) (Figure 8).

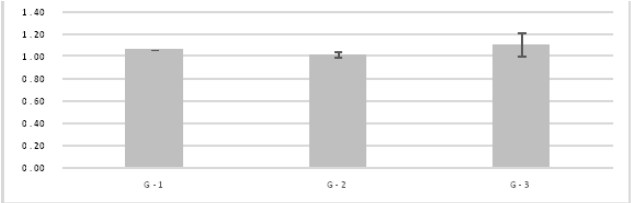

Figure 8: Mean (SD) of the distance from 3 to 7 days

Days from 7 to 15

The mean distances from day 7 to 15 were: (1.15 ± 0.07 mm, 1.09 ± 0.05 mm, 1.47 ± 0.13) for groups I, II and III respectively.

The rate of tooth movement was highest in group III then I then II. Statistically, there is no significant difference between group I and II (p1 > 0.05 (0.67). There is a significant difference between group II and III (p2 < 0.05 (0.001). There is a significant difference between group I and III p3 < 0.05 (0.002) (Figure 9).

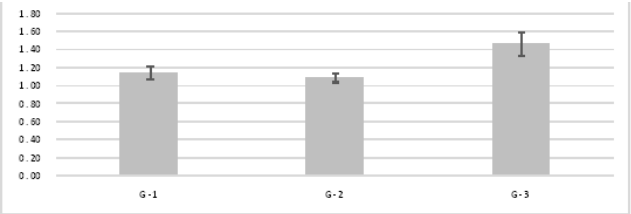

Figure 9: Mean (SD) of the distance from 7 to 15 days

Days from 15 to 21

The mean distances from day 15 to 21 were: (1.10 ± 0.05 mm, 1.11 ± 0.08 mm, 1.40 ± 0.07 mm) for groups I, II and III respectively.

The rate of tooth movement was highest in group III then II then I. Statistically, there is no significant difference between group I and II (p1 > 0.05 (0.93). There is a significant difference between groups II and III (p2 < 0.5(0.0004). There is a significant difference between groups I and III (p3 < 0.5 (0.0003) (Figure 10).

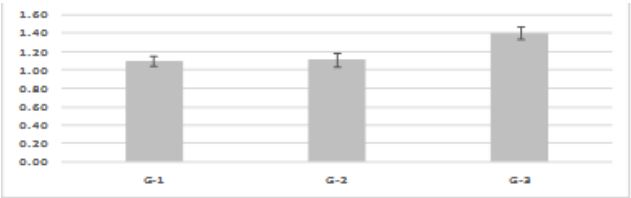

Figure 10: Mean (SD) of the distance from 15 to 21 days

Relapse: (After appliance removal)

Days from 21 to 28

The mean relapse distance from day 21 to 28 was: Group I: -0.41 mm, Group II: -0.6 mm, Group III: -0.74 mm.

Mean percentage relapse: Group I: 46%, Group II: 46.88%, Group III: 56% (Figure 11).

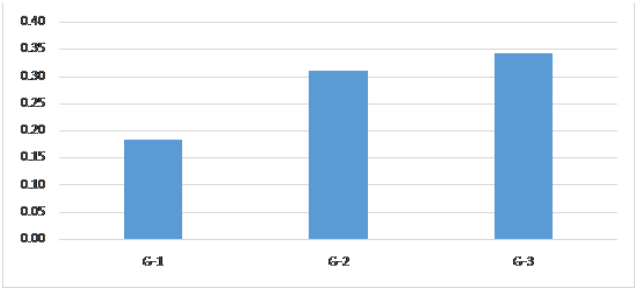

Figure 11: Coefficient of variation between the three groups during relapse

Discussion

It is beneficial to decrease the duration of orthodontic treatment as reflectively this can decrease the side effects as root resorption and white spots. Furthermore, it can increase the patient's compliance and satisfaction.

Orthodontic literature presents different methods to stimulate bone remodelling such as, drug injections like prostaglandin [19] and osteocalcin [20] that could be associated with pain and discomfort [21]. Electric stimulation [22], and ultrasound application are other methods [23] which need sophisticated apparatus and demands applications for the long term to achieve its therapeutic effects [24].

LLLT, a non-invasive and simple method has been recently investigated in literature for accelerating tooth movement and shows promising results [25]. There are only a few experimental studies concerning the optimal laser dose for biostimulation of orthodontic tooth movement.

The present study examined the biological response to LLLT on the speed of orthodontic tooth movement using two different dosages. Recently, a systemic review compared 11 studies of LLLT in animal models of OTM rates and concluded that different laser protocols could increase OTM rates [14], with total energy per tooth ranging from 0.5 J to 204.5 J and energy density ranging from 0.05 J/cm^2 to 4821.4 J/cm^2. In our study, two doses were examined which were the high dose given to group II (10 J per tooth and energy density 5000 J/cm^2), whereas the low dose given to group III was (5 J per tooth and 2500 J/cm^2).

Rabbits were used as the experimental animals in this study. Rabbits were chosen as they have lower central incisors of a good size so that the orthodontic appliance can be installed easily. Also, the structure of their oral tissues has close similarity to

humans. Amongst various strains, New Zealand white strains of rabbits are commonly being used for research activities. These strains are less aggressive and have fewer health problems as compared with other breeds [26]. They are also genetically closer to humans than rodents.

The fixed orthodontic appliance used in this study was designed to produce a reciprocal force on the right and left lower first incisors, producing distal tipping movement of both incisors. This was by Altan 2012 [16] and Eldakroury 1998 [17].

The reciprocal force used on the lower incisors of each rabbit was 30 cN force so that it would be 15 cN on each incisor and this was by Altan 2012 [16] and Ren 2004 [18]. The split-mouth technique was not used in this study to avoid the systemic effect of phototherapy [27] that may give misleading results.

The activation of the orthodontic appliance was done once at the beginning of the study (just before the appliance fixation), and no further activation was done throughout the study period, and this meant a decrease in the amount of force throughout the study period.

Indium Aluminum Arsenide (In-Al-As) semiconductor diode laser (940 nm) was used in this study as currently, most commercially available lasers are characterized by a wavelength located in the near infrared band (790-1064 nm) very versatile - although most studied lasers are those that emit the visible red spectrum (635-685 nm) [14].

In a penetration test done by (Li-Fang Hsu 2018) [27] showed that at this longer wavelength 970 nm infrared light provides deep penetration, without being completely absorbed by 2 mm bone slice before reaching periodontal ligament and deeper bone tissue.

The results of the rate of tooth movement were compared among the three groups for the period of tooth movement and then the relapse period.

The tooth movement period lasted for 21 days then the orthodontic appliances were removed and the teeth allowed to relapse till day 28 which is the last day of the experiment.

Although there is a widespread acceptance for the use of LLLT in the clinical setting, there is still a lack of scientific evidence and insufficient guidelines in the use of the most effective parameters for different laser treatments. The laser biostimulation effect depends on laser units and doses that are continuously changed to reach the optimal treatment parameters in both in-vitro and in-vivo conditions [28]. Karu conducted a series of experiments that demonstrated that biologic stimulation followed dose dependency [29]. This was exhibited through bell-shaped curves where every laser wavelength produced a maximum stimulation at a specific dose. Doses greater and less than the optimal resulted in less stimulation. These bell-shaped curves are indicative of bio-stimulation's dose dependency. Application dose measures the amount of energy applied at any one given treatment and is measured in joules (J). To determine the application dose, or energy dose, delivered during one treatment session, the power output is multiplied by the time of application. Also, the treatment dose, or total energy dose, is an additive or accumulative value combining the energy delivered over the entire length of treatment. If a patient receives an application dose of 1 J every week for eight weeks, their total treatment dose would be 8 J [25].

The present findings suggest that low-level laser therapy with low dose significantly increased the rate of orthodontic tooth movement compared to the LLLT with a high dose and the control group that did not receive any laser therapy.

Twenty-one studies have been reported on how LLLT affects the rate of orthodontic tooth movement. Nine of them were clinical studies [30], [31], [32], [33], [34], [35], [36], [37], [38] and twelve were on experimental animals [15], [16], [25], [39], [40], [41], [21], [27], [42], [43], [44], [45].

However, making a direct comparison between studies is complicated by some factors. The varying experimental designs, varying laser parameters including laser wavelength, power output, mode of delivery, power density, energy density, the number of applications, the time separating each application, and the length of the experiment. In addition to the different animal models used as cats, rats, rabbits, dogs, and humans. So in our discussion, we will discuss our results in comparison with the twenty-one studies according to the total energy dose given in periods 3, 7, 15 and 22 days.

Experimental animal studies

Days from 0 to 3

Group II received 20 J total energy dose while group III received 10 J.

There was no significant difference between the 3 groups at this period, although the rate of tooth movement was highest in group II (High dose) than group I (control) then group III (low dose). These results agreed with Marquezan 2010 [42] and Rowan 2010 [25] and disagreed with Kawasaki and Shimizu 2000 [21], Duan 2012 [39], Yamaguchi 2010 [45] and Fujita 2008 [40] who found a significant increase in the rate of OTM. The reason for this disagreement may be because the total energy dose (20 J and 10 J) was still insufficient to cause biostimulation in comparison to these studies where the total energy dose was (162 J and 216 J) except for the study of Duan 2012 [39] who had 6.48 J total dose and still caused significant increase.

Days from 3 to 7

Group II has received total energy dose 40 J while group III 20 J.

Statistically, there was no significant difference in the rate of OTM between the three groups although it was highest in group III then I then II. These results agreed with Altan 2012 [16], Marquezan 2010 [42] and Rowan 2010 [25].

These results disagreed with Yamaguchi 2010 [45], Fujita 2008 [14] who found a significant increase in the rate of OTM (1.3 fold) at day 7 with total energy dose 432 J. And also disagreed with Duan 2012 [39] who found significant increase at day 7 with 6.46 J.

Days from 7 to 15

Group II has received total energy dose 80 J while group III has received 40 J.

Statistically, there is a significant increase in the rate of OTM III (low dose) and group II (High dose) and between group III and the control group, while there is no significant difference between group III and the control group although the rate was higher in the control group. These results agreed with Duan 2012 [39], Shirazi 2013 [44] and Kawasaki 2000 [21]. These results disagreed with Seifi 2007 [43], Rowan 2010 [25] and Li-Fang Hsu 2018 [27] who found no significant difference at the same period of the experiment.

Days from 15 to 21

Group II has received total energy dose 110 J while group III has received 55 J.

Statistically, there is a significant increase in the rate of OTM between group III and group II and the control group. There is no significant difference between group II and the control group. These results agreed with Goulart 2006 [41] and disagreed with Rowan 2010 [25].

These disagreements may be related to many factors, the different animal models used as rats, rabbits and dogs, the different study designs used, some tried to move molars, and others worked on upper incisors or lower incisors, the different designs of the orthodontic appliance used and the different amount of forces used. Moreover, the different laser parameters used as different wavelengths, energy densities, power densities, total energy dose, time of application and points of application, the duration of the experiment, the frequency of LLLT sessions and the different methods of measurements used.

Clinical Studies

Our results agreed with Gene 2012 [34] where the patient received at day 3: 4 J, at day 7: 6 J, at day 14: 8 J and at day 21: 10 J. And he found significant increase at days: 7, 14, 21, 28 and 35. Also agreed with Youssef 2008 [38] who found a significant increase at day 21 with total energy dose 32 J.

For the following studies, the study lasted for more than one month, and the measurements were done after 1 month. Our results agreed with Dosh-Mehta 2011 [33], Sousa 2011 [37], Cruz 2004 [31] and Dominguez 2013 [32] who all found a significant increase with total energy dose 320 J, 18 J, 8 J, 648 J per month respectively. And disagreed with Kansal 2014 [35], Altan 2014 [30] where all didn't find any significant change with total energy dose 69 J, 8.4 J and 30 J per month respectively.

The second part of our study aimed to investigate the effect of LLLT on the rate of relapse of orthodontically moved teeth. It started at day 21 of the experiment. The orthodontic appliance was removed at day 21, and the teeth were allowed to relapse till day 28 of the experiment. The amount of relapse was measured every day.

In group I the relapse was rapid at the first day after removal of the appliance, while in group II the relapse was rapid at the third day of appliance removal and in group III the relapse was rapid at day 2 and more rapid at day 3 of appliance removal.

At the end of the relapse period; by day 28 the mean percentage of relapse was measured as Group I (46%), Group II (46.88%) and Group III (56%). These results agreed with Kim 2010 [3] and disagreed with Franzen 2014 [46].

Relatively few studies have been carried out on the effect of LLLT on orthodontic relapse. Kim et al. studied the effects of LLLT on relapse and retention of rat molars and concluded that LLLT administered with retention facilitated collagen synthesis contributing to faster repair of damaged PDL tissue and better retention, while irradiation performed without retention in place would lead to an increased rate of relapse due to increased catabolic metabolism of collagen. So, LLLT appears to decrease orthodontic relapse but cannot inhibit it, so the use of retainers is mandatory after removal of the orthodontic appliance with the help of LLLT may decrease the period required for retention.

In conclusion, acceleration of orthodontic tooth movement using LLLT is dose-dependent, very low dose or very high dose will not cause acceleration**.** LLLT with the optimum dose can be used during retention to decrease the retention period, but a retainer should be used. Otherwise the relapse tendency would increase.

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The Efficacy of Acceptance and Commitment Therapy (ACT) Matrix on Depression and Psychological Capital of the Patients with Irritable Bowel Syndrome

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Abstract

BACKGROUND: Irritable bowel syndrome (IBS) is one of the most common functional gastrointestinal disorders, worldwide. Psychological disorders are common among patients with IBS.

AIM: This study aims to investigate the efficacy of acceptance and commitment therapy (ACT) matrix on depression and psychological capital of patients with IBS.

MATERIAL AND METHODS: In a quasi-experimental study, a total number of 30 patients with IBS were selected using convenience sampling. Those patients who meet the inclusion criteria were randomly assigned to control and experimental groups (15 patients in each group). Data were collected using the Beck Depression Inventory (BDI) and the Psychological Capital Questionnaire (PCQ). The experimental group was subjected to the acceptance and commitment therapy (ACT) amtrix, but the control group do not receive this treatment. Questionnaires were completed before (pre-test) and after (post-test) the intervention by patients in two groups. All patients in two groups responded to the questionnaires and returned them to the researcher. Data were analyzed using chi-square test, independent t-test, analysis of covariance (ANCOVA) and multivariate analysis of covariance (MANCOVA).

RESULTS: Analyzing the data showed that there were significant differences regarding depression and psychological capital between experimental and control group, before and after the study ($p < 0.05$).

CONCLUSION: Using ACT matrix is a useful modality to improve the depression and psychological capital among patients with IBS.

Introduction

Irritable bowel syndrome (IBS) is one of the most common functional gastrointestinal disorders which is characterised by bloating, abdominal pain or discomfort and mixed symptoms of constipation or diarrhoea [1], [2]. Other digestive symptoms of this disorder consist of abdominal distension, early satiety and dyspepsia. Non-digestive symptoms of this disorder consist of a chronic and migraine headache, menstrual pains, chronic fatigue, backache, agitation and depression [3]. There are several hypotheses about the aetiology of this disorder, but almost none of them is accepted by all. Also, none of the therapeutic approaches to this disorder such as drug therapy, diet therapy and psychological therapy were completely successful or ineffective [4]. In one hand, uncertainties regarding the best treatment approach to IBS and on the other hand, an expansion the field of psychology of health attract the attention of researchers with a psychological approach. It has been demonstrated that psychological disorders don't cause IBS directly, but they affect the feeling of pain, duration and influence of suffering IBS [5]. Depression, as a psychological disorder, is one of the common comorbid diseases in patients with IBS. Depression is a state of mind that is associated with a reduction of self-esteem, sense of incompetence and wrong perception of oneself [6]. Depression is the natural response of human beings to the pressures of life. Depression is considered abnormal only when it is not fit with the incident that has happened or continues beyond the point which is a starting point for most people. Many environmental stressing stimuli such as financial problems, new jobs, legal issues, retirement

or other changes can contribute to the development of depression [7], [8], [9]. Previous studies showed that there is a relationship between depression and IBS. The results of a study which were done in 3153 IBS patients showed that 30.5% of the patients with IBS suffered from depression [10]. Also, it was shown that there is a significant relationship between depression and occurring of symptoms and their severity in patients with IBS [11]. Also, it was shown that anxiety is significantly higher in people suffering IBS, in comparison to healthy people [10], [11].

On the other hand, psychological capital is one of the components in health psychology. Psychological capital is one of the indicators of positivism psychology which is defined with characteristics such as the person believing in his/her abilities to achieve success, being diligent in pursuing goals, creating positive perceptions about himself/herself and tolerating the problems [12]. Thus, psychological capital consists of self-perception, having a plan to achieve success and tolerance against problems [13]. Psychological capital is a complex and congruent structure that includes four perceptual-cognitive components that are hope, self-efficiency, resiliency and optimism [14]. These components give meaning to the life of the person, stabilise the person's efforts to change the stressing situations and prepare him/her to enter the action through a cooperative and evaluative process [15], [16], [17]. It is believed that there is a relationship between each of psychological capital components and physical diseases [17], [18].

The relatively high prevalence of IBS and high co-occurrence of this disorder with psychological problems have encouraged the researchers to evaluate the effectiveness of various psychological therapies on this disorder. Acceptance and commitment therapy (ACT) is one of the newfound psychological therapies in Iran which has become very beneficial recently. This context-based therapy, emphasises on building a valuable and meaningful life instead of reducing and omitting symptoms such as annoying thoughts and emotions. About one decade ago, Hayes introduced the third generation of behavioural therapies as a representative of the therapies formed in last 20 years and emphasised on the role of acceptance and conscious attention for making a change [2], [18].

Acceptance denotes to the active inclination to experience emotions, physical senses and thoughts without trying to control or change them [19]. In ACT, the main intention is to form psychological flexibility [17]; that is creating the ability to choose an action which is more appropriate among various options. The flexibility formed by acceptance and mindfulness helps the patient to react less to his/her physical senses [18]. The final goal of ACT is to help the person gain a purposive sense of life, in a way that includes his/her life values, and then working towards those values [20]. Different researches have studied the ACT in different conditions. Wetherell et Al. evaluate the efficacy of ACT and behavioural-cognitive therapy in patients with chronic pain. The findings showed that pain, depression and pain induced anxiety had been reduced significantly in ACT group [19]. Nowadays, ACT is known as an effective therapy for depression and anxiety which are both common in IBS patients. Also, it has been previously shown that ACT has a positive effect on quality of life and mental state of cancer patients [8].

Hence, with regards to the high co-occurrence of IBS with psychological problems such as depression, and the probable effect of ACT matrix on this group of patients, and lack of published study to asees the efficacy of this therapy on IBS patients in Iran, the current study aims to evaluate the efficacy of ACT matrix on depression and psychological capital of the patients with IBS.

Material and Methods

This study was performed using the quasi-experimental method and pretest-posttest design with the control group. Intervention group underwent ACT matrix. The statistical population of this study consisted of all of male and female IBS patients who were introduced to the researcher in three clinics in Tehran by gastroenterology specialist, from October 2016 to March 2017. Using convenience sampling, among 57 patients who were introduced to the researcher, 40 met the inclusion criteria. These patients were randomly assigned to intervention and control groups. The inclusion criteria were a definite diagnosis of IBS by a gastroenterologist, having the minimum literacy for reading and writing to fill the questionnaires and inclination of the person to take part in the study. The exclusion criteria included the history of attending ACT-related courses and also more than 2 session's absence in the held courses. The data were collected using the short-form of Beck Depression Questionnaire and Psychological Capital Questionnaire (PCQ), which were completed by the patients. The intervention group, underwent the ACT, in addition to the common pharmacotherapy; but the control group did not receive this therapy and only underwent the common pharmacotherapies. Both groups have received and completed the related questionnaires before the therapy (pretest).

Beck Depression Inventory (BDI) short form: This questionnaire has 13 self-report propositions that express specific symptoms of depression. Each proposition of this questionnaire consists of a four-optioned scale, the domain of which is sorted from 0 to 3. Maximum and minimum of its scores are respectively 39 and 0. This questionnaire has been developed to measure different typology of depression (emotional, cognitive, motivational and physiological).

Cronbach's alpha and split-half coefficients were 0.89 and 0.82 respectively for the whole questionnaire [22]. Psychological Capital Questionnaire (PCQ) of Lothans has 24 items which consist of 4 components: hope, tolerance, optimism and self-efficiency [23]. The items of the questionnaire are in the form of six-point Likert scale (completely disagree to agree completely). To get the psychological capital score, first the score of each component was calculated, and the some of the scores of these components was considered as the total score of psychological capital. The reliability of this Questionaire was previously evaluated and confirmed by Cronbach's alpha 0.85 [24].

The ACT matrix package consists of 6 principles (cognitive defusion, acceptance, being present, observing self, values clarification and committed action) which were presented to the IBS patients during six 90 minutes' sessions. In the first session, the psychological flexibility viewpoint was introduced by drawing a matrix. This matrix consisted of 2 intersecting vertical and horizontal lines which form a four-room. At the top of the vertical line, realities are put, which are sensible through five senses. At the bottom of the vertical line, the mental experiences are put. These mental experiences can be the person's negative experiences and worries which are written at the left bottom section of the matrix. But in the other section which is the right bottom, the values (important things or people) of the person are put. In the first stage, it is necessary for the person to understand the difference between reality and mental experience and distinguish them from each other (cognitive defusion). The second difference which a person should pay attention to is his/her behaviors, so that he/she can determine if his/her behaviour is in regards to getting away from the worries or approaching the values. Paying attention to these two differences leads to awareness, and awareness prepares the ground for a change. The second session is focusing on finding awareness on the efficacy of thoughts and behaviours. In a way that to what extent the set of thoughts, feelings, physical senses and behaviour in last week's practices have reduced the person's worries and led him/her towards the values. Usually, people are stuck and in a false cycle of negative mentality along with worry, and eventually are unable to move towards the values. In the third session, the attention-stealing hooks that concern the person's mind were explained, and the patients learnt to get free of their continual mental-harassment. In the fourth session, the verbal Aikido was practised with the patients. Aikido is an oriental martial art which uses the opponent's force to overcome him/her, without any serious harm done to them. This technique is, in fact, a kind of peace and friendship, without anyone getting hurt. This method is useful for facing worries, without avoidance or fighting. In the fifth session, self-compassion was taught, so that the person can have compassion on and respect himself/herself. In the sixth session, the viewpoint selection force inhibition was taught, so that it would lead to preventing mental conflicts in the future. At the end of each session, some homework was given, and they were evaluated and investigated at the beginning of the next session [27]. After the end of the intervention, both groups completed the related questionnaires (posttest).

Results

The results of the Shapiro-Wilk Test, which was used to assess the normality of the distribution of each variable, clearly indicates the normality of data. In this study, the sample consisted of 30 IBS patients, which 19 of them were females (63.3%) and 11 of them were males (36.7%). The participants' age ranged from19 to 60 years old, and the mean of their ages was 31.93. Seventeen of these patients were single, 10 were married, and 3 have lost/divorced their spouses.

Table 1: Gastrointestinal symptoms in the two studied groups

Symptom		Groups		Total
		Experimental	Control	
Diarrhoea	Frequency	5	4	9
	Percent	16.7	13.3	30
Constipation	Frequency	4	5	9
	Percent	13.3	16.7	30
Both	Frequency	6	6	12
	Percent	20	20	40
Total	Frequency	15	15	30
	Percent	50	50	100

There was not a significant statistical difference between the two studied groups from the aspect of age, gender and marital status ($P > 0.05$). From the aspect of suffering diarrhoea or constipation or both of these conditions, there was not a significant statistical difference between the two groups of patients ($p > 0.05$) (Table 1).

Table 2: Depression raw scores in two studied groups

Variables		Groups	
		Experimental	Control
Depression scores (pretest)	Mean	28.93	27.86
	Standard Deviation	7.86	8.62
Depression scores (posttest)	Mean	4.46	31.53
	Standard Deviation	3.20	4.82

The mean and standard deviation of raw scores of depression and psychological capital is presented in Tables 2 and 3.

Table 3: Psychological capital in the two studied groups

Variables	Experimental Group		Control Group	
	Mean	Standard Deviation	Mean	Standard Deviation
Psychological capital pretest scores	32.86	8.08	10.26	14.34
Psychological capital posttest scores	104.20	12.10	38.13	13.27

Data analysis showed that there is a significant difference between the modified mean of depression and psychological capital scores in the

control and experimental groups in the posttest stage. This means that ACT matrix based on acceptance and commitment is effective on depression and psychological capital of the patients suffering IBS. As is observed in table 4, the group effect is significant for psychological capital and depression scores ($\eta 2 = 0.959$ and $p < 0.0001$).

Table 4: Results of multivariate analysis of covariance (MANCOVA)

Change resources	Wilks Lambda	F-statistic	The degree of freedom 1	The degree of freedom 2	Significance	η2
Psychological capital	0.70	2.20	4	21	0.10	0.29
Depression	0.91	0.50	4	21	0.73	0.08
Group	0.04	123.18	4	21	< 0.0001	0.95

This significance confirms that significant general changes have happened in psychological capital and depression scores. It is observed in covariance's' part that pretests of none of the variables had any effect on posttest score.

Table 5: Results of analysis of covariance (ANCOVA) in MANCOVA

Component	Change resources	Sum of squares	Degree of freedom	Mean of squares	F-statistics	significance	η2
Depression	Group	4392.51	1	4392.48	2.34	< 0.0001	0.91
	Error	419.44	24	17.48	51		
Psychological capital	Group	2785.22	1	2785.24	2.19	< 0.0001	0.90
	Error	277.54	24	115.48	41		

The effect size is calculated to be 0.959 for change resources which is a strong amount and shows the effects of therapy (Table 5).

Table 6: Modified means and paired comparison of the mean of depression scores

Experimental group	Control group	Mean difference	Standard deviation	significance
4.195	31.80	-27.61	1.742	< 0.0001

Data analysis using follow-up test showed that treatment based on acceptance and commitment has had a significant positive effect on depression and psychological capital in IBS patients and have caused them to heal (Tables 6 and 7).

Table 7: Modified means and paired comparison of the mean of psychological scores (Toki's follow-up)

Experimental group	Control group	Mean difference	Standard deviation	Significance
105.92	36.40	69.52	4.47	< 0.0001

Discussion

The research background shows that the obtained results in this study are consistent with results of Mohammadi et al., [24], Baghban et al., [28], Blanchard et al., [1], Lotsson et al., [29] and Gillanders et al., [30]. Depression is one of the psychological factors which is characterised by a loss of sense of control over emotion and behaviour, and the person suffers a lot [31]. These changes almost always cause the experience of negative feelings, disruption of interpersonal, social and occupational functions of the patient and are followed by continual physiological stimulation of the nervous system [32]. The bowel nervous system is very sensitive to the changes mental status, in a way that negative emotions can cause changes in movement activities of the bowel and create bowel symptoms such as symptoms occur in IBS [33]. To explain the effectiveness of the matrix of ACT on the reduction of depression in IBS patients, several possibilities can be considered. ACT can target the avoidance pattern. During the interventions, reduction of the experimental avoidances are considered as a mediator of change in the symptoms of depression [7]. Avoidance is defined as an effort to escape depressing thoughts and memories, which are brought to the depressed person's awareness [20]. In this treatment, the accepted practices and discussions about values and goals of the person cause reduction of depression in IBS patients. This treatment teaches people how to let go of their avoidance beliefs and accept them instead of trying to control them [17]. Even though experimental avoidances have reducing effects on the unpleasant experience in short-term, but have many destructive effects in long-term and can lead to psychological inflexibility and functional deficiency. In this treatment, persons are encouraged to evaluate their behaviour based on the success of the strategies they use. Successful strategies are usually the ones that help people act based on their values. Creating flexibility through a focus on field related to thoughts and emotions leads to desirable behaviours and also leads to a reduction of credibility in the depressed [34].

Additionally, one of the reasons for depression is the negative cognitions related to one's abilities [31]. In the ACT, participants are guided towards this direction so that they see themselves detached from their thoughts and emotions. This, in turn, causes a reformation in negative cognitions [32].

In ACT, increasing of psychological flexibility is the mediator for healing the psychological problems such as depression. The depressed patients are usually looking to bring reasons for their depression, which leads to increasing of rumination, and they criticise their experience negatively [33]. In ACT, the change and relationship reformation of the person with his/her feelings are strengthened through mental awareness practices, self-regard and awareness of body conditions. In this regard, efforts are made to increase acceptance of thoughts, beliefs, emotions and sentimental, physical perceptions [8]. Thus, the patients with IBS observe their depressing thoughts and detach themselves from the ruminations caused by depression. Hence, the content of thoughts is experienced regardless of threatening aspects and

ruminations that are being experienced currently. Also, the acceptance component in ACT makes the IBS patients able to accept their unpleasant internal experiences, and this causes the experiences to seem less threatening, and the symptoms are alleviated in the person. On the other hand, there is the psychological capital which can be explained in this way; it has newly found its place in Iranian researches and has been considered by the researchers of health psychology. Psychological capital consists of 4 components: self-efficacy, hope, tolerance and optimism. Regarding self-efficacy component, Masuda et al., [34] evaluate the effect of cognitive defusion on self-directed negative thoughts, which an example of these thoughts are the thoughts related to self-efficacy.

The results showed a significant reduction in the amount of these thoughts by using cognitive-defusion techniques. It is probable that self-knowledge based on the values and rebuilding them has been effective on reducing the negative self-directed thoughts. As Dikelmente claims, persons who are less sure of their abilities in doing behaviours such as compatibility with new situations, sense of efficacy, usefulness, etc., may get stuck in pre-contemplation or unawareness stage and feel desperate about the probability of change. For the frustrated person to reach the stage of decision and preparation to stage of action and maintenance from the current stage, the sense of self-efficacy and self-confidence should be strengthened in him/her. One of the important stages in most treatments, especially ACT, is assigned to the taking responsibly of treatment by the participants [35]. Self-efficacy is in a way the answer to this question that "Will I be able to do this action or not?" The ACT can increase the self-efficacy in person by assigning suitable homework and working on the values. The ACT can be effective on increasing of self-efficacy and taking the responsibility of treatment by the participants through emphasising on flexibility, clarification of values, and discussion on the matter that change is possible and also doing various practices successfully.

Thus, in this study, it seems that ACT matrix helped the IBS patients to reflect their past experiences happily and gain mental stability. Hope is another component of psychological capital. Hope is a cognitive process but has emotional consequences. Thus, hope is not a passive emotion that appears in only dark moments of life; but is a cognitive process which people actively try to reach their goals through it. Hence, hope is a process through which the person determines his/her goals, makes approaches to reach them and creates and maintains the necessary motivation to perform these approaches [36].

It should be considered that patients who are hopeful towards healing and health, get treated faster due to positive beliefs because it causes positive physiological changes to take place in the human. ACT causes the person to find his/her life rich, meaningful and significant, which leads to enhancement of daily functions, mental and psychological health. Thus, ACT matrix can increase the hope in IBS patients.

Another component of psychological capital is tolerance. The tolerant people have a different viewpoint towards stressing condition, do not surrender to it and do not become passive, but they fight against the conditions and confront it, they do not avoid it [37]. This necessitates an effort to know the environmental conditions, and ACT helps people to give their entire attention to the existing conditions without judging through increasing mental awareness; because some of the factors that affect the mental health of IBS patients negatively are emotions like guilt, deficiency and frustration [38]. Using ACT can increase efficacy and tolerance as well as reducing mental symptoms, because of some hidden mechanism existing in it such as acceptance, an increase of awareness, sensitivity elimination, being present and observation without judging. The therapists, instead of focusing on changing the content of the patient's problem, help the participants in accepting and having the inclination to experiencing thoughts and emotions and emphasise on reforming the attention and awareness of the referee by using mental awareness techniques. This approach is a behavioural therapy which used the skills of mental awareness, acceptance and defusion-knowing to increase psychological flexibility. ACT caused the increasing of the referee's ability to form a relationship with their experiment at present and based on what is possible for them at present, and this leads to increase of tolerance in IBS patients. In the end, the optimism component is introduced. ACT can give the persons the ability to pay attention to the current experiences optimistically, through having freedom in their choices and the responsibility they accept to do a job. Pessimism causes the person to experience negative feeling and emotions. On the opposite, is pessimism that expands the positive feelings and emotions. To explain the results of this study, it can point out that ACT matrix teaches people that we all are suffering in life. All of our thoughts, emotions, physical signs, urges and memories are clean sufferings, but if we value them too much and highlight them, they create unclean sufferings which are unpleasant for us. Thus, by defusing and not doing cognitive mix-ups, one can live happily and be optimistic.

Optimism is not the superficial positivity, but the optimists are problem solvers and plan for their activities, and then act according to them [39]. Hence, it can be said that optimistic people act more successfully in solving their problems and in their marriage, and do not take a passive position towards the problems and have a more positive viewpoint towards others [40], [41].

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Losing Years of Human Life in Heavy Polluted Cities in Macedonia

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Abstract

INTRODUCTION: The urban air pollution will deteriorate globally, by 2050 outdoor particulate air pollution and ground-level ozone is projected to become the top cause of environmentally related deaths worldwide.

AIM: To assess the burden of diseases due to particulate air pollution and health benefits form the pollution reduction policies.

METHODS: Environmental burden of disease methodology has been applied. Environmental data for population exposure, total years of life lost from all causes, and relevant concentration-response functions have been used in estimation.

RESULTS: The estimated disease burden from all causes (excl. external) is 30,256 YLL (19,436-40,625 95% CI) in Skopje Region, and 10,343 YLL (6,224-14,785 95% CI) in Tetovo. In terms of cardiopulmonary mortality, the estimated disease burden is 9,282/100,000 in Tetovo, in the Skopje Region 3,784/100,000 respectively. Annually in Tetovo 1,645 years of healthy life are lost, while in the Skopje Region 3,936 due to lung cancer premature mortality. The estimated health gain is significant, for the three selected health outcomes if EU limit values are reached, 41-42% of the estimated burden in Skopje Region will be eliminated, and 74-77% in Tetovo.

CONCLUSION: the estimated impact of particulate air pollution on mortality is significant and not negligible. The same applies to the health and well-being of the population if the EU or WHO limit levels are reached.

Introduction

The Global Burden of Diseases Study (GBD; 2015) estimates that pollution-related disease was responsible for 9 million premature deaths in 2015, presenting 16% of total global mortality. Out of it, 4.2 million (3.7–4.8) premature deaths are attributable to ambient particulate air pollution, whereas 2.9 (2.2–3.6) due to indoor air pollution [1]. According to the World Health Organization (WHO), in the European region of the WHO, the exposure of suspended particles is responsible for reducing every person's life expectancy by an average of almost a year, mainly due to the increased risk of cardiovascular, respiratory diseases and lung cancer [2].

Air pollution emphasizes the inequalities, disproportionately affecting mostly the poorest and vulnerable group's particularly children. Children are affected even at low-dose exposures to pollutants during the windows of vulnerability in utero and early infancy as well. Disease caused by pollution is most prevalent among minorities and the marginalised [3]. Air pollution often serves as an indicator of the economically sustainable development of a country because sources of air pollution are also the main

producers of substances that are major climate modifiers such as carbon dioxide, black carbon etc. Sustainable Development Goal (SDG 11) refers to the target to make cities inclusive, safe, resilient and sustainable. Annual mean levels of fine particulate matter in cities indicates the sustainable urban development. Also, SDG3 refers to the provision of healthy living and well-being for all. Mortality rate attributed to household and ambient air pollution is a direct indicator of the progress toward this target [4]. Achieving the pollution control, the health and well-being benefits will be significant, climate change pace will be slowed down, and social justice promoted.

However, the urban air pollution will deteriorate globally, by 2050 outdoor particulate air pollution and ground-level ozone is projected to become the top cause of environmentally related deaths worldwide [5]. Primarily, at risk are people in urban areas. 30% of the urban population in EU are exposed to air pollution levels exceeding EU standards and 98% citizens living in European cities that exceed more stringent WHO guidelines for air quality [6], [7]. There is no evidence of a safety thresholds of PM concentration [8], and EU standards are not sufficiently stringent to protect human health [9].

The large scientific evidence for health impact of air pollution is already built, starting with the Harvard Six-Cities study [9], ACS (American Cancer Society) study [10] from the beginning of 1990s that have established a permanent positive association between long-term exposure to air pollution and total cardiovascular mortality (mainly due to coronary artery disease), and continuing with the European studies such as the Dutch study and following ones [11]. Approximately 71% of the total burden of disease account to non-communicable diseases (NCDs) [12]. Deaths related to cardiovascular disease (ischaemic heart disease, stroke), chronic obstructive pulmonary disease and lung cancer poses a great burden to the health systems and societies in general [13], [14], [15], [16], [17], [18].

Still, there is insufficient evidence from the countries of the Former Yugoslav republics and region in general. Converting lost years of life and disabilities to DALYs (or Disability-adjusted Life Years), the World Bank Study in 2011 estimated health effects of air pollution in Macedonia that represent an annual economic cost of €253 million or 3.2 per cent of GDP. Savings from reducing the levels of particulate matters in the country will be significant, 151 million per year will be saved, and over 800 deaths and thousands of days in lost productivity will be avoided [19]. The latest study indicates 1205 attributable deaths (819-1538 95% CI) due to $PM_{2.5}$ exposures in the Skopje Region, and 265 attributable deaths (187-327 95% CI) in Tetovo, the most polluted cities in the country [20].

Based on the decade long monitoring of air pollution concentration, nowadays it is well known that urban air pollution is a significant problem in the country. The mountainous terrain and meteorological conditions cause extra challenges for the national air pollution management. Many sources have been identified: traffic (poorly maintained vehicle fleet), domestic heating, energy production relies mostly on poor-quality lignite in old thermal power plants, the absence of proper waste management etc. [21]. Based on the broad scientific evidence and knowledge, is expected that current exposures on particulate air pollution will lead to further deterioration of the health status of population, causing a variety of adverse and harmful health effects and great economic losses due to increased need of health care services, increased medication use, absenteeism from work and school, restricted activity and losses due to premature deaths and active years of life.

Material and Methods

Aiming to assess the burden of diseases due to particulate air pollution and most importantly to assess the health benefits form implementation of effective and consistent measures and policies that would reduce the concentration of the stressors to the EU limit values (EU LV), we have applied a method for assessing disease burden that was firstly developed in the 1980s by WHO and other HIA studies [22], [23], [24], [25], using a YLL (Years of Life Lost) as a selected metrics for quantification of the disease burden. The data for the YLL due to premature mortality are obtained from the WHO Global Health Estimates (WHO GHE, 2016) database. Environmental data for population exposure of $PM_{2.5}$ obtained from the National Air Quality Monitoring Network run by Ministry of Environment and Physical Planning (MoEPP) have been used, and relevant Concentration-response Functions (CRFs) have been applied.

The epidemiological concept *Population Attributable Fraction (PAF)* assess "the proportional reduction in the outcome incidence if all risk factors of interest were simultaneously eliminated from the target population" [26], [27]. It has been applied and calculated using the following formula:

Equation 1. Calculation of PAF

$$PAF = \frac{f\ x\ (RR - 1)}{f\ x\ (RR - 1) + 1}$$

Then we assess the environmental burden of disease due to $PM_{2.5}$ exposures using the following formula:

Equation 2. Calculation of environmental burden of disease

$$EBoD = PAF\ x\ BoD$$

Eventually, we have estimated the years of life lost that could be avoided if the EU LV is achieved by the implementation of the consistent policies and actions, using EU LV for $PM_{2.5}$ as a cut-off value (25 $\mu g/m^3$).

Basic demographic data

Distribution of the population by the age groups and sex were obtained from the State Statistical Office (SSO). In the study, we have used the Population Projections of the SSO for 2017 [28] (Table 1). The total population of the selected cities (Skopje Region and Tetovo) is considered as a target population. The City of Skopje is a capital city and separate unit of the local self-government, consisting 10 urban municipalities, while the Skopje Region comprised seven more rural municipalities. Regarding Tetovo, the total population of the urban and 19 rural municipalities is included in this study [29]. We have selected those two cities due to the highest population exposures on particulate air pollution that exceed EU significantly and WHO limit values particularly during the cold season.

Table 1: Distribution of the population by the age groups in the selected cities

Age groups	Skopje region		Tetovo		Macedonia	
	#	% of total population	#	% of total population	#	% of total population
0-24	190,266	30.3	30,469	33.0	605,255	29.2
25-64	345,637	55.1	52,634	57.1	1.186,597	57.2
65 and over	91,595	14.6	9,098	9.9	283,256	13.6
Unknown	60	0.01	15	0.0	193	0.01
TOTAL	627,558		92,216		2.075,301	

Source: MAKStat database. SSO, 2018.

Environmental data

We have used an annual mean concentration of PM_{10} and $PM_{2.5}$ from six monitoring stations for the Skopje Region and one located in Tetovo. For calculation of the national mean value of $PM_{2.5}$, the average of all seventeen monitoring stations has been used. To avoid the variation of the concentration of selected stressor due to some partial policies or meteorological factors, we used the five-years average for the period 2012-2016 (Table 2).

Table 2: Annual mean concentration of PM_{10} for the period 2012-2016

		PM_{10} ($\mu g/m^3$)					
	Monitoring point	2012	2013	2014	2015	2016	5-year average
Skopje region	Karpos (UB), Centar (UT), Gazi Baba (SUB), Lisice (I) Rektorat (UT) and Miladinovci (R)	86.8	73.4	68.7	66.6	65.7	72.2
Tetovo	Тетово (UT)	133.3	140.9	133.8	147.2	96.9	130.4
MKD		83.9	68.8	62.5	63.8	54.0	66.6

UB-urban background; UT-urban traffic; SUB-suburban background; I-industrial, UT-urban traffic; R-refinery*; Source: Macedonian Environmental Information Center (MEIC), MoEPP. 2017.*

Only two monitoring stations in the Skopje Region continuously monitors the concentration of $PM_{2.5}$. The annual mean concentration of $PM_{2.5}$ in Tetovo and the national average is based on calculation (Table 3). HRAPIE study (Health risk of air pollution in Europe) formula is applied, whereas $PM_{2.5}$ are calculated as a fraction of the total mass of measured PM_{10} ($PM_{2.5}/PM_{10}$ ratio = 0.65)

Table 3: Annual mean concentration of $PM_{2.5}$ for the period 2012-2016

	$PM_{2.5}$ ($\mu g/m^3$)					5-year average
	2012	2013	2014	2015	2016	
Skopje region	51.4	40.7	39.2	44.4	33.0	41.8
Tetovo[e]	86.7	91.6	87.0	95.7	63.0	81.7
MKD[e]	56.8	54.0	44.7	45.2	38.2	45.9

e (estimation); Source: Macedonian Environmental Information Center (MEIC), MoEPP. 2017.*

Years of Life Lost, from the WHO database (GHE; 2016)

The data for the YLL due to premature mortality (for the age group 30 and over) have been obtained from the WHO Global Health Estimates (GHE; 2016) database. Three health outcomes were selected for the burden quantification and assessment of the health benefit: All-cause (natural) mortality (excluding external causes); cardiopulmonary and lung cancer mortality.

Concentration-response functions (CRFs)

Health Impact Assessment approach, in general, apply CRFs mostly expressed in terms of Relative Risk (RR) for a unit change in concentration, estimated in the numerous epidemiological studies. These functions are used to link the exposure to air pollution and scenarios for changes in the air quality of the population at risk, as well as its basic health status [30]. The CRFs used in this paper for the selected health outcomes with Confidence Intervals of 95% (95% CI) are shown in Table 4.

Table 4: Summary of the relative risk (RR, 95% CI) applied in the study for $PM_{2.5}$ as a selected stressor

Stressor	Health endpoint	Age group	ICD10 code	Unit of exposure	Point estimate of CRF	LCL (95% CI)	UCL (95% CI)	Reference(s)
$PM_{2.5}$	All-cause (natural) mortality	Adult (>30 yr)	A00-R99	10 $\mu g/m^3$	1.062	1.040	1.083	Heroux et al. 2015 [31] Meta-analysis of 13 cohort studies: Hoek et al. (2013) [32]
$PM_{2.5}$	Cardiopulmonary mortality	Adult (>30 yr)	I00-I99 J00-J99	10 $\mu g/m^3$	1.0797	1.0202	1.1401	Pope et al., 2002 [33], WHO, 2006a
$PM_{2.5}$	Lung cancer	Adult (>30 yr)	C32-C34	10 $\mu g/m^3$	1.1267	1.0407	1.2190	Pope et al., 2002 [33] WHO, 2006a

Results

Using YLL due to premature mortality as a selected metrics in this study, we aimed to assess not only the environmental burden of disease due to $PM_{2.5}$ but estimation of the benefits of implementation of targeted public health policies and actions and policies in other sectors was from our great

importance. The ultimate objective will be a creation of the targeted public health policies and policies in the other sectors that would reduce the disease burden due to current exposures.

For that purpose, several tables (Table 5, 6, and 7) were prepared for three selected health outcomes: all-cause (natural) mortality where external causes have been excluded; mortality from cardiorespiratory diseases, and lung cancer deaths. The estimation uses YLL data for the age group of 30 years and more that corresponds to the relative risk estimates in Table 4, expressed for the same group.

Table 5: Years of life lost due to premature mortality in terms of all-cause (natural) mortality

Health endpoint - All-cause mortality (excl. External causes of death), stressor $PM_{2.5}$								
	Estimated YLL ('000) (WHO GHE 2016), >30 yr.	5-yrs mean $PM_{2.5}$ ($\mu g/m^3$)	RR Macedonian exposures (10 $\mu g/m^3$)	Population Attributable Fraction (PAF)	Estimated burden of disease -YLL			Years of life saved if the annual mean of $PM_{2.5}$ is reduced to 25 $\mu g/m^3$ (EU LV)*
					#	*LCL 95%CI*	*UCL 95%CI*	
Skopje region	380.7	41.8	1.29	0.08	30,256	19,436	40,625	12,470
Tetovo	380.7	81.7	1.64	0.03	10,343	6,224	14,785	7,644
MKD	380.7	45.9	1.32	0.24	91,851	62,720	116,679	38.696

**EU LV (EU Limit values,* EU Directive 2008/50/EC on ambient air quality and cleaner air for Europe*).*

The estimated disease burden from *all causes* (ICD10 code *A00-Y89, excl. external causes of death V01-Y89)* expressed as YLL is highest in Tetovo, where 10,343 years of healthy life are lost annually due to current exposure to $PM_{2.5}$ (11,216/100,000). In the Skopje Region, the rate is 4821/100,000 while the national average is 4426/ 100,000 inhabitants (Table 5). The estimated *health benefits* observing all-cause natural mortality (except external), the number of saved years of healthy life in Tetovo will be 8,289/100,000, in the Skopje Region by 1987 and Macedonia with 1865/100,000 inhabitants.

Regarding the second selected health outcome - *Mortality from cardiopulmonary diseases (ICD10 code I00-I99 and J00-J99)*, the estimated disease burden is 9,282/100,000 inhabitants in Tetovo, in the Skopje Region 3,784 whereas the national average is 3,315/100,000 inhabitants (Table 6).

Table 6: Years of life lost due to premature mortality in terms of cardiopulmonary mortality

Health endpoint - Cardiopulmonary mortality, stressor $PM_{2.5}$								
	Estimated YLL ('000) (WHO GHE 2016), >30 yr.	5-yrs mean $PM_{2.5}$ ($\mu g/m^3$)	RR Macedonian exposures (10 $\mu g/m^3$)	Population Attributable Fraction (PAF)	Estimated burden of disease -YLL			Years of life saved if the annual mean of $PM_{2.5}$ is reduced to 25 $\mu g/m^3$ (EU LV)*
					#	*LCL 95%CI*	*UCL 95%CI*	
Skopje region	231.8	41.8	1.38	0.10	23,749	5,941	41,874	9.840
Tetovo	231.8	81.7	1.87	0.04	8,559	1,795	18,052	6,423
MKD	231.8	45.9	1.42	0.30	68,792	20,312	104,829	28,344

**EU LV (EU Limit values,* EU Directive 2008/50/EC on ambient air quality and cleaner air for Europe*).*

Regarding cardiopulmonary mortality expressed per 100,000 population, the number of years of healthy life that would be saved is 6965, 1568 and 1366 for Tetovo, Skopje region and Macedonia.

Due to the linear association between the concentrations of $PM_{2.5}$ and the observed health outcome (in this case, *lung cancer mortality (ICD10 code C32-C34)*), again the highest in the rate of years of healthy life lost per 100,000 populations in Tetovo (1784); following Skopje Region and Republic of Macedonia (627 and 490/100,000 respectively). Annually in Tetovo 1,645 years of healthy life are lost, while in the Skopje region 3,936 (Table 7).

Table 7: Years of life lost due to premature mortality in terms of lung cancer mortality

Health endpoint - Lung cancer mortality, stressor $PM_{2.5}$								
	Estimated YLL ('000) (WHO GHE 2016), >30 yr.	5-yrs mean $PM_{2.5}$ ($\mu g/m^3$)	RR Macedonian exposures (10 $\mu g/m^3$)	Population Attributable Fraction (PAF)	Estimated burden of disease -YLL			Years of life saved if the annual mean of $PM_{2.5}$ is reduced to 25 $\mu g/m^3$ (EU LV)*
					#	*LCL 95%CI*	*UCL 95%CI*	
Skopje region	24.1	41.8	1.65	0.16	3,936	1,253	6,750	1,648
Tetovo	24.1	81.7	2.65	0.07	1,645	402	3,639	1,269
MKD	24.1	45.9	1.73	0.42	10,161	4,035	14,389	3,947

**EU LV (EU Limit values,* EU Directive 2008/50/EC on ambient air quality and cleaner air for Europe*).*

In Tetovo (about lung cancer), the number of saved years of healthy life will be five times greater when it is expressed on 100,000 population (Tetovo-1376, Skopje Region 263 and Macedonia 190 YLLs). The results of this methodological approach are summarised in Figure 1, where the association between the exposure concentrations of $PM_{2.5}$ and the observed health outcomes is demonstrated.

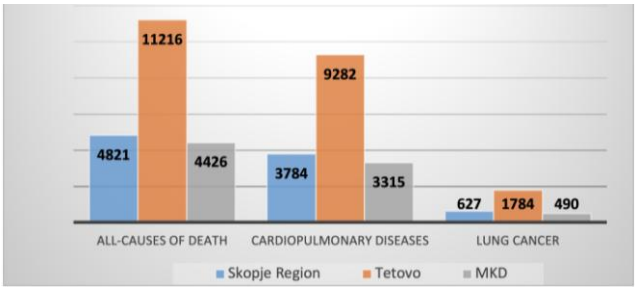

Figure 1: Years of life lost (YLL) due to current exposures to $PM_{2.5}$ for the selected health outcomes

Health benefit estimates are graphically presented in Figure 2. As is expected, the health gain will be significantly higher in Tetovo in comparison to the Skopje Region and national estimates. The estimated health gain for the three selected health outcomes if EU limit values are reached, is 41-42% of the estimated burden in Skopje Region will be eliminated, and 74-77% in Tetovo.

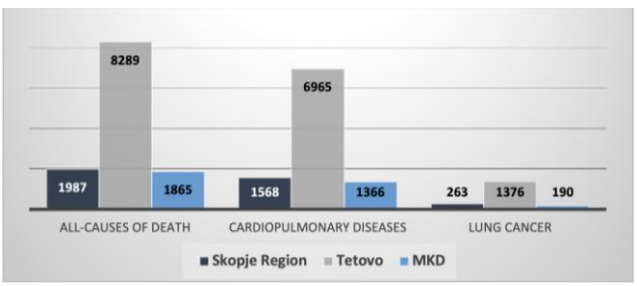

Figure 2: Health gains if the EU Directive limit values are met

Discussion

The use of the metrics for quantification of the environmental burden of diseases (DALY, YLL, YLD) was introduced by Murray and Lopez in 1996 for the World Bank GBD study [34]. In the first GBD study was estimated that almost a quarter of all diseases worldwide were caused by environmental exposure [35]. In Europe, national assessments of the environmental burden of disease (EBoD) have been carried out in several countries. The work by RIVM (the Netherlands) was one of the first systematic works in Europe in the field, which used the new DALY as a measure to compare the burden of different health outcomes in terms of population exposure to environmental stressors [36].

According to the European Environmental Agency (EEA) data for 2014, the number of premature deaths attributed to $PM_{2.5}$ in the EU-28 is 4,278,000. In relative terms, when considered as YLL per 100,000 population, the biggest influences have been observed in the countries of Central and Eastern Europe where the highest concentrations of $PM_{2.5}$ have been monitored such as Kosovo, Bulgaria, Macedonia, Poland, Serbia, Hungary, Romania, Greece, the Czech Republic and Slovakia [37]. The EBD study of six European countries assessed the total environmental burden of diseases related to nine stressors. The study estimated that particulate matters caused a loss of 1.8 million DALY annually, including 1.3 million healthy years of life lost due to premature death (73% of total DALYs). A total 67% of the estimated environmental burden of diseases in the EboD study is explained by the exposure of $PM_{2.5}$, making particulate air pollution the most important environmental risk factor affecting public health [38]. The study conducted for 2012 for the Skopje Region (without Sopiste, a rural municipality within Skopje agglomeration), estimated 16,209 attributable YLLs due to exposure of $PM_{2.5}$ which represent a sum of years of life lost as a result of deaths that occurred before their expected lifespan. The number of years of life that could be prevented through appropriate policies to meet EU's limit values is 306 [39].

In this study, we estimated that at national level 91,851 years of healthy life (4426/100,000 population) are lost annually due to exposure of $PM_{2.5}$ (the annual $PM_{2.5}$ mean 45.9 $\mu g/m^3$), which is significantly above the EEA estimates for 2014 (32,600 YLLs, expressed as a rate 1,578/100,000). This difference is primarily due to the difference in the population exposure concentrations. In 2014, the EEA estimates that the annual average of $PM_{2.5}$ is 27.4 $\mu g/m^3$, an average taken from urban background station only, while in our study calculations have been made for concentrations of $PM_{2.5}$ as a fraction of PM_{10} from all measuring stations in the country, as a five-year average (45.9 $\mu g/m^3$ national mean, 41.8 $\mu g/m^3$ for the Skopje Region and high 81.7 $\mu g/m^3$ for Tetovo (Table 2)).

Regarding the obtained differences with the Sanchez and collaborators study, the difference is due to the different methodological approach for estimation of YLL, were YLLs are calculated using a calculator for life tables developed by Spadaro J., which was later integrated into the AirQ + software used for the calculations. In our study, the global burden of disease due to all risk factors expressed through YLL has been obtained from the WHO database (WHO GHE DB, 2016). As well, we have included all rural municipalities in the estimates.

Estimated disease burden for all-cause natural mortality is highest in Tetovo, where 10,343 years of healthy life are lost annually because of the current exposure of $PM_{2.5}$ (11,216/100,000). The Skopje Region estimates 30,256 YLL (4821/100,000) compared to the national average 91,851 YLL (4426/100,000 population) (Table 5). Calculated how many years of life are lost in terms of one lost life (in 2018 we estimated 1205 for Skopje Region and 265 for Tetovo), the average loss of life expectancy (expressed in years) is 25.1, while in Tetovo 39.0 [20].

The study has some limitations. Missing environmental data have been calculated from the averages in the respective months from the other years, as well as using a formula to estimate PM concentration as a fraction of PM_{10} could be considered as a limitation of this study. The long-term effect of particulate air pollution observed the only through the YLL could also be a limitation because the burden of the years of life spent with some disability (expressed as YLD) is also significant.

These estimates could be a good basis for conducting a further study (economic analysis) to assess economic losses and expenditures associated with air pollution, as mortality or YLL represent the largest burden from the pollution, while DALYs as recommended by WHO is better to use for cost-effectiveness of a particular project or measure [40]. In terms of *health gains*, according to the COMEAP study the health benefits of reducing concentrations of the particulate matters analyzed through mortality are perceived immediately through a visible reduction in the number of deaths in the first or several years after some policy has been taken; or through long-term benefits that will be perceived by increasing the life expectancy of the population that will results with increment of the fraction of the elderly population [41]. Similarly, the benefits of policies that will reduce the exposure of the population to the PM reaching EU limit values will be most pronounced in cities with the highest concentrations of $PM_{2.5}$. Proportionally, for the three selected health outcomes reaching the EU Directive limit values will remove 41-42% of the estimated workload in the Skopje Region (expressed as YLL), 74-77% in Tetovo and 39-42% of the disease burden in the Republic of Macedonia.

In conclusion, the estimated impact of

particulate air pollution on mortality expressed with YLLs is significant and not negligible. The same applies to the health and well-being of the population if the EU or WHO limit levels are reached. The results of this type of assessments should be the basis of the decision-making process at all levels-from a local to a central level. The crucial element is national and local authorities to become aware of the magnitude of the problem and to take responsibility for prioritising activities, strengthening monitoring and control of ambient air pollution and consistently to enforce legislation without exceptions.

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Public Health

Current Knowledge, Attitudes, and Practice of Medical Students Regarding the Risk of Hepatitis B Virus Infection and Control Measures at Qassim University

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Abstract

BACKGROUND: Medical students are exposed to occupational health hazards in hospitals during their studies and lack sufficient education about infection control measures. Injury to medical students is a substantial problem and students have an increased risk of hepatitis B virus (HBV). To understand how medical students think about infection control, it is important to identify the strengths and weaknesses of their education.

AIM: To assess current knowledge, attitudes, and practice of medical students regarding HBV infection and control measures at Qassim University, Saudi Arabia.

MATERIAL AND METHODS: A cross-sectional study was conducted at a medical college. Participants completed a 39-item self-administered questionnaire assessing demographics, knowledge, attitudes, and practice. Item response frequencies were calculated. Responses were recorded into yes (strongly agree and agree) and no (neutral, disagree, and strongly disagree) answers. Correct responses were totalled and categorised as good or poor performance. A scale cut-off of less than 75% correct responses was considered poor, and 75% or more correct responses was considered good. Odds ratios and 95% confidence intervals were calculated, and the chi-square test was used for analysis.

RESULTS: A total of 21%, 41%, and 8% of students expressed good knowledge, attitudes, and practice, respectively. There was no statistically significant difference between males and females on knowledge ($p = 0.089$), attitudes ($p = 0.829$), and practice ($p = 0.248$). There was a statistically significant difference between academic years on knowledge ($p = 0.0001$), attitudes ($p = 0.0001$), and practice ($p = 0.0001$).

CONCLUSION: Most medical students have poor knowledge, attitudes, and practice regarding the risk of HBV infection. It is recommended that a policy is implemented for training on infection prevention for all medical students before they start clinical practice. Prevention programs about HBV infection should be instituted, and existing programs must be strengthened.

Introduction

Hepatitis B virus (HBV) infection is a major public health concern in any health care institution. The risk of occupational exposure to HBV among health care workers (HCWs), particularly students in health professions, is a major public health issue [1]. Medical students are exposed to occupational health hazards in hospitals during their academic and clerkship years but lack sufficient education about infection control measures. Injuries to HCWs, including medical students, remains a substantial problem in the health care systems of many countries and leads to increased risk in acquiring infections such as HBV [2], [3], [4], [5]. Global studies have demonstrated that every HCW has a chance to acquire injury related to work four times a year [6], [7]. Exposure to infected blood as a consequence of injury means that HCWs (including medical students) are at great risk of infections from the human immunodeficiency virus, HBV, and hepatitis C [8], [9], [10], [11]. Studies showed that HBV demonstrate huge social and economic burden on the society and the authority, with high prevalence rates in some countries around the world, which explain the urgent need to develop more sophisticated infection control procedures training [12], with implementing efficient public health measures and infection control practices to help control the issue [13], moreover, introducing

successful public health policies and guidelines will lead to a great decrease and improvement of the HBV status in any community [14].

Prospective studies on HCWs have found that the average risk for HBV transmission is 6% to 30% [15]. Some studies indicate that students lack understanding and do not use isolation precautions and personal protective equipment [16]. Although there are many studies on professional HCWs, few studies have focused on undergraduate medical students. This study aimed to determine the knowledge, attitudes, and practice of medical students regarding the risk of HBV infection and control measures.

This study was conducted at Unaizah College of Medicine, Qassim University, which will be expected to graduate their first batch in mid of 2019. Meanwhile, there has not been any study that investigates this issue in the Qassim region. This study is important to understand how medical students think about occupational health hazards, and the first step is to identify the strengths and weaknesses of their current educational curriculum to help evaluate the infection control training and improve the teaching process.

Material and Methods

A quantitative observational cross-sectional study was conducted in Unaizah College of Medicine, Qassim University, Saudi Arabia. All medical students were recruited by contacting the student affairs office and academic year group leaders. All students from the second to the sixth years were included; students in their preparatory year and their internship year were excluded, no other inclusion/exclusion criteria were applied.

A 39-item self-administered questionnaire was constructed that measured demographics, knowledge, attitudes, and practice. To ensure the face validity of the questionnaire, a pilot study was conducted with 30 medical students at Qassim University. This pilot study was used to validate the logistics of data collection, to establish the clarity of the questionnaire, and to estimate the timing for data collection. The questionnaire took about 10 to 15 minutes to complete. To ensure content validity, the questionnaire was reviewed by community medicine physicians, and amendments were made based on their recommendations. We calculated frequency scores for all items. Then, for each question, we recorded the strongly agree and agreed responses into a YES response, and recorded the neutral, disagree, and strongly disagree responses into a NO response. The number of correct responses was totalled and categorised as good or poor performance. Scores on knowledge, attitudes, and practice were calculated by scoring correct responses as 1 and incorrect responses as 0. A scale cutoff of less than 75% correct responses was considered poor, and 75% or more correct responses was considered good.

Statistical analysis

IBM SPSS Statistics for Windows (SPSS statistical software V.21 (IBM Corp. Released 2012) was used for data entry, data management, and analysis. Odds ratios (ORs) and 95% confidence intervals (CI) were calculated, and chi-square was used for analysis. Logistic regression analysis was used to test interactions among variables. All p-values were based on two-tailed tests. The minimum significance level will be 0.05, with all p values based on two-tailed tests.

Institutional review board (IRB) approval was obtained from Unaizah College of Medicine, Qassim University, for this study before study execution.

Results

The response rate was 92%. Table 1 shows the demographic data of our sample, with the number of students according to gender and year of education.

Table 1: Demographic data

Characteristics			Frequency	Percentage
Gender		Male	164	51.1
		Female	157	48.9
Year	Pre-clerkship	First year	136	42.4
		Second year	44	13.7
	Clerkship	Third year	63	19.6
		Fourth year	54	16.8
		Fifth year	24	7.5
Total		Total	321	100

Table 2 shows the number of responses for each question on knowledge, attitudes, and practice for HBV and control measures. Correct responses to each question varied between 41.1% to 81.9%, 34.6% to 79.8%, and 31.5% to 75.7% for knowledge, attitudes, and practice, respectively.

Table 3 shows that 21% of the students achieved 75% or more correct responses on knowledge and 79% showed poor knowledge. There were 41% of the students who achieved 75% or more correct responses on attitudes and 59% who scored poorly on attitudes. There were 8% of the students who achieved 75% or more correct responses on practice and 92% who scored poorly on practice. The knowledge, attitude, and practice scoring percentages were not statistically significant between males and females. Finally, a comparison of clerkship students with pre-clerkship students showed that clerkship

students scored significantly higher than pre-clerkship students on knowledge, attitudes and practice.

Table 2: Participant responses to a questionnaire on the risk of hepatitis B virus infection and control measures

Part I (Knowledge)	Yes n (%)	No n (%)	Correct answers n (%)
HBV infection control issues have been addressed in the teaching courses.	207 (64.5)	114 (35.5)	207 (64.5)
HBV is the most contagious blood-borne pathogen acquired through accidental exposure to blood.	206 (64.2)	115 (35.8)	206 (64.2)
HBV can be spread through sharing injecting equipment, such as needles and operation tools.	251 (78.2)	70 (21.8)	251 (78.2)
Needlestick injuries are considered a mode of HBV transmission.	233 (72.6)	88 (27.4)	233 (72.6)
An infected mother may transmit HBV to her newborn baby during delivery.	165 (51.4)	156 (48.6)	165 (51.4)
Chronic HBV infection can lead to liver cirrhosis.	233 (72.6)	88 (27.4)	233 (72.6)
Chronic HBV infection can lead to liver cancer.	168 (52.3)	153 (47.7)	168 (52.3)
Most chronic HBV infection cases are symptomatic.	169 (52.6)	152 (47.4)	152 (47.4)
Sterilization (e.g., surgical instruments) is considered a measure to prevent HBV transmission.	197 (61.4)	124 (38.6)	197 (61.4)
Routine blood screening for HBsAg is considered a measure to prevent HBV transmission.	164 (51.1)	157 (48.9)	164 (51.1)
Ensuring safe injection practices is considered a measure to prevent HBV transmission.	217 (67.6)	102 (31.8)	217 (67.6)
Drinking and eating from contaminated drinks is a risk factor for HBV.	120 (37.4)	201 (62.6)	201 (62.6)
HBV test is conducted before marriage.	176 (54.8)	145 (45.2)	176 (54.8)
The minimum number of doses for a complete primary HBV vaccination is three.	132 (41.1)	189 (58.9)	132 (41.1)
Pregnancy is a contraindication for the use of the HBV vaccine.	120 (37.4)	200 (62.3)	200 (62.3)
People who are carriers of HBV are at risk of infecting others.	145 (45.2)	176 (54.8)	145 (45.2)
Can HBV be caught through casual contact such as holding hands?	85 (26.5)	236 (73.5)	236 (73.5)
Can HBV be spread through contact with open wounds/cuts?	194 (60.4)	126 (39.3)	194 (60.4)
Can HBV vaccine prevent HBV?	217 (67.6)	104 (32.4)	217 (67.6)
Can HBV be transmitted by unsterilized syringes, needles, and surgical instruments?	227 (70.7)	92 (28.7)	227 (70.7)
Can HBV be transmitted by unsafe sexual contact?	198 (61.7)	123 (38.3)	198 (61.7)
Do you think that there is a laboratory test for HBV?	263 (81.9)	58 (18.1)	263 (81.9)
Is HBV curable/treatable?	173 (53.9)	147 (45.8)	147 (45.8)
Do you think that post-exposure prophylaxis is available for HBV?	135 (42.1)	186 (57.9)	135 (42.1)
Part II (Attitudes)			
Do you think you are at risk of acquiring HBV infection during practice training?	207 (64.5)	114 (35.5)	207 (64.5)
Do you think you are at a higher risk of HBV infection than the general population?	189 (58.9)	132 (41.1)	189 (58.9)
I feel that I do not have the skills needed to effectively and safely deal with occupational HBV risk in health care settings.	111 (34.6)	210 (65.4)	111 (34.6)
I do not believe in the HBV vaccine.	66 (20.6)	255 (79.4)	255 (79.4)
I believe that changing gloves during blood collection and testing is a waste of time.	64 (19.9)	256 (79.8)	256 (79.8)
I believe that all patients should be tested for HBV before they receive health care.	124 (38.6)	197 (61.4)	197 (61.4)
I do not like treating people with HBV.	72 (22.4)	248 (77.3)	248 (77.3)
I believe that following infection control guidelines will protect me from being infected with HBV at work.	188 (58.6)	133 (41.4)	188 (58.6)
Part III (Practice)			
I get rid of tools and objects contaminated with blood in a medical waste bag, regardless of the presence of the source of infection.	187 (58.3)	133 (41.4)	133 (41.4)
I perform needle recapping for needles after giving injections or using needles.	152 (47.4)	169 (52.6)	169 (52.6)
Have you conducted screening for HBV?	101 (31.5)	219 (68.2)	101 (31.5)
Have you been vaccinated against HBV?	133 (41.4)	188 (58.6)	133 (41.4)
I always change gloves for each patient during blood taking.	194 (60.4)	127 (39.6)	194 (60.4)
Have you ever had a needlestick injury?	78 (24.3)	243 (75.7)	243 (75.7)
I always report needlestick injuries.	145 (45.2)	176 (54.8)	145 (45.2)

Discussion

A major occupational hazard for HCWs is HBV infection. There have been few studies in Saudi Arabia on undergraduate medical students' knowledge, attitudes, and practice of HBV infection. Such data are important in designing health intervention methods and public health policies. Assessing knowledge, attitudes, and practice is a useful step in assessing how much individuals are receiving medical training adopt risk-free disease behaviours for contagious diseases. Medical students should be aware of the risks involved in dealing with patients and should adopt the appropriate management procedures and precautions during training on infectious diseases. Our study involved 321 participants and included almost equal proportions of male and female students: 51.1% and 48.9%, respectively.

Table 3: Multivariate analysis of factors associated with knowledge, attitudes, and practice regarding the risk of hepatitis B virus infection and control measures among medical students

Variables		Knowledge Good n = 69 n (%)	Knowledge Poor n = 252 n (%)	AOR (95% CI) P value	Attitudes Good n = 133 n (%)	Attitudes Poor n = 188 n (%)	AOR (95% CI) P value	Practice Good n = 25 n (%)	Practice Poor n = 296 n (%)	AOR (95% CI) P value
Gender	Male	29 (42%)	135 (53.6%)	1.59 (0.92-2.72) 0.089	67 (50.4%)	97 (51.6%)	1.05 (0.67-1.63) 0.829	10 (40%)	154 (52%)	1.62 (0.70-3.73) 0.248
	Female	40 (58%)	117 (46.4%)		66 (49.6%)	91 (48.4%)		15 (60%)	142 (48%)	
Year	Pre-clerkship	34 (49.3%)	209 (82.9%)	1	87 (65.4%)	156 (83%)	1	10 (40%)	233 (78.7%)	1
	Clerkship	35 (50.7%)	43 (17.1%)	5 (2.81-8.89) < 0.001	46 (34.6%)	32 (17%)	2.57 (1.53-4.34) < 0.001	15 (60%)	63 (21.3%)	5.54 (2.37-12.94) < 0.001

We first categorised data according to individual academic years; however, some years contained very small numbers of students. We, therefore, categorised data by clerkship status, which produced a better test of differences. There was no significant difference in knowledge, attitudes, and practice between males and females, but there were differences between academic years. We found that 21% of students achieved 75% or more correct responses on knowledge (which we considered as indicating a good level of knowledge), whereas 79% had poor knowledge. We found that 41.1% of medical students knew that the minimum number of doses for complete HBV vaccination is three, and 42.1% knew that post-exposure prophylaxis is available for HBV. These results are compatible with findings from a study at Haramaya University in Ethiopia, which showed an overall low knowledge score [17]. Studies of medical students in Erbil city in Iraq [18] and in Qatar [19] demonstrated similar poor knowledge of HBV and its infection control measures. Also, a study at Aljouf University, Saudi Arabia, found that students had inadequate overall knowledge of the occupational risks of HBV infection; although 63.0% considered the vaccine safe and 52.2% had been vaccinated against HBV, 72% of participants did not know post-exposure prophylaxis for HBV [20]. A study of the attitudes of medical students at Tanta University, Egypt, toward hepatitis B and C revealed that only 57.8% of participants had sufficient knowledge; 63.3% had incorrect knowledge of modes of transmission of infection, 49.7% did not know that there is a treatment for B and C viral hepatitis, and 13.5% did not know that there is a vaccine for B viral hepatitis [21]. In contrast, a study in northwest Ethiopia [22], and a study at Northern Border University, Saudi Arabia, [18] showed that medical students had good knowledge of the mode of transmission and prevention of HBV, findings inconsistent with those of the present study. In addition, a study of medical

students in Hail, Saudi Arabia, demonstrated that most participants had adequate knowledge of HBV infection and its mode of transmission, 73.6% knew that HBV infection was associated with liver cancer, and 81.4% were aware of the HBV vaccine and that it provides protection against HBV infection [23]. Differences in the number of questions on knowledge could explain why our findings differ from those of these previous studies. We found that 41% of students achieved 75% or more correct responses on attitudes (which we considered as indicating good attitudes), whereas 59% showed poor attitudes; these figures are compatible with those of the Hail study, which found that pre-clinical students showed unfavourable attitudes toward HBV prevention compared with clinical year students [23]. In contrast, the studies of medical students in northwest Ethiopia [22], Qatar [19], and Northern Border University [18] identified good attitudes toward HBV infection in medical students. Also, the Tanta University study showed that more than 75% of participants had a positive attitude toward viral hepatitis B and C; although a small proportion of respondents had negative or unsure attitudes, most (81.6%) did not share objects of personal use with others [21]. We found that 25 (8% of the sample) students achieved 75% or more correct responses on practice (which we considered as indicating good practice), whereas 296 showed poor practice. A study in Mazandaran Province, Iran, showed that low knowledge scores were related to lack of regular post-employment education [24], and the study at Haramaya University in Ethiopia [17] indicated that adequate knowledge of the subject matter could lead to good infection control practices. The poor practice results found here are by findings from a study of medical students in northwest Ethiopia [22], and a study at Northern Border University [18], both of which showed poor practice toward HBV risk. In the Tanta University study, 68.1% of participants achieved good practice scores [21], and the Hail study showed good practice scores for the prevention of HBV infection [23]. The low knowledge scores among our students can be explained by the inadequate tuition on infection control measures in the curriculum. Lack of post-employment education about infection control affects health care personnel as well as students [24]. Although we found that knowledge, attitudes, and practice improved as students progressed through their academic years, this could be a result of students' increased awareness of infection control measures when they start their clerkship years. Despite these positive findings, our study revealed that most participants had poor practice regarding HBV risk. These findings demonstrate the urgent need to address the gap between student knowledge and practice by strengthening health educational programs on infection control precautions. Concerted efforts are needed to understand the reasons underlying poor knowledge, attitudes, and practice regarding HBV, and to determine if these stem from current medical college curricula. More in-depth investigations are recommended [25]; also, a comprehensive ongoing occupational hazard training program should be implemented as a mandatory course for all medical students. More attention should also be paid to post-educational studies on infection control precautions as practised in clinical settings [22]. As medical students are at increased risk of exposure to hospital-acquired infections in their training practice at hospitals, they should be vaccinated against HBV as they enter the medical college. Also, preventive health departments should be established in medical colleges, which would take responsibility for implementing a well-planned program of vaccination for all newly enrolled medical students. Before starting clinical training, it is recommended that policy is implemented for complete vaccination and training on infection control prevention measures for all medical students [17]. This study had some limitations. The use of a self-report questionnaire may have resulted in recall bias. Also, the study sample was restricted to medical students from one college in one city; therefore, the results cannot necessarily be generalised to other health college students from the same university who are also exposed to the risk of HBV infection.

In conclusion, medical students showed poor knowledge, attitudes, and practice regarding the risk of HBV infection and control measures. Implementation of an occupational hazard course for undergraduate medical students is highly recommended.

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Construct and Psychometric Properties of a New Version Quality of Life Scale Based on Choice Theory

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Abbreviations: QOLSCT = quality of life scale based on choice theory; GHQ = general health questionnaire; SF36 = short form 36; SD = standard deviation; RMSEA = The root mean square error of approximation; CFI = comparative fit index; NFI = Normed Fit Index

Abstract

BACKGROUND: Choice theory, as a psychopathological model, postulated five basics, genetically and universally, needs. Accordingly, the rate of everybody happiness and quality of life is depended on the number of his needs fulfilling. Although some scales have been constructed to assess basic needs, they have proposed unity of needs for all human.

AIM: The present study was designed to construct a new scale, considering individualisation needs for each person; quality of life scale based on choice theory (QOLSCT).

METHODS: Using cluster sampling, six hundred (49% female and 51% male) postgraduate students were selected. One hundred fifty participants also filled SF-36 and GHQ, and 80 participants refilled QOLSCT with four weeks' interval again.

RESULTS: Cronbach's alpha, split-half and test re-test (one month) reliability scores were 0.78, 0.75, and 0.92 successively. The correlation between items and total scores range from 0.36 to 0.72, all with P values ≥ 0.0001. Confirmatory factor analysis showed satisfactory values of goodness-of-fit indices, RMSEA, CFI, NFI and GFI were, 0.05, 0.99, 0.99 and 0.99 respectively. Convergent and divergent validity also showed significant correlations.

CONCLUSION: The result of the present study showed that the new version of quality of life scale based on choice theory could be confirmed. The good level of reliability, fairly goodness of fit indexes, and very good convergent validity support this idea.

Introduction

Quality of life is a concept and term that has been discussed in various guises throughout history [1]. However, it is a debatable, broad, and multi-level term and appears to be a complex collection of amorphous objective and subjective dimensions [2]. In health psychology literature, there are a variety of definitions about the quality of life [3]. Satisfactory with life and fulfilling of needs are the most important domains in the majority of these definitions[4], [5], [6], [7], [8]. For example, Shin and Johnson [6] defined the quality of life as a concept consists of possession resources needed to satisfy individual needs, wants and desires, participation in an activity that improve personal development, self-actualisation, and satisfaction with self in comparison to others.

Several scales have been developed to measure the concept of quality of life [1], [2], [4], [9], [10], [11], [12], [13]. For developing a scale, at first, each investigator established a model and accordingly selected appropriated items [9]. These scales, generic or specific, they have been applied as an instrument mostly for medical practice, improving the doctor-patient relationship, assessing and comparison of different effectiveness treatments, mental and physical health evaluations, and research and policy makings [12].

Choice theory, as a psychopathological model, postulated a variety idea about human nature, mental health, psychotherapy, working with couples,

and school children [14]. Definitively, for an investigation in this area (especially for assessment of treatment, mental health and every interventional program) merit and valid instrument have to be needed. As mentioned above, each scale and its items will be derived from the fundamental ideas in every model.

Glasser [14] as the founder of choice theory proposed that human being possesses five basic genetically and universally, needs [15], [16], [17], [18]. All of the human behaviours are driven by these five needs: survival, power, belonging, freedom and fun. Accordingly, behind all behaviour is an attempt to satisfy one or more of these needs [19]. Failure to satisfy these needs is equal to symptoms or mental health abnormities. In contrast, effective and successful satisfactory of these needs resulted in a feeling of control, happiness and quality of life [18], [19]. In other words, the rate of everybody happiness and quality of life is depended on the amount of his/her needs fulfilling. Therefore, for assessment of everybody quality of life, according to choice theory, we need to assess his/her degree of needs satisfaction and fulfilling. A few scales have been developed and used to measure basic needs satisfactory according to choice theory [16], [17], [20], [21], [22], [23], [24], [25], [26], [27]. Glasser [15] believes that every person has a unique profile of basic needs. In another hand, in attention to the unity of each person quality of life, more recent approach to quality of life is individualised measures [5], [28], [29], [30], [31], [32]. The most important problems with previous scales, especially in choice theory area, could be none serious attention to this individualisation. In other words, all of these scales have posited the same degree of needs intensity in everybody. Also, a tendency toward brief and short form scale, especially in structural equation models, has been increasingly popular in recent years [30], [31], [33], [34].

This research was established to develop and assess psychometric properties of a new quality of life scale according to choice theory with adaptation a new system of individually based on the quality of life inventory [28], [29], [35]. Ultimately the aim was to construct an instrument to use in the assessment of the quality of life in people according to choice theory (QOLSCT) with mentioned proposes.

Methods

Participants

After confirming the research proposal by the Research Ethics Committee of Yasuj University of medical sciences, six hundred (49% female and 51% male) postgraduate students were selected by cluster sampling. Informed consent was obtained from all individual participants included in the study. Participants' mean age was 28.62 years (SD = 7.34; range: 18-53 years). According to gender, the mean age was 27.01 ± 6.7/for female and 30.16 ± 7.61 for a male. Of the initials sample, 150 participants also filled SF- 36 and GHQ. All of the participants were studying at postgraduate levels (MA and PhD) in Azad University of Kohgiloyeh and Boyer Ahmad (a province in the south of Iran).

Short-form 36 health survey (SF-36)

SF-36 is a generic and self-administrated instrument widely used to measure health-related quality of life [36]. It consists of 36 questions (items), and the scores are summarised into eight subscales, and a total score of quality of life ranged 0 to100. In this scale, the higher scores indicating higher levels of function and/or better health. The Iranian version of the SF-36 used in this study has been provided for use in the Persian language [37].

General health questionnaire (GHQ-28)

The GHQ-28 was included to test the divergent validity of the translated version of QOLSCT. The GHQ-28 is a self-administered screening instrument aimed at detecting those with diagnosable psychiatric disorders [38, 39]. The GHQ-28 provides four scores on somatic symptoms, anxiety and insomnia, social dysfunction and severe depression (seven items for each dimension). Items may be scored using 0-1-2-3 Likert scores (at present study), or they may be scored 0-0-1-1, which indicates whether a symptom is absent or present.

Procedure

At the first step, we needed to prepare some items for sale, according to Choice theory basic needs (survival, belonging, power, freedom, and fun), using Brown Model [40] and Spector design [41]. A literature review was done on Glasser books [14], [16], [19], [22], [42], Wubbolding book [18], LaFond [25], Burns et al., [21], Mason et al., [26]and Sing and Jusoh [27] papers to determine basic needs in choice theory and history of works done to make measurable Glasser idea about basic needs. Using the Brown Model [40] and Spector design [41], items were built. In the calculation, a total of ten items (five pairs) were constructed for the assessment of basic needs.

We adopted The Quality of Life Inventory [28], [29], [35] method to overcome previous biases [5], [35] in present scales. Accordingly, we advocated two items for each basic need. In first group items (n1-1, n2-1, n3-1, n4-1, and n5-1) respondents rated how important each need is for their life (0 = not important to 5 = very important). In second group items (n1-2,

n2-2, n3-2, n4-2 and n5-2) then they rated how satisfied they are in that area ((0 = not satisfied at all to 5 = very satisfied; (appendix 1)).

For content validity, the draft version of items was presented to a five expert panel, and after some minor corrections, the final version was prepared. The importance ($n_{(1,2,3,4,5)}$-1) and satisfaction ($n_{(1,2,3,4,5)}$ – 2) ratings for each item were multiplied to calculate the final score (N1 to N5) for each need. Therefore, we had finally one score for each need (totally five items). For assessment of test re-tests, reliability 80 participants refilled QOLSCT four weeks later.

Data were analysed using SPSS version 22 (for descriptive, reliability and correlation data analysis) and confirmatory factor analysis (CFA) was carried out to determine the QOLSCT structure. The LISREL program version 8.8 [43] was used for this analysis. This study used the Root Mean Squared Error of Approximation (RMSEA) [44], the Standardized Root Mean Squared Residual (SRMR), as well as the Comparative Fit Index (CFI). Hu and Bentler [45] recommend that good model fit is indicated when RMSEA < 0.05, SRMR < 0.08, and CFIP > 0.95.

Results

The sample size was 600 (293 females and 307 males). The mean age of the group was 28.62 years (SD = 7.34; range 18-53 years). The mean total score in QOLSCT was 70.83 ± 21.09 (71.73 ± 20 for female and 69.96 ± 22.08 for male). The descriptive statistics of the scale are presented in Table 1.

Table 1: The descriptive statistics of the scale

Items	Mean (SD)			T	Sig
	Female	Male	Total		
1-1	4.20 (0.79)	4.15 (0.85)	4.18 (0.82)	0.71	0.42
1-2	3.36 (0.97)	3.24 (1.0)	3.30 (0.99)	1.45	0.14
2-1	4.24 (0.76)	4.04 (0.95)	4.14 (0.87)	2.88	0.004
2-2	3.62 (1)	3.38 (1)	3.50 (1.00)	2.95	0.003
3-1	4.15 (0.81)	4.05 (0.92)	4.10 (0.87)	1.39	0.16
3-2	3.37 (1.0)	3.35 (0.95)	3.36 (0.98)	0.34	0.74
4-1	4.1 (0.83)	4.07 (0.93)	4.09 (0.88)	0.38	0.70
4-2	3.26 (1.06)	3.29 (1.04)	3.30 (1.05)	0.77	0.44
5-1	4.14 (0.87)	4.19 (1.4)	4.17 (1.19)	0.50	0.61
5-2	3.31 (1.06)	3.41 (1.05)	3.36 (1.06)	0.79	0.25
N1 (Survival)	14.31 (5.26)	13.70 (5.43)	14.00 (5.35)	1.29	0.16
N2 (Belonging)	15.69 (5.82)	14.01 (5.84)	14.83 (5.88)	3.53	0.0001
N3 (Power)	14.25 (5.5)	13.83 (5.53)	14.04 (5.51)	0.94	0.34
N4 (Freedom)	13.51 (5.56)	13.74 (5.79)	13.63 (5.67)	0.48	0.63
N5 (Fun)	13.95 (6.06)	14.68 (7.05)	14.33 (6.59)	1.35	0.18
Total	71.73 (20.01)	69.96 (22.08)	70.83 (21.09)	1.02	0.30

From the degree of important in life point of view (items 1-1, 2-1, 3-1, 4-1 and 5-1) the highest mean was for item 1-1 and the lowest mean was for 4-1 in total scores. Among the satisfaction items, the highest and lowest means were 3.50 (2-2) and 3.30 (1-2 and 4-2) respectively in total. The mean of final scores (important × satisfaction) ranged from 13.70 (N1) to 14.68 (N5). These sequences were changed in some items when we look to scores according to gender (male and female).

Data analysis with five items showed acceptable alpha values. Mean of inter-items correlation was 0.43 and for corrected item-total correlation was 0.55. For inter-item correlation, the highest was between N1 and N2 (0.51), and the lowest was between N2 and N5 (0.36). The highest and lowest means in corrected item-total correlation were N2 (0.59) and N5 (0.50) respectively. Cronbach's alpha, split-half and test re-test (one month) reliability scores were 0.78, 0.75, and 0.92 successively. Also, the correlation between items and total scores are presented in the same table. The highest and lowest correlations were between N1 and N2 (0.51) and N1 an N5 (0.36) respectively.

Table 2: Inter items correlations and, Pearson correlations of QOLSCT with GHQ-28 and SF-36

	N1	N2	N3	N4	N5	total
N2[1]	0.508**					
N3[1]	0.44**	0.47**				
N41	0.37**	0.39**	0.41**			
N51	0.36**	0.39**	0.37**	0.40**		
Total[1]	0.72**	0.76**	0.73**	0.71**	0.72**	
GHQ-28[2]	-0.004	-0.26**	-0.23**	-0.10	-0.06	-0.20*
SF-36[2]	0.100	0.30**	0.39**	0.31**	0.14	0.31**

* Significant at 0.05 level; ** Significant at 0.01 level; N1, N2, N3, N4, N5 are for survival, belonging, power, freedom and fun respectively.

Spearman correlations, used to evaluate convergent and divergent validity, are presented in Table 2. All items (except N1) and total scores of scale showed a positive and significant correlation with the SF-36 scale. The highest correlation is related to item N3 (0.39), and N1 (1) was at the bottom of these correlations. The total scores of the scale also showed a negative and significant correlation with GHQ-28 scores, as a divergent validity.

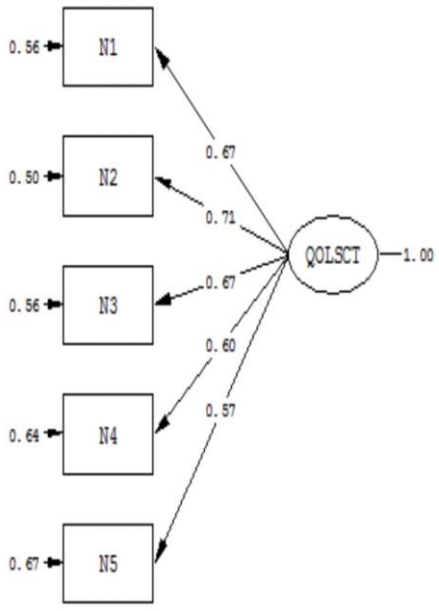

Figure 1: Confirmatory Factor analysis based on the postulated model (Standardized factor loadings); N1-N5 are questionnaire Items; N = (important × satisfaction), quality of life scale based on choice theory (QOLSCT)

The one-factor model showed satisfactory values of goodness-of-fit indices, despite a significant χ^2 ($p < 0.02$). However, RMSEA, CFI, NFI and GFI were at an acceptable range, 0.05, 0.99, 0.99 and 0.99 respectively. Standardised factors loading and T values are presented in Figures 1 and 2.

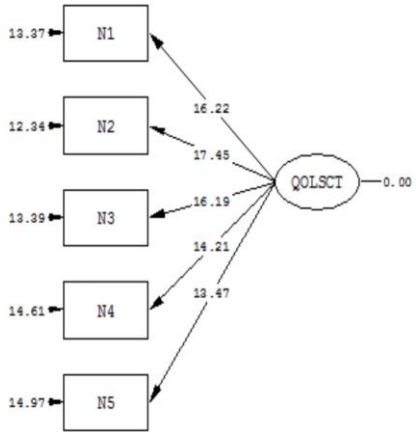

Figure 2: Confirmatory Factor analysis based on the postulated model (T values); N1-N5 are questionnaire Items; N = (important × satisfaction), quality of life scale based on choice theory (QOLSCT)

All items loaded significantly on their original factors in the acceptable range [46], with the highest loading of item N2 (0.71) and the lowest loading of item N5 (0.57). All T values, as shown in Figure 2, were at significant ranges.

Discussion

The current study was designed to construct a new model of quality of life scale based on choice theory concepts. Analysing the data, psychometric properties of this scale were evaluated. The scale was designed so that to consider an individual's differences in the degree of importance of each need.

The coefficient alpha for the total scale and the test-retest reliability coefficients all exceeded .75 that shows an acceptable range of reliability [46], [47], [48], [49], [50]. These results are in consistent with previous studies in this area [20], [23], [24], [26]. Also, the correlations between five needs scores all were at a significant level ($P > 0.001$ and over). The strongest correlation was between N1 and N2, and the weakest was between N1 and N4.

In attention to RMSEA, CFI and NFI scores, the model showed well goodness of fit conceptual model. The acceptable scores in RMSE, CFI and NFI (according to MacCallum et al., [51], and acceptable loadings for all items on their factors, significant T values for all items and theoretical basic supported an appropriate fit for the model. In other words, all of the indices supported a fair model for this scale with this structure. Although a significant χ^2 ($p < 0.02$) resulted in the analysis, the large size of the sample could be accounted for this significant result [52], [53]. The strong loading values on confirmatory factor analysis and high and significant T-values supported the validity of scale items in the Iranian population.

Evidence for convergent validity of QOLSCT was supported by significant positive correlations between QOLSCT, and total scores of-of-36 and negative correlation of total scores with GHQ-28 supported divergent validity for this scale. As would be expected, high scores in the QOLSCT scale were accompanied by high scores in of-36, and low scores were accompanied with high scores in GHQ-28. These results represent additional support for the postulated model, especially for validity.

This study contains limitations that are important to acknowledge. First, the population consisted of university students, and therefore cannot be generalised to other populations. Second, in the present study, we used convergent, divergent and factor analysis for assessment of scale validity. Therefore, it is recommended to use this scale for other population (especially clinical population) and assess by another type of validity methods (for example discriminate validity).

In conclusion, the result of the present study showed that the new version of quality of life scale based on choice theory (QOLSCT) could be confirmed. The strong reliability, fairly goodness of fit indexes, and very good convergent validity support this idea. In addition to construction a tool for clinical and research proposes based on choice theory and health quality of life research, the new method of calculating of scores (degree of importance and degree of satisfaction) could compensate the defects of previous quality of life scales. Also, the brief form of a scale (10 items) could respond to the increasing tendency toward this kind of scales [30], [31], [34]

Ethics approval and consent to participate

The research proposal was approved in the research ethics committee of Yasuj University of Medical Sciences and accordance with the ethical standards of the 1964 Helsinki declaration. The reference letter is 23.2.1948. Informed consent was obtained from all individual participants included in the study.

Availability of data and materials

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

Authors' contributions

AS was the main investigator, who designed the study, analysed the data and wrote the paper. SKh contributed to the study design and writing of the paper. MM contributed to the analysis of the data and writing the result of the manuscript. SM contributed to the analysis of data, helped in writing and contributed to the interpretation of the data. All authors read and approved the final manuscript.

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Review Article

Evaluation of Observational and Behavioural Pain Assessment Tools in Nonverbal Intubated Critically Adult Patients after Open - Heart Surgery: A Systematic Review

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Keywords: Behavioral Pain Assessment Tools; Intubated; Open-Heart Surgery

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Abstract

BACKGROUND: Over 70% of patients hospitalised in an intensive care unit (ICU) often experience moderate to severe pain due to pre-existing diseases, trauma, surgery, aggressive procedures, and routine ICU care. Many patients hospitalised in ICU are not able to speak and express their pain due to various causes, including mechanical ventilation, reduced consciousness, and administration of sedative drugs. Therefore, the use of observational and behavioural pain tools is recommended in this group of patients given their inability to express pain.

AIM: To examine the existing observational and behavioural tools for assessment of in Nonverbal Intubated Critically Adult Patients after Open-Heart Surgery.

METHODS: A systematic review of available observational and behavioural tools for assessment of pain was undertaken using the COSMIN checklist. A literature search was conducted using the following databases: Ovid, Science Direct, Scopus, PubMed, and CINHAL databases, Google Scholar search engine as well as Persian resources Sid, Magiran, Iran doc, and IranMedex up to the end of 2017 were reviewed.

RESULTS: A total of 47 studies that had examined five tools used in intensive care units after cardiac surgery in patients under mechanical ventilation were reviewed. Each of the five tools included behavioural and observational items, and only one tool had physiological items. All the tools had been evaluated regarding validity and reliability. In the three tools, sensitivity, specificity, responsiveness, and satisfaction were considered.

CONCLUSION: Based on available evidence and investigations, CPOT and BPS tools have good validity and reliability to be used in pain assessment in Nonverbal Intubated Critically Adult Patients after Open-Heart Surgery. The NVPS tool requires more studies to be further confirmed before the assessment of pain in this group of patients.

Introduction

Pain is an unpleasant sensation recognised as an important physiological and psychosomatic stressor, which is experienced by many patients in some degree during hospitalisation [1]. More than 71% of patients admitted to hospital wards have memories of pain [2]. For example, in the intensive care unit, over 30% of patients experience a degree of Pain during rest, and more than 50% of them feel significant pain during routine care, including position change, endotracheal suction, and dressing [3], [4]. Untreated pain can have negative effects on various systems of the body such as endocrine, cardiovascular, immune, neurological, and musculoskeletal systems as well as affecting the mental health of patients admitted to ICU [5].

In addition to the mentioned physiological and mental consequences, failure to control pain leads to delayed postoperative recovery, prolonged hospitalisation, restlessness due to inability to communicate [1], chronic pain, and decreased the quality of life after the operation, leading to increased medical expenses for the patient and society [6]. Moreover, poor or inadequate assessment of pain is associated with increased mortality in ICU [7].

Pain after open-heart surgery has various reasons, including sternum incision, tissue excision, pain in the site of saphenous vein removal, the presence of chest tube as well as various factors

related to the type of operation [8], [9]. The occurrence of acute pain can trigger autonomic reactions and lead to the emergence of stress responses causing an imbalance between the supply and demand of oxygen in cardiac tissue, ischemia, infarction and eventual increase in morbidity and mortality [10].

Pain assessment is the first and most important step in the care and cure of these patients [11]. According to a general definition, the expression of pain by a patient is known as the golden standard for pain assessment. However, a large number of patients admitted to ICU cannot speak and report their pain for various causes like mechanical ventilation, decreased consciousness, and administration of sedative drugs which has placed this group of patients at maximum risk of inadequate pain assessment and management [12]. For this reason, some tools have been proposed as an alternative for assessment of pain in patients who cannot speak. American Society for Pain Management Nursing (ASPMN) recommends observational and behavioural pain tools as an alternative to self-reported pain in patients who are not able to communicate verbally for any reason. Studies on observational and psychometric tools for pain assessment in patients who are not capable of verbal communication have been the focus of research in recent years [10], [13]. Vital signs, which can be easily quantified in intensive care units, are another criterion to assess pain in patients admitted to intensive care units. According to a study, more than 70% of nurses use vital signs to assess pain in patients [14]. However, current evidence does not support the validity of vital signs to assess pain in these patients. ASPMN guidelines in 2006 stated that the application of changes in vital signs is not recommended for pain assessment in patients who are not able to speak [15] and that vital signs should not be considered as the only pain assessment tool but rather can be used as a guide when other pain assessment tools are not applicable in the long term [16], [17].

In this study, we aimed to review the studies on behavioural and observational pain assessment tools in patients admitted to ICU (especially after open-heart surgery) that were not able to communicate verbally.

Methods

Research questions

1. What behavioural and observational do tools have appropriate validity and reliability to be used in intensive care units for patients under mechanical ventilation?

2. Which of these tools has been used in the intensive care unit after cardiac surgery?

3. What are the best tools used in the intensive care unit after cardiac surgery?

Objective

The goal of this review study is to investigate and explain existing observational pain tools and compare them with each other to assess pain in patients not capable of verbal communication admitted to intensive care units. Also, in reviewing these tools, studies evaluating patients admitted to intensive care units after cardiac surgery have been emphasised.

Search method

In this review study, the keywords of pain assessment, behavioural pain tool, nonverbal pain scale, observational pain tool in intensive care, and post-cardiac surgery pain assessment were searched in Ovid, Science Direct, Scopus, PubMed, and CINHAL databases, Google Scholar search engine as well as Iranian resources such as Sid, Magiran, Iran doc, and IranMedex up to the end of 2017. Based on the significance of papers for the research topic and comments expressed by research team members, appropriate and relevant papers were selected and evaluated. Papers citing pain assessment indicators or non-verbal pain assessment in intensive care units, especially among non-verbal patients, will be subject to final analysis. The inclusion criteria of papers are as follows:

1. The language of the texts in English or Persian.

2. The words pain, non-verbal pain, tool or instrument, index, scale, non-conscious patient, patients with an endotracheal tube, and open-heart surgery are present in the title, abstract, and keyword.

3. The study is of quantitative, qualitative, combined, and instrumental type.

4. The study is conducted on patients over 18 years of age.

5. Tools are investigated in at least one study concerning the intensive care unit following cardiac surgery in unconscious and mechanically ventilated patients.

6. The studies published in languages other than English and Persian, editorial and commentary papers, as well as a book review, will be eliminated.

7. The number of samples study is > 30.

Search results

A total of 1216 papers were found in the initial search of databases based on keywords. After studying the titles, 70 papers were selected. The difference in the clinical conditions of patients and the presence of one keyword in the title without the proximity of the content to this review study resulted in the exclusion of papers. Then, 54 studies were selected from among the nominated papers according to a review of abstract and methodology. Finally, considering the number of patients under study and other inclusion criteria, 47 papers were selected from among the tools as follows: BPS (14 papers), CPOT (22 papers), NVPS (8 papers), FLACC (2 papers), and PBAT (1 paper).

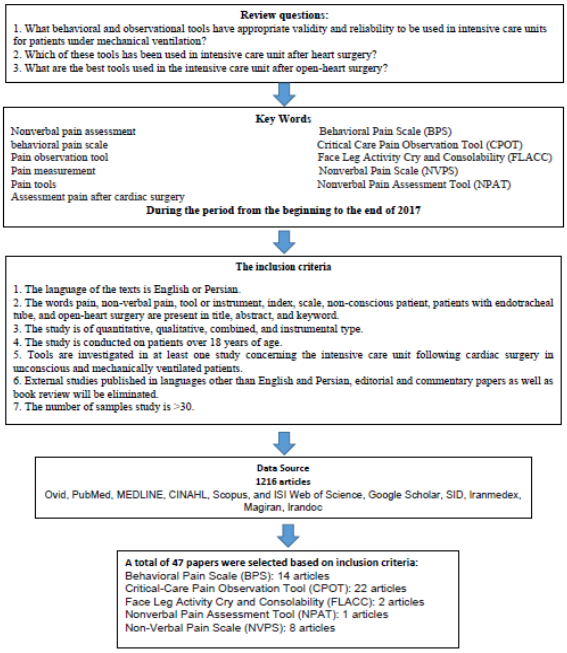

Figure 1: Flow chart of the review process

Design

This study was designed to systematically review the published papers on observational and behavioural pain assessment tools, progression and testing of these tools, as well as evaluation of their validity and reliability for pain assessment among patients with endotracheal tube admitted to ICU who were not able to communicate. In this research, the quality of studies and their methodology have been assessed and reported based on the COSMIN checklist [18]. Following consideration of the reported psychometric properties of the tools alongside COSMIN ratings, conclusions are drawn as to the established psychometric properties of each tool.

Quality of Methodology

Each reported study was assessed for methodological quality by an author using the COSMIN checklist with 4-point [18]. A rating of excellent, good, fair or poor was assigned separately to the evaluated measurement properties of each study.

Results

Behavioural Pain Scale

The Behavioral Pain scale was originally in the French language, which was translated into English [19], Chinese [20], Finnish [21], Brazilian Portuguese [22], Swedish [23], and Persian [24]. The behavioural pain scale has three parts: facial expression, upper limb movement and Compliance with mechanical ventilation, each graded with scores 1-4. The sum of minimum scores is 3 from 3 parts (no pain) and a top score of 12 (maximum pain). In 14 studies investigated, the validity and reliability of this tool were assessed on 1082 mechanically ventilated adult patients in the intensive care unit [19], [20], [24], [25], [26], [27], [28], [29], [30], [31], [32], [33], [34], [35].

Reliability

BPS reliability was investigated in 13 studies [19], [20], [24], [25], [26], [27], [28], [29], [30], [31], [32], [33], [35]. Of these, in test-retest procedure the reliability of tool was re-tested in three studies [19], [20], [24], and in all of them satisfactory results relative to primary test were obtained ($r = 0.50$-0.84, $p < 0.001$). Internal consistency of the tool was calculated in 8 studies [24], [27], [29], [30], [31], [32], [33], [35], and Cronbach's alpha in the range of 0.59-0.80 indicated moderate to high internal reliability. The inter-rater agreement percent was over 85% in studies [19], [20], [30], which was statistically ideal. However, in one study, when the patient felt no pain, the inter-rater agreement percent was 82-91%, and after a non-painful stimulation (oral care), the agreement percent was 64-73%, which was 36-46% during painful stimulation (change of position) [35]. Based on Kappa coefficient, the inter-rater agreement percent was in the range of $k = 0.67$-0.83 [19], [25], [26], [29], which was good because it was > 0.60. The inter-rater agreement percent for each item of tool was investigated in one study according to Kappa coefficient, and the results were as follows: face expression item ($k = 0.78$-0.80), upper limb movement item ($k = 0.67$-0.72), and Compliance with mechanical ventilation item ($k = 0.61$-0.62)(25). ICC ranged from 0.74 to 0.95 in the studies [24], [27], [31], [32], [33]. In one study, the highest value of ICC was related to face expression item (ICC = 0.90) [27]. also, ICC had a higher value at the time of painful stimulation (suction and position change) in the upper limb

movement item (ICC = 0.85-0.94) [27]. In Chinese version of the tool, ICC was reported 0.98-1.00 [30]. Inter-rater reliability was reported in the range of r = 0.65-1.00 (p < 0.001) based on Pearson correlation coefficient [20], [28]. In correlation analysis of items with total score, the highest and lowest correlation was related to face expression and Compliance with mechanical ventilation, respectively (r = 0.92, 0.65).

Validity

BPS validity was evaluated across in 13 studies [19], [20], [24], [25], [26], [27], [28], [29], [30], [31], [32], [33], [34], [35]. To assess construct validity, average changes in final BPS scores were evaluated at the time of stimulation compared to the base time. In these studies, significant changes were observed in an average score of the tool at the time of painful stimulation (change in position, endotracheal suction), and the average changes were reported in 3.0 versus 6.8 range. The significant difference in BPS score at the time of painful stimulation (3.0 vs. 6.8) compared to the time of non-painful stimulation (3.0 vs. 3.5) in studies confirmed a good differential validity of the tool [19], [25], [27], [31], [33], [35]. Moreover, in two studies, the increase by two points in the score of the tool was reported at the time of painful stimulation compared to baseline [32], [33]. In three studies, the criterion validity was also examined [20], [24], [30]. Higher BPS scores in subjects confirming the presence of pain indicated the validity of the tool (P < 0.001). In one study, BPS score was observed to increase in 97.1% of patients under painful stimulation (endotracheal suctioning), but only 2.9% of subjects underwent non-painful stimulation (body temperature measurement) [30]. For convergent validity, BPS tool was investigated using the NVPS tool, and the correlation between the two tools at rest and during the painful procedure was ρ = 0.69 and ρ = 0.77, respectively [31]. According to the analysis performed in two studies, 55-65% of pain expression variance is determined by the initial factor (face expression: 0.78-0.90, upper limb movement: 0.79-0.85, Compliance with mechanical ventilation 0.63-0.64) [19], [27]. Also, in one study, the correlation between tool items was reported, which indicated moderate to high correlation between the items (face expression with upper limb movement: 0.70, face expression with ventilation challenge 0.40, and upper limb movement with ventilator challenge 0.29) [27]. Concurrent validity was instated by comparison between BPS scores and patients' self-reports of pain intensity [25], and showed a positive and statistically significant correlation (ρ = 0.67; *p* = < 0.001).

Responsiveness

Responsiveness refers to the ability of the tool to detect important changes in measurement unit over time, even if the changes are small [36]. In four studies, BPS has been found to be responsive [25], [27], [28]. The effect size on responsiveness was large for the scores of three tool items as well as for total BPS score. The responsiveness for the final score of this tool has been reported to be excellent (1.8-3.4). The largest effect size was related to face expression item in the range of 2.3-5 [27]. Also, in a study, the responsiveness of BPS, CPOT, and NVPS tools was compared, indicating the size effect factor and therefore the response of BPS tool (1.99 vs 1.55 and 1.46, respectively). Moreover, in one study, the highest responsiveness was reported during painful stimulation (suctioning r = 1.20, position change r = 1.87) [28].

Feasibility

The Feasibility and utility of the tool were investigated in two studies [19], [29]. In the first study, 24 out of 28 nurses participating in the research were satisfied or highly satisfied with the use of tool [19]. In the second study, out of 20 nurses participating in the study, the scores 7-8 were assigned regarding accuracy, usability, and convenience from a score range 0 (worst) to 10 (best). Also, 33% of participants preferred BPS to other study tools [29].

Sensitivity and specificity of the tool were also investigated in two studies [20], [34]. In the Chinese version of the tool, the cutoff point was reported to be 6.5, and it was stated that when BPS score was > 6.5, 75.9% of the area under the curve had a splitting property with a sensitivity and specificity of 52.4% and 87.5%, respectively [20]. Another study also found that in a score > 5, 76% of the area under the curve had splitting property, with a general sensitivity of 84.8% and specificity of 52.3% for the tool. At the time of painful stimulation, the sensitivity and specificity of the tool was 62.8% and 91.7%, respectively [34].

Limitations

There were still limitations to the tool after reviewing and verifying the validity and reliability of BPS in several studies, including the lack of a practical definition of some items such as upper limb movement that may be interpreted differently between nurses. In different studies, repeated and frequent observations of patients under study may lead to more results, so the results should be interpreted with more cautiousness [37], [38].

Critical-Care Pain Observation Tool (CPOT)

Critical-Care Pain Observation Tool (CPOT) is designed to assess pain in both verbal and non-verbal adult patients admitted to ICU [39]. The tool has four items of facial expression, body movements, muscle

tension, and Compliance with a ventilator in intubated patients or Vocalization in non-intubated patients. Scoring range of this tool is 0-2 per item with a total score of 0-8. The pain observation tool in intensive care was originally provided in French and then translated into English [40], Spanish [41], Danish [42], Finnish [21], Swedish [43], Dutch [44] and Turkish [45].

This tool has been reviewed and investigated in 22 studies on 1249 patients in various intensive care units such as medical, surgical, open-heart surgery, and neurosurgery [10], [15], [28], [29], [32], [33], [34], [39], [40], [41], [42], [43], [44], [45], [46], [47], [48], [49], [50], [51], [52], [53].

Reliability

The reliability of the tool was examined in 18 studies [28], [29], [32], [33], [34], [39], [40], [41], [42], [43], [44], [45], [47], [49], [50], [51], [52], [53]. A test-retest procedure was then performed in one study with non-parametric Spearman correlation coefficient of 0.81-0.93, indicating the reliability of the primary test [49]. Internal consistency of the tool was investigated in 10 studies [28], [29], [32], [33], [42], [43], [44], [45], [49], [51], with Cronbach's alpha reported in 0.31-0.81 range. However, in one study, Cronbach's alpha was 0.95 [28]. In the Dutch version of the tool, the internal consistency of the tool was 0.56, which increased to 0.60 by eliminating the Compliance with ventilator item [44]. The inter-rater agreement per cent has been reported 97-100%. Moreover, the kappa coefficient has been calculated in 0.79-0.94 range in one study [50]. The inter-rater agreement per cent was reported in the range of 0.52-0.88 using weighted kappa coefficient [39]. Moderate to high measures of inter-rater reliability from two or more raters were found was calculated using the Kappa coefficient in three studies, which ranged 0.79-0.94 [29], [43], [50]. In the Turkish version of the tool, inter-rater reliability has been reported in the range of 0.55-1.00 [45]. inter-rater reliability of the CPOT was found to be lower during patient turning as painful procedures when compared to the BPS (0.90) and NVPS (0.92) [29]. ICC rate was studied in eight studies [32], [33], [42], [43], [44], [49], [52], [53], which was reported in the range of 0.62-0.93, and in the Dutch version of tool, ICC has been recorded in 0.56-0.98 range. In the Danish version, the ICC was also reported to be > 0.90. In a study, the agreement between CPOT and facial expression after open-heart surgery was considered, and the highest level of agreement was reported during consciousness of patients (k = 0.787) [39]. In a research conducted on patients admitted to intensive care units after cardiac surgery, CPOT tool was compared with BIS monitoring and vital signs, in which there was positive and strong correlation between CPOT and BIS before ($r = 0.666$, $p < 0.001$), during ($r = 0.612$, $p < 0.001$), and after painful stimulation ($r = 0.738$, $p< 0.001$) [46].

Validity

The validity of the tool was evaluated in 17 studies [28], [29], [32], [33], [34], [39], [40], [41], [42], [43], [44], [45], [49], [50], [51], [52], [53]. To assess content validity, in the study by Gelinas et al., [39], 4 physicians and 13 nurses in ICU reviewed the tool as specialists and scored the tool in 0.88-1.00 range based on four-point Likert scale. Content validity in the translated version into Turkish [45] was also evaluated by five experts in the field of intensive care using a four-point Likert scale and was reported to be satisfactory. Also, in another study, content validity was approved by experts [52]. In order to investigate the construct validity, changes in average CPOT scores during painful stimulation (repositioning, endotracheal suction) were investigated compared to baseline in the studies, and it was shown that the average CPOT score was significantly increased during painful stimulation (0.48 vs 3.38) [39], [43], [45]. Discriminant validity of tool was also reviewed in 11 studies [29], [32], [33], [34], [39], [40], [42], [43], [45], [49], [53], and significant increases in pain during painful stimulation relative to non-painful stimulation indicated a good Discriminant validity of the tool. In one research, average CPOT score at painful stimulation (position change) was 3.04, that is higher than baseline, while at non-painful stimulation (dressing change), only 0.25 points increase compared to baseline was observed, which indicated a non-significant finding [50]. Furthermore, in two studies, it was stated that the average score increase during painful stimulation compared to baseline was 2 points, but it was 0-0.5 points during non-painful stimulation [32], [33]. The criterion validity was measured by comparing CPOT and self-reporting of pain by the patient [39], [53]. Moderate correlation with Spearman correlation coefficient of ($r = 0.40$-0.49, $p < 0.05$) was observed as well as higher correlation ($\rho= 0.59$, $p < 0.001$) during painful stimulation among 105 patients [39]. In another study, Spearman correlation between CPOT and mean arterial pressure was $\rho = 0.35$ ($p < 0.001$) during painful stimulation [43]. In one research on 55 patients, criterion validity was reported $r = 0.71$ ($p < 0.05$) by comparing CPOT and self-reported pain by the patient during painful stimulation [40]. A strong correlation was found between CPOT and VAS tools ($r = 0.48$, $p < 0.0001$) [26]. CPOT was also compared with PIAND and NVPS tools ($r = 0.86$, $p < 0.001$), and in all the three tools, the increase in score was observed in the case of painful stimulation compared to baseline [51]. In a comparison of the Turkish version of CPOT with BPS tool, the correlation between the two tools at painful stimulation was 0.89. Moreover, in a study on patients admitted to intensive care units after cardiac surgery, CPOT score increased by 3 points during painful stimulation (change of position) compared to baseline [45].

Responsiveness

The responsive rate of the tool was reviewed in one study, in which the effect size of CPOT between baseline and during the painful procedure was reported to be 1.55 [29].

Feasibility

The Feasibility and utility of the tool were investigated in two studies [29], [39]. In the study of Gelinas et al., over 90% of 33 nurses participating in the study described the use of CPOT as satisfactory regarding understanding, learning, and ease of use. Moreover, 72.7% of the participants in the study considered the use of CPOT as useful and recommended it. In the study of Chanques et al., 20 nurses participating in the study gave the score 7-8 from 0 (worst) to 10 (best) range regarding accuracy, utility, and ease of use. Also, 24% of these people preferred BPS tool to other study tools.

Sensitivity and specificity of the tool

In two studies, the sensitivity and specificity of the tool were studied [44], [47]. During painful stimulation, the sensitivity and specificity was 66.7-86% and 78-83.3%, respectively, with a cutoff point of 2-3 scores [47]. Also, in the Dutch version of the tool, at a cut-off point of > 2, sensitivity and specificity of the tool was 39% and 58%, respectively [44].

Limitations

Limited review of some items of the tool (including Vocalization item) is a limitation of CPOT, which has been studied only in patients without endotracheal tube after heart surgery, while the purpose of this item has been to assess pain in non-intubated patients who are not able to express their pain. Furthermore, in some studies, it has been stated that the face expression item in patients with brain injuries or face trauma is different from other patients hospitalised in ICU. However, it seems that the validity and reliability of CPOT should be considered in various patient groups, including delirium patients as well as those with mental problems and brain injuries [37], [38], [54].

Non-Verbal Pain Scale (NVPS)

In 8 studies, non-verbal pain scale (NVPS) was investigated [28], [29], [31], [55], [56], [57], [58], [59], which was originally designed based on FLACC tool. This tool has 5 items for assessment of pain: face expression, activity (movement), and guarding as the item's behavioral section, and physiological section in original version of NVPS, including physiological item I (blood pressure, heart rate, respiratory rate) and physiological item II (pupil size, skin color and temperature, sweating) [57], and in the revised version includes a physiological item (blood pressure, heart rate) and a respiration item (based on SPO2 and Compliance with ventilator). Each item is scored in 0-2, with the score range calculation of 0 (painless) to 10 (maximum pain). The revised version of the tool is based on studies by Odhner et al., in which the respiration item has replaced the physiological item II. To assess validity and reliability, the original and revised versions of the tool were reviewed among 213 and 401 patients admitted to ICU, respectively [28], [29], [31], [55], [56], [57], [58], [59].

Reliability

The reliability of tool was examined in seven studies [28], [29], [31], [55], [56], [57], [58], and A test-retest procedure was then performed in one study. The re-test was conducted 8-12 hours after the initial test among 37 out of 60 patients, and the correlation coefficient between the two tests for original and revised versions of the tool was r = 0.51-0.75 and r = 0.55-0.86, respectively(55). Internal consistency of the tool was reported using Cronbach alpha, which was 0.62-0.78 during painful stimulation [31], [55], [56], [57], [58]. Moreover, in the revised version of NVPS, Cronbach alpha value was reported to be in 0.72-0.86 range during painful stimulation [28], [55], [56]. Also, in a study by Odhner et al., if FLACC and the original version of NVPS were combined, the internal consistency of the tool would increase to 0.90 [57]. Inter-rater agreement percent for both tools was reported > 90% (90.8-94.7%) in 72 observations(56). Inter-rater reliability was 0.71 for the final score using kappa coefficient [29], and the highest value of it was related to the face expression item (k = 0.70) and the lowest for the physiological item II (k = 0.02) [29]. ICC has also been reported at a range of 0.62-0.95 at different times of study [28], [31]. The inter-rater reliability based on the Pearson correlation coefficient was also reported 0.89-0.96 for the revised version and 0.80-0.87 for the original version [55]. The highest correlation level of items with a final score was reported to be r = 0.708 during the painful stimulation associated with face expression item in the original version and guarding item (r = 0.663) in the revised version [56].

Validity

The validity of the tool was also reviewed in seven studies [28], [29], [31], [55], [56], [57], [58]. The discriminant validity was confirmed through increasing average score by at least 2 points at painful stimulation (endotracheal suction, position change) compared to basic time (P < 0.001) [28], [55], [58]. To evaluate the convergent validity, NVPS was compared with FLACC tool, which showed a correlation between the two tools (r = 0.86, p < 0.05) [57]. Comparison of

patient self-reports to NVPS scores demonstrated a moderate statistically correlation (ρ = 0.313; *p* = < 0.001) [59].

Also, to assess the criterion validity of the tool, NVPS was measured using self-report by the patient (yes/no). The higher NVPS scores in patients who confirmed the presence of pain indicated the validity of the tool (p < 0.001) [55]. The correlation of NVPS with numerical scores of pain (NRS) was ρ = 0.559 during painful stimulation and ρ = 0.405 during non-painful stimulation [58].

Responsiveness

In a study, the Responsiveness of the tool was investigated by reporting the effect size [29]. The effect size for average final score was 1.01 during suction, and it was 1.20 during repositioning. The maximum effect size was related to respiration item during suction (0.22), and the maximum effect size was related to the face expression item (1.68) during repositioning.

Feasibility

In a study, the utility and usefulness of this tool were evaluated before and after implementation in the intensive care unit [59]. 78% of participants (out of 32) stated that the application of this tool was easy or very easy. Also, 80% were satisfied with the training and implementation of this tool. 81% of nurses also stated that NVPS was a reliable tool for pain assessment in non-conscious patients, while 57% of nurses participating in the survey were ensured of the tool to assess pain in non-conscious patients before NVPS training and implementation (from 53 people) [59].

Sensitivity and specificity

In one study, the sensitivity and specificity of the tool were reported to determine the cut-off point of pain. In the original version of the tool, cutoff point in 1.5 scores had 95.6% sensitivity and 97.4% specificity. In the revised version of the tool, the pain cutoff point in 1.5 scores had sensitivity and specificity of 95.6% and 96.3%, respectively [55].

Limitations

There are limitations in NVPS design and study. For example, in physiological item II, the definition of pupillary dilation and sweating is not standardised [38]. In behavioural items, the expression of a smiley state or normal position of body and hands cannot indicate a painless situation [54]. In the study of Topolovec-Vranic et al., the number of nurses participating in a post-implementation survey of the tool (32 subjects) was less than those participating before the implementation of the tool (53 subjects), which could affect the final results [54].

Faces, Legs, Activity, Cry and Consolability Scale

This tool was originally designed to assess pain among children with cognitive impairment [60]. Each item of the tool is scored 0-2, and finally, for the five items, the score range of 0-10 is expected. This tool has been evaluated in two studies on 88 adult patients admitted to intensive care units [57], [61].

Reliability

The reliability of the tool was examined in both studies [57], [61]. Internal consistency of the tool was measured using Cronbach's alpha coefficient, which was high in both studies and was reported in 0.84-0.88 range, which would increase to 0.934 by eliminating the cry item [61]. However, internal consistency was reduced by eliminating any of the other items. Inter-rater reliability had a high final score (k = 0.98), which was lower for the cry item (k = 0.72). Also, the inter-rater agreement per cent for tool items ranged 84-93%. Moreover, to calculate the variance of score, participation of each tool item was calculated using factor analysis method, with minimum level related to cry item (68.9%) compared to the items of face (0.86), legs (0.94), activity (0.90), and consolability (0.95) [57], [61].

Validity

A significant reduction in the score of FLACC after administration of an analgesic drug or decrease in scores in non-painful situations relative to painful situations (mean, 5.27; SD, 2.3 vs mean, 0.52; SD, 1.1; P < .001) indicates construct validity [61]. Also, to verify the criterion validity, the correlation between FLACC and Checklist of Nonverbal Pain Indicators (CNPI) was investigated, indicating high correlation as a sign of excellent reliability of tool (ρ = 0.963; P < 0.01). Furthermore, the high correlation of final FLACC score with that of NVPS tool represents excellent validity of the tool (r = 0.86, p < 0.0001). The highest correlation among FLACC and NVPS items is related to the face expression item (r = 0.78, p < 0.0001). The correlation of FLACC items with the final score of NVPS is in r = 0.65-0.75 range (p < 0.0001) [57].

Responsiveness/Accessibility

In none of the studies conducted on adult patients admitted to the intensive care unit were responsiveness, accessibility, and utility of the tool examined.

Limitations

The number of samples used in the studies is limited, and the tool should be used in more patients and in other wards to achieve better and more robust results. Moreover, for further verification of validity, the comparison of the tool with the patient's self-report seems to be more beneficial [38].

Non-Verbal Pain Assessment Tool (NPAT)

This tool was developed and introduced in 2010 to assess pain in people who could not communicate verbally [2]. NPAT has been studied in patients hospitalised in internal intensive care, general surgery, cardiac and chest surgery units. The initial version of this tool has five items, each of which scored 0-2, with 0 representing the lowest score and 10 the top score. The tool includes emotion (effective response to a situation), movement (change in the placement and positioning of the body), verbal cues (vocalization from the patient other than speech), facial cues (expressions of the face), and positioning/guarding (body response that imply a protection of the body from contact with external touch), which has been studied in three phases in the initial research. The first and second phases of the study were conducted to verify the validity and reliability of the tool, and the third phase focused on assessment of criterion validity in comparison with patient's self-reported pain. In the first and second phases of the study, five teams each involving two nurses were present, and the third phase was attended by a team of two nurses [2], [54].

Reliability

In the first phase of the study, 68 non-verbal patients in intensive care units were evaluated. To assess the inter-rater reliability, the concordance correlation coefficient was used that was reported 0.69 in this study, indicating a moderate to high reliability among assessors. The internal reliability of the tool for the final score was reported using Cronbach α, which was 0.82. The internal reliability of the tool items was also reported at 0.79-0.77. The correlation of the average score of each item with the total score is also in 0.60-0.63 range, except for the verbal item, which is equal to 0.55. Also, in this phase of the study, the kappa value was 0.35, indicating minimal strength of agreement.

In the second phase of the study, 39 patients who were not able to communicate verbally were evaluated. The concordance correlation coefficient was 0.72 (95% confidence interval), demonstrating strong interrater reliability [2], [54].

Validity

In the second phase of the study, the criterion validity of the tool was evaluated in 42 patients, showing tool validity of 0.21 indicative of poor validity and concern over the utility of the tool. In the third phase of the study, to review the criterion validity of the revised NPAT version, 50 patients admitted to a post-operative ward who were able to self-report pain were examined. In this phase of the study, two nurses separately and blindly attempted to record the degree of pain among patients based on the patient's self-report as well as the revised version of NPAT. The correlation coefficient was 0.66 ($P < 0.05$), which showed a moderate to high correlation [2].

Limitations

In this study, the responsiveness, satisfaction, sensitivity, and specificity of the tool were not considered. In this research, the time to evaluate pain has not been clearly expressed, which is a major weakness. Furthermore, unlike the first and second phases of the study, the tool was investigated in patients undergoing surgery, the results of which are not reliable in our case [2], [54].

Discussion

In this study, a review of psychometric and appropriateness of tools as well as the adequacy of tools has been used to assess pain. In this systematic review, five pain assessment tools for patients admitted to post-cardiac surgery ICU have been investigated.

At least 100 samples have been recommended for review according to instrumentation and psychometric papers [18]. However, in most studies, the number of samples is usually < 100 patients, and in some studies, < 50 patients have been evaluated [27], [29], [35], [43], [61] and in some studies > 100 [2], [11], [26], [30], [31], [34], [39], [44], [47], [62]. Nevertheless, in most studies, at least two assessments were done for patients. The test-retest procedure was conducted to express the per cent of missing items as well as data management for CPOT, BPS, and NVPS tools, and a majority of studies were related to BPS tool [19], [20], [24]. One of the limitations in validity assessment of tools was that in a small number of studies, the tools were compared with self-reported pain. In these studies, BPS, CPOT, and NPAT tools were compared in different groups with patients' self-report of pain. NPAT tool was evaluated only in one study [2] in which the validity and reliability of tools were assessed in three phases; however, further studies are needed to ensure the validity of the tool. In the third phase of the study, in contrast to the first and second phases, the tool was examined among patients in the surgical ward, the

results of which were not reliable for our review. Also, the validity of the tool cannot be verified, and there seems to be a need for further studies [54].

An important point in this review was nurses' attitude to the need for observational tools and how to use these tools. In previous studies, there has been a positive correlation between the ability of nurses to assess pain in patients admitted to intensive care units with the adequacy of treatment and pain control. Furthermore, the lack of timely diagnosis of pain or accurate assessment of patient's pain as well as following a treatment course not based on patient's actual pain hurts improvement of the disease and clinical and psychological conditions of the patient. Therefore, implementation of tools among nurses can be effective in changing the viewpoints of nurses in this regard [63].

Based on our analysed in all of the tools examined, immobilisation of patient was considered as indicative of no pain in the patient, while in some painful situations, immobilisation of patient is regarded as a measure to reduce and control pain [54]. Another probable drawback of observational pain tools is that various items of these tools could be a function of causes other than pain such as restlessness. Another problem with these tools is the lack of proper performance in patients with spinal cord injuries, patients receiving muscle relaxants, and those having facial trauma.

In NVPS, physiological signs (including vital symptoms) have been used as items assessing the tool. In the original version of the tool, there is no standard definition of pupil dilation and sweating in physiological item II. Also, the use of vital signs, including heart rate, is unlikely to be effective in pain assessment among patients [38].

According to research, over 70% of nurses use vital signs to assess pain in patients [62]. However, current evidence does not support the validity of critical signs to assess pain among the patients. American Society for Pain Management Nursing (ASPMN) in a 2006 guideline stated that the use of vital signs changes is not recommended for the assessment of pain in patients not able to speak [15] and that vital signs should not be considered as the only pain assessment tool but rather as a guide when other pain assessment tools are not applicable for a long time [17]. In some studies, the convergence of CPOT and MAP tools has been shown [28], [46], which represents the convergence of MAP and CPOT changes. However, further supplementary studies can be helpful in this respect. Overall, considering the clinical conditions of each patient, general conditions, the course of the disease, and provision of treatment based on individual changes of vital signs should not be ignored among patients admitted to the intensive care unit.

In one study [57], the FLACC tool was considered as the gold standard to evaluate the validity of NVPS, while FLACC was originally designed to assess pain among children. Based on our study FLACC tool was implemented in two studies on adults with a low sample number [57], [61], which does not seem to be able to confirm the validity of tool in adults. Moreover, the responsiveness rate and utility of the tool have not been reviewed, which is a disadvantage by itself. Another possible drawback is that FLACC has not been evaluated and compared with self-reported pain of adults. In the FLACC tool, the cry item looks non-specific for adults, which reduces the value of this tool to assess pain among adults. It seems to Comparison of CPOT and NVPS tools in patients admitted to ICU after cardiac surgery indicated that CPOT tool in these patients was superior for assessment of pain and that the nurses participating in the study had more consensuses on scoring and use of CPOT. Also, the items have not been clearly explained in the CPOT tool.

Among these tools, BPS and CPOT had the highest adequacy to be used in pain assessment among adult patients admitted to these wards. According to our research, CPOT and BPS tools have higher validity and reliability and appear to be the best choice for pain assessment in post-cardiac surgery intensive care units among patients with mechanical ventilation. In the case of BPS tool, the base score (painless) is 3, which is sometimes misleading [54]. It seems that changing the painlessness score from 3 to 0 eliminates the wrong impression of the base score. CPOT tool is likely to be an appropriate tool for pain assessment in patients admitted to post-cardiac surgery intensive care units under mechanical ventilation not able to express their pain according to examined validity, reliability, responsiveness rate, and utility. Nevertheless, to confirm this conclusion, further studies in these patients and comparison with other available tools are warranted.

The results obtained in this systematic review are in line with those related to previous studies [37], [38], [54]. Examining the versions of tool translated to various languages have confirmed similar findings in previous studies, although further confirmation of the reliability and validity of the investigated tools requires more studies. More clinical studies with higher sample sizes and elimination of limitations in previous studies are needed to develop more functional and specialised observational tools, which can be used as a stable component in patient assessment and recording by nurses as well as treatment based on these assessments.

Limitation: In our research, studies examining < 30 patients, those written in a language other than English and Persian, and letters to the editor were excluded, although there were researchers among them that were noteworthy to be considered. Another limitation of our study was that only one researcher extracted data from studies, which was error-prone, although the overall review was under the supervision of other researchers. Similar to any systematic review,

other relevant tools subject to thorough and unannounced testing may not be included in this review.

Conclusion

Application of behavioural and observational pain tools in unconscious and non-verbal adults patients in an intensive care unit is essential for pain assessment and timely treatment. In patients undergoing open-heart surgery, lack of well-timed diagnosis and treatment of pain can cause irreparable consequences and complications, increase hospitalisation time, lead to mental health problems, raise medical expenses, and eventually result in death. Therefore, familiarisation and training of nurses working in intensive care units with behavioural and observational tools of pain assessment are necessary. Nurses' skills and experience in using these tools to identify and cure pain promptly can lead to a new experience by patients when admitted to intensive care units.

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Review Article

Hepatitis E Virus Infection in Bulgaria: A Brief Analysis of the Situation in the Country

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Abstract

BACKGROUND: Over the past two decades, more thorough investigations for hepatitis E virus (HEV) infection have been done in the world. Reports from Southeast European countries have increased.

AIM: The current article presents a critical analysis of all studies for HEV in Bulgaria.

MATERIAL AND METHODS: A literature search was done using available medical databases. We analysed the literature in PubMed databases and Bulgarian medical databases for English and Bulgarian languages sources. Preference was given to the sources published within the past 24 years (January 1995 – September 2018).

RESULTS: Two thousand two hundred and fifty-seven blood serums were tested for the analysed period (1995 – 2018), and 13.1% of them were positive for acute HEV (Mean ± SD: 20.38 ± 25.77%; 95% CI: 1.29 – 39.47%). The following subtypes were established in the country – HEV Subtype 3e, HEV Subtype 3f, HEV Subtype 3c, HEV Subtype 3i, HEV Subtype 3hi and HEV Subtype 1.

CONCLUSION: We hope that the National Health Organizations will take adequate and timely measures to increase the knowledge and research for HEV among Bulgarian citizens.

Introduction

Hepatitis E virus (HEV) infection is an infectious disease which thorough investigation has realised over the past twenty years [1] [2]. First studies of HEV were from the late 1970s and the early 1980s [3], [4]. In Bulgaria, the first human cases were reported by Teoharov et al., in 1995 [5]. The infection has been investigated thoroughly in the country since 2008 [6], [7]. Some Bulgarian authors presented data for HEV infection among Bulgarian patients [8], [9]. However, there are still many unknown questions about this infection in our country.

The aim of this study is a critical analysis of all available articles for HEV infection in Bulgaria.

Materials and Methods

Study Design

A literature search was done using available medical databases. We analysed the literature in PubMed databases and Bulgarian medical databases for English and Bulgarian languages sources using the following keywords "Hepatitis E virus" AND "Bulgaria" AND "HEV" AND "Hepatitis E virus infection" AND "HEV infection". No age restriction was observed. All studies were written in the English or Bulgarian language. The critical analysis included original articles, brief reports and case series. The critical analysis excluded articles with missing data for the number of investigated individuals, case reports

and letters to the editor. Preference was given to the sources published within the past 24 years (January 1995 – September 2018).

Ethics Statement

The study was performed by the principles of the Declaration of Helsinki. Participation in the study was fully voluntary and anonymous and written informed consent was obtained from each person before the medical examination.

Statistical Analysis

The results obtained from the majority of studies do not allow detailed statistical analysis. Therefore, basic statistical indicators such as confidence interval (CI), standard deviation (SD), etc. were applied. Statistical analysis was performed by Excel 2007 (Microsoft, Redmond, Washington, USA) and SPSS Statistics 19.0 (IBM Corp., Armonk, New York, USA). A *P*-value < 0.05 was considered statistically significant.

Results

We found 23 papers (PubMed databases – 5; Bulgarian medical databases – 18). Seven articles fulfilled the inclusion and exclusion criteria and were included in the final data analysis (Table 1) [5], [8], [9], [10], [11], [12], [13]. All articles described hospitalised patients with clinical presentation of acute HEV infection. Two thousand two hundred and fifty-seven blood serums were tested for the analysed period (1995 – 2018), and 13.1% of them were positive for acute HEV (Mean ± SD: 20.38 ± 25.77%; 95% CI: 1.29 – 39.47%). Individuals have been tested for anti-HEV IgG and/or anti-HEV IgM. The following subtypes were established in the country – *HEV Subtype 3e* (GenBank accession number 77A 2012-BG, 2588 2011-BG, 2308 2011-BG, et al), *HEV Subtype 3f* (GenBank access. num. 762 2011-BG, 855 2011-BG, 688A 2010-BG, et al.,), *HEV Subtype 3c* (GenBank access. num. ISS75 Plov 2014, ISS100 Paz 2014, ISS2 Sof 2013, et al.,), *HEV Subtype 3i* (GenBank access. num. 1785 2011-BG, 905 2012-BG), *HEV Subtype 3hi* (GenBank access. num. ISS62 Paz 2014), and *HEV Subtype 1* (GenBank access. num. ISS78 Haskovo – travel Afghanistan 2014) [9], [13]. In Bulgaria HEV infection affected predominately male gender (male/female – 80.0/20.0%). The mean age of HEV-positive people varied between 50.7 ± 23.0 years (95% CI: 32.26 – 69.06) for Sofia district [8], and 53.6 ± 14.0 years (95% CI: 45.66 – 61.49) for Plovdiv district [10].

Table 1: Studies on acute hepatitis E virus infection in Bulgaria

First author and reference	Year of Publication	Methodology	Target group	Diagnostics	Investigated, (n)	HEV positive, (n)
Teoharov et al. [5]	1995	Retrospective	Hospitalized	ELISA	53	4
Dikov et al. [8]	2012	Retrospective	Hospitalized	ELISA	117	6
Teoharov et al. [9]	2014	Retrospective	Hospitalized	ELISA HEV RNA	741	67
Petrov et al. [10]	2015	Retrospective	Hospitalized	ELISA	112	12
Baymakova et al. [11]	2016	Retrospective	Hospitalized	ELISA	806	20
Stoykova et al. [12]	2017	Retrospective	Hospitalized	ELISA	325	111
Bruni et al. [13]	2018	Retrospective	Hospitalized	ELISA HEV RNA	103	76

Discussion

HEV infection more frequently affects male than female (61-69%) [14]. Similar data were found in Bulgarian studies for HEV gender distribution (male/female – 80.0/20.0%). But opposite data were reported from Romania (male/female – 40/60%) [15], and Turkey (21.4/78.6%) [16].

The highest incidence of HEV infection is observed in people over fifty years [14]. Similar data are reported from Romania (28% HEV-positive in the age group of 45-65 years) [15], Turkey (50.9 ± 16.8 years) [16], and Albania (35% HEV-positive in the age group over 50 years) [17]. In Bulgaria, HEV infection occurs mainly in people over 50 years of age.

Up to now, there are only two Bulgarian studies for HEV phylogenetic analysis of human samples [9], [13]. The studies established *HEV Subtype 3e*, *HEV Subtype 3f*, *HEV Subtype 3c*, *HEV Subtype 3i*, *HEV Subtype 3hi* and *HEV Subtype 1* [9], [13]. It is known that *HEV Subtype 3e* occurs mainly in swine, *HEV Subtype 3f* is human subtype, and *HEV Subtype 3i* is mainly wild boar subtype. The established *HEV Subtype 3e* in Bulgaria is close to the reference sequence AB248520 Japan 3e. The established *HEV Subtype 3f* showed high similarity with reference sequence EU495148 France Hu 3f. The third found *HEV Subtype 3i* in Bulgaria is familiar to reference sequence FJ705359 Germany wild boar 3i. These results indicate that the virus might have an autochthonous character in Bulgaria with zoonotic potential and main reservoirs as domestic pigs and wild boars.

In Bulgaria, HEV infection is poorly known and diagnosed. The realised human studies are focused on the clinical manifestations and characteristics of acute clinical form. There are many uncertain things about HEV infection in Bulgaria. First, a basic problem has no established HEV case definition in Bulgaria. Second, has not enough investigations on HEV RNA among Bulgarian patients. Third, there is no licensed diagnostic laboratory for hepatitis E sequencing (HEV RNA analysis). Fourthly, there are no morbidity registers for HEV in Bulgaria.

Therefore, in some areas of the country, it is not known what is the real prevalence of HEV among the population. Fifth, the mortality of HEV is unknown among Bulgarian citizens. Sixthly, HEV seroprevalence is unknown for risk groups (HIV-positive patients, immunocompromised persons, transplanted patients and pregnant women). Seventh, animals' investigations are limited in the country. There is one study for HEV seropositivity among pigs in industrial farms in Bulgaria [18]. The results from this project found 40% HEV-positive pigs [18].

There are some reasons for those missing information and knowledge for HEV in Bulgaria. First, there is a lack of coordination and collaboration between scientific societies of infectious diseases specialists, microbiologists, virologists and gastroenterologists. Second, the researches in the country are poorly financed. Third, the Ministry of Health does not record the incidence and mortality of HEV infection in Bulgaria. Fourthly, National Health Authorities do not conduct a screening programme for blood and blood products for hepatitis E virus. Fifth, the veterinary services in the country do not search for HEV infection routinely in animals (domestic pigs, wild boars, bears, dogs, cats, etc.). Sixthly, lack of efficiency of some hospitals. Seventh, poor diagnostic capacity of some hospital laboratories. Eighth, poorly trained medical staff is seeking, recognising and investigating HEV infection.

In conclusion, the combination of these facts leads to the national position of insufficient evidence of HEV infection in our country. The notified weaknesses could be revised, and the local situations could be improved. We hope that the National Health Organizations will take adequate and timely measures to increase the knowledge and research for HEV among Bulgarian citizens.

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Review Article

Systematic Evaluation of Efficacy and Safety of Acupuncture Treatment for Patients with Atrial Fibrillation

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Keywords: Atrial Fibrillation; Acupuncture; Meta-analysis

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Abstract

BACKGROUND: Atrial fibrillation (AF) is one of the most common types of arrhythmia diagnosed in clinical practice. Due to its negative effects on people's physical and mental health, it is necessary to prevent and treat AF. Recently, scholars have found that acupuncture can be used to treat AF, but some scholars have questioned its therapeutic efficacy.

AIM: Therefore, this study was performed to assess the efficacy and safety of acupuncture treatment for AF patients.

METHODS: Previously published research articles were retrieved from six databases, and the data was analysed using RevMan5.3 software with a statistically significant difference defined as $P < 0.05$.

RESULTS: A total of 8 relevant kinds of literature were retrieved containing 633 AF patients (323 in the treatment group and 310 in the control group). Acupuncture treatment increased the total efficacy and the rate of AF cardioversion to sinus rhythm (RR: 1.38; 95% CI: 1.25 to 1.53 vs RR: 1.40;95% CI: 1.16 to 1.69; each $P < 0.05$), and decreased the time of AF cardioversion to sinus rhythm, the heart rate and incidence of adverse effects (RR: -3.95; 95% CI: -4.98 to -2.91 vs RR: -14.54; 95% CI: -24.09 to -5.00 vs RR: 0.48; 95% CI: 0.21 to 1.11, each $P < 0.05$). There was difference between retention time more and less than 30 minutes ($I2 = 74.9\%$, $P = 0.05$). The funnel plot displayed a symmetrical and funnel-form shape, indicating low bias.

CONCLUSION: Acupuncture has a good therapeutic effect and safety profile on patients with AF, and its application in clinical practice should be considered.

Introduction

Atrial fibrillation (AF) is one of the most common types of arrhythmia diagnosed in clinical practice. Recent data shows that its incidence rate, prevalence rate, disability rate and mortality rate are increasing every year globally [1], depending on the country's geographical region, race, and gender [2]. The hemodynamics and thromboembolism caused by AF seriously affect people's physical and mental health, so it is necessary to search for treatments for AF patients.

The main treatment strategies for AF are rhythm control, rate control and anticoagulation [3], [4]. Rate control requires the use of antiarrhythmic drugs (AAD) to adjust the AF patient's heart rate to a reasonable state, while rhythm control is the attempt to restore and maintain sinus rhythm. Studies have shown that rate or rhythm control could reduce the risk of too fast AF rate and the harm caused by heart failure as well as a thrombus. It can also improve the quality of life of the patients [5], [6]. However, rhythm or rate control is not satisfactory in treating AF patients, such as AAD having obvious proarrhythmia, AF recurrence rate in electrical cardioversion, Cox-Maze and catheter ablation. All these influences the therapeutic effects of AF.

Acupuncture is a treatment method which has been used in Chinese traditional medicine for over 3000 years, and it is defined as the needling of specific points of the body. In many countries, acupuncture has become one of the most widely used complementary therapies in recent years. Some

studies reported that acupuncture seems to be effective in preventing cardiac arrhythmias [7]. In traditional medicine, a study has shown that wrist-ankle acupuncture has an obvious effect on managing patients with paroxysmal supraventricular tachycardia [8]. However, some scholars also have cast some doubts over the safety of acupuncture in disease treatment [9].

In this study, we evaluated the efficacy and safety of acupuncture in treating AF patients [10], [11], [12], [13], [14], [15], [16], [17], so as to provide a basis for better treatment of AF.

Methods

Literature Search

Published reports were searched using the retrieval words "atrial fibrillation" OR "AF" and "acupuncture" OR needling and ("database "[PDAT] : "2018/01/31"[PDAT]) from PubMed, Cochrane Library, Web of Science, Scopus, China National Knowledge Internet, and Chinese Biomedical Literature Database. Crossover and parallel RCTs were selected if they focus on the acupuncture treatment for patients with AF, regardless of blinding. RCTs were selected if they included at least one group receiving acupuncture and one control group receiving another active treatment. There were no language restrictions in the search strategy.

Selection criteria

Inclusion criteria: 1) adult AF patients, paroxysmal AF was defined as self-terminating within seven days ; Persistent AF was defined as any AF episode either lasting longer than seven days or requiring drug or direct current cardioversion. 2) In addition to the intervention measures, acupuncture or electrical acupuncture were basically regarded as the same treatment. 3) with definite control groups and treatment groups; 4) with basic disease treatment, the record showed a mean ± standard deviation or effective rate; 5) with RCTs. Exclusion criteria: the article was excluded if 1) the same data was republished twice; 2) it had no control group; 3) it involved animal and basic experimental literature; 4) it was a thesis, conference or essay articles.

Data extraction and quality assessment

Data extraction and quality assessment were assessed by two reviewers independently and crosswise. Any differences were settled through discussion or consultation with a third party. Evaluation of the content was as follows: 1) random method; 2) concealment of allocation method; 3) blind method; 4) integrity of the data; 5) selective reporting of the results; 6) other bias sources [18]. A grade: if meeting 4 of the above, the study showed that there was little possibility of the existence of bias; B grade: if there were one or more of the requirements which were only partially satisfied, it meant that there was a moderate bias in the study; C grade: if one or more of the requirements were not completely satisfied, it meant that there was a high degree of bias in the study.

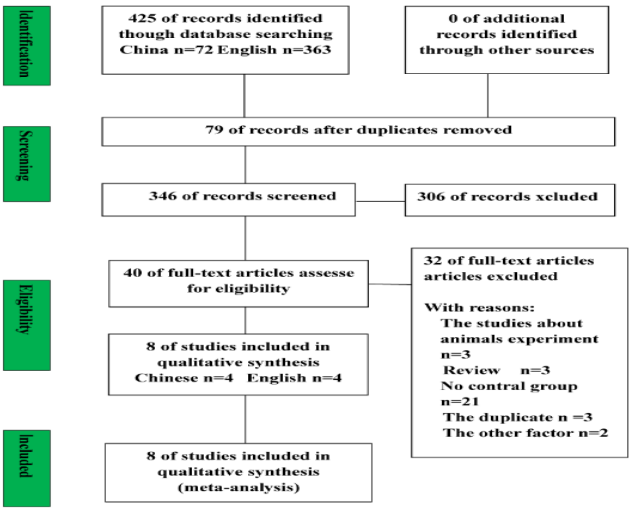

Figure 1: Flow of information through the different phases of a systematic review. The procedure led to the identification and inclusion of eligible studies in our analysis. Eventually, a total of 8 studies were selected for the analysis

Statistical analysis

The data analysis was done using RevMen5.3 software. Dichotomous data were expressed as relative risk (RR), and continuous outcomes were presented as mean differences (MD), while 95% confidence intervals (CI) were calculated for both. Random effect mode was used when the I squared (I^2) value exceeded 50% or $P < 0.05$; data was pooled using the fixed effect mode, when $I^2 < 50\%$ or $P \geq 0.05$. Bias analysis was evaluated using the funnel plot. The differences were considered significant if $P < 0.05$.

Results

Original data description

A total of 425 relevant literature were retrieved, 79 duplicates and 306 irrelevant articles were excluded. 8 articles were finally included in this study after reading the abstract and full texts [10], [11], [12], [13], [14], [15], [16], [17].

Table 1: Baseline characteristics of the trials included in the meta-analysis

Author	Year	Nationality	Group	Total case (m/f)	Age	AF Type	Drug	Treatment course (d)	Acupuncture points	Hold the needle time (min)	Quality grade
[10] Xu HK	2007	China	T	40 (25/15)	58.9 ± 10.5	Paroxysmal	A	-	PC6, CV17, CV6, CV12, ST36,SP10,ST40	60	C
			C	40 (27/13)	57.4 ± 9.4		a				
[11] Lomuscio A	2011	Italy	T	17 (10/7)	65 (62-67)	Persistent	A	70	PC6,HT7,BL15	15-20	C
			C	24 (17/7)	65 (63-67)		-				
[12] Han BD	2012	China	T	62 (35/27)	64.3	Paroxysmal	A+D	-	PC6,PC4,SP6,SP10	-	C
			C	52 (29/23)	65.7		D				
[13] Zhang XL	2013	China	T	30 (19/11)	59 ± 4	Paroxysmal	A+W	31	PC6,HT7,PC4	30	C
			C	30 (16/14)	55 ± 7		W				
[14] Xia YS	2014	China	T	50 (-)	62.8 ± 5.5	Paroxysmal	A+D	-	PC6,PC4,SP10,SP6	-	C
			C	40 (-)			D				
[15] Yan YH	2014	China	T	30 (18/12)	52.1 ± 6.7	Paroxysmal	A+D	-	PC6, HT7, BL14, CV17,BL15,BL17	30	C
			C	30 (16/14)	51,7 ± 5.8		D				
[16] Xu BZ	2015	China	T	54 (30/24)	63 ± 7	Paroxysmal	A+W	14	PC6,PC4,SP6,SP10	30	C
			C	54 (28/26)	64 ± 7		W				
[17] Park J	2015	Korea	T	40 (-)	-	Persistent	A	70	PC5,PC6,ST36,ST37,HT7	20	B
			C	40 (-)			-				

T: Treatment group; C: Control group; A: Acupuncture; a: amlodipine; D: Digitalis; W: Wenxinkeli; -: undescribed; m: male; f: female. PC4: Ximen; PC5: Jianshi; PC6: Neiguan; HT7: Shenmen; ST36: Zusanli; ST37: Shangjuxu; ST40: Fenglong; CV6: Qihai; CV12: Zhongwan; CV17: Tanzhong; SP6: Sanyinjiao; SP10: Xuehai; BL14; Juyinshu; BL15: Xinshu; BL17; Geshu.

There were 4 Chinese and 4 English articles, having a total of 633 AF patients (323 in the treatment group and 310 in the control group). All the 8 articles mentioned “random”, but four articles mentioned digital random method [10], [13], [16], [17]. Except for one B grade, the others were C grade. The minimum age of the patients in the literature was 43 years old, and the maximum age was 79 years old, while the proportion of men to women was not mentioned [14], [17]. The literature was published between 2007 and 2015. There were 6 Paroxysmal AF cases [10], [12], [13], [14], [15], [16], and 2 cases of persistent AF [11], [17]. There were 3 articles that highlighted the time of AF cardioversion to sinus rhythm [10], [13], [16]. 3 articles mentioned the heart rate after treatment [10], [13], [16] and the adverse effects were discussed in 2 articles [10], [13]. All literature refer to “NeiGuan”(PC6) [10], [11], [12], [13], [14], [15], [16], [17]. The needling time was for more than 30min [10], [13], [15], [16] Figure 1, and Table1.

Outcome Measure

The total efficacy. The studies showed no heterogeneity (Chi^2 = 7.14, I^2 = 2%, P > 0.05); the combined effects using a fixed-effects model showed RR of 1.38 (95% CI: 1.25 to 1.53) and Z of 6.21 (P < 0.05).

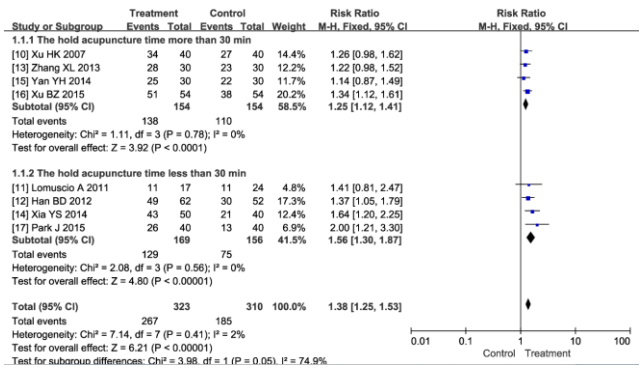

Figure 2: Forest plot of the total efficacy

Compared to the control group, the total efficacy of the treatment group was significantly enhanced. There was difference between retention time more and less than 30 minutes (Chi^2 = 3.98, I^2 = 74.9%, P = 0.05) (Figure 2).

The rate of AF cardioversion to sinus rhythm: The studies showed no heterogeneity (Chi^2 = 2.03, I^2 = 0%, P > 0.05); the combined effects using a fixed-effects model found an RR of 1.40 (95% CI: 1.16 to 1.69) and Z of 3.49 (P < 0.05). Compared to the control group, the rate of AF cardioversion to sinus rhythm of the treatment group was significantly enhanced (Figure 3).

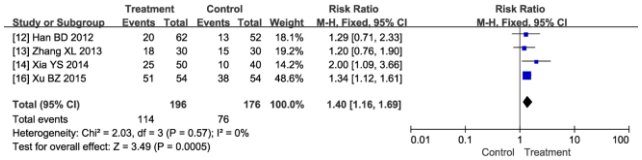

Figure 3: Forest plot of the rate of AF cardioversion to sinus rhythm

The time of AF cardioversion to sinus rhythm: The studies showed heterogeneity (Chi^2 = 4.61, I^2 = 57%); the combined effects using a random-effects model showed an MD of -3.95 (95% CI: -4.98 to -2.91) and Z of 7.46 (P < 0.05). Compared to the control group, the cardioversion time of treatment group was significantly decreased (Figure 4).

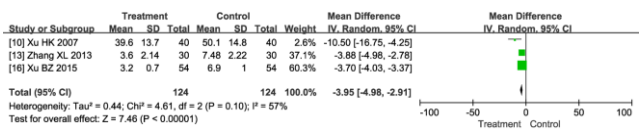

Figure 4: Forest plot of the time of AF cardioversion to sinus rhythm

Heart rate after acupuncture treatment: The studies showed heterogeneity (Chi^2 = 66.55, I^2 = 97%, P < 0.05); the combined effects using a random-effects model showed MD of -14.54 (95% CI: -24.09 to -5.00) and Z of 2.99 (P < 0.05). Compared to the control group, the heart rate of the treatment group

was significantly reduced (Figure 5).

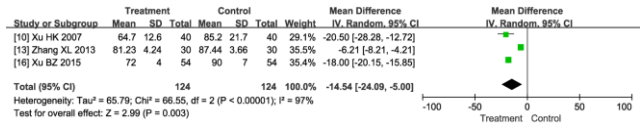

Figure 5: Forest plot of the heart rate after acupuncture treatment

Incidences of adverse effects: The studies showed no heterogeneity (Chi^2 = 0.9, I^2 = 0%, P > 0.05); the combined effects using a fixed-effects model showed RR of 0.48 (95% CI: 0.21 to 1.11) and Z of 1.72 (P < 0.05). Compared to the control group, the adverse effects incidence of the treatment group was significantly reduced (Figure 6).

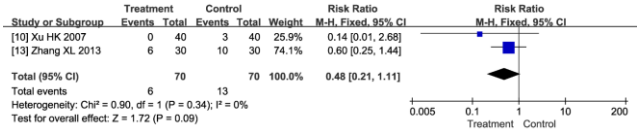

Figure 6: Forest plot of the adverse effects

Assessment of risk of bias

The funnel plot displayed a symmetrical and funneled shape, indicating little bias (Figure 7).

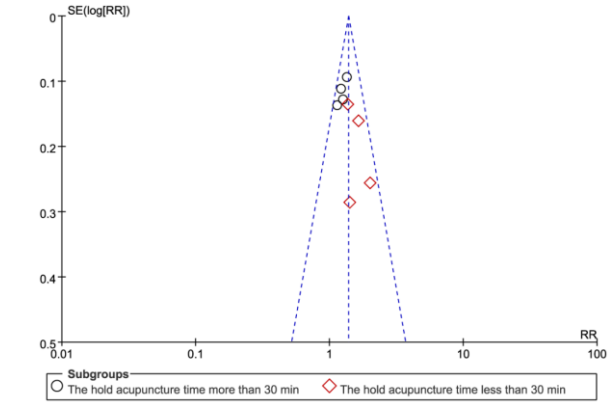

Figure 7: Funnel plot of the risk bias

Discussion

The treatment efficiency of 633 AF patients treated with acupuncture was analysed in 8 RCTs. It was confirmed that the total efficiency was improved after acupuncture treatment of AF. Further study found that the rate of restoration of sinus rhythm was enhanced, and the time for restore of AF sinus rhythm, heart rate and adverse effects incidence after treatment was reduced. There was a difference between retention time more and less than 30 minutes. Van Wormer et al. analysed 8 kinds of literature about needles treatment for arrhythmia (including SVT, AF, and ventricular extrasystole etc.), the results showed that 87% patients were converted to sinus rhythm, and there was 100% decrease in the frequency of arrhythmia [19]. Dilber et al. showed that when a 57 years old patient with paroxysmal AF was treated with acupuncture after other methods failed to convert AF, it successfully converted AF to sinus rhythm. During 30 days follow up, there was no AF recurrence in the patients after sinus rhythm was established by acupuncture combined with peroral propafenone [20]. These experiments illustrated that acupuncture was effective in treating AF patients, but they were not completely randomised controlled trials (RCTs), and the cases of AF were few. Therefore, the conclusion had some limitations.

AF is a trial tachyarrhythmia characterised by rapid and disordered atrial electrical activity. A fast and irregular ventricular rate is the main cause of discomfort symptoms such as palpitation and chest distress. The rapid ventricular rate can make the ventricular diastole shorten, cardiac output reduce, blood pressure drop and coronary blood perfusion decrease, which induce or aggravate myocardial ischemia, and may lead to tachycardia cardiomyopathy. Rate control and rhythm control can relieve AF patients [5], [6]. This study showed that the rate of restoration of sinus rhythm was enhanced, and the time for restore of AF sinus rhythm and heart rate after treatment were reduced, which further indicated that acupuncture had an obvious therapeutic effect on patients with AF.

In this study, two literature referred to the adverse effects of acupuncture in treating AF patients. In the control group, adverse effects were shown through blood pressure decrease, vomiting, gastrointestinal reactions, bradycardia, and QT interval extension. There were bradycardia, gastrointestinal reactions, dizziness and dry mouth in the treatment group. The results showed that the adverse drug reactions occurrence rate of the treatment group was significantly reduced. Therefore, it was believed that acupuncture was safe to treat AF patients.

Acupuncture basic theory is based on the meridian and the tendon theory, the meridian theory states that the human body surface and internal organs are associated with twelve meridians, twelve tendons, twelve skins, fifteen collaterals and countless small collaterals, which unite the body, skull, musculoskeletal and trunk. Many acupoints and meridians have antiarrhythmic effect, such as the Meridian of Minister of Heart, the energy produced by acupuncture can be transferred to other areas of the heart through the related nerve fibers continuously, which can balance Yin and Yang [21], [22], [23], adjust ion channel [24], anti-inflammation [25] and modulate cardiac autonomic function [26]. The needle retention is a period of time when the needle is injected into the acupoints, acupuncture curative effect is closely related to the retention time. Because the time of needling is determined by the

pathogenesis, the course of illness, the patient, and the meridians, the retention time is different for each disease [27], [28], [29]. The effect of acupuncture treatment time on the effect of AF therapy was also compared and differences between different acupuncture times were noticed. This result suggests that the acupuncture treatment for AF patients should be administered for at least 30 minutes. Presently, there are few clinical and experimental studies on the relationship between acupuncture effect and needle retaining time among patients with AF. In view of Chinese traditional medicine, taking the randomness of the uncertain time of “qi arrival” as a reference, it is feasible to set the needle retention time at 30 minutes [30]. However, there is a need to prove whether longer acupuncture time translates to better effects.

The results of the experiment are often influenced by heterogeneity. There was heterogeneity in this study, and the reasons were as follows: 1) the origin of AF disease in the 8 articles were as follows: rheumatic heart disease [10], [11], [12], [14], coronary heart disease [10-12, 14], hypertensive heart disease [10], [12], [14], dilated cardiomyopathy [11]; 2) the original disease treatment drugs were different, for example, for coronary heart disease lipid-lowing drug and antiplatelet drugs were used. For dilated cardiomyopathy, myocardial nutrition drugs were administered, while for hypertension, pressure decrease drugs were taken, etc.; 3) For the diagnostic criteria of AF, two cases come from a 2005 book on the practice of internal medicine [10] and 2000 Emergency internal medicine science [12]. Two cases come from literature guidelines [15], [16], whiles others did not describe the source; 4) the treatment course and the position and time of acupuncture were different; 5) the quality of the literature was low, and all mentioned randomly in this study, but not all of them were described in detail. Only one case referred to double blindness; 6) the number of samples was small, and most of the literature came from China, which brought about unavoidable language and location bias. Therefore, it is necessary to follow the basic guidelines for reporting clinical trials conducted with clarity.

In conclusion, although fewer factors may affect the results of this meta-analysis, we believe that acupuncture can treat for AF, through improving conversion rate, slowing the heart rate, by reducing adverse drug reactions and the time of AF cardioversion to sinus rhythm. It is therefore worthy of clinical application.

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Review Article

Transverse Skeletal Effects of Rapid Maxillary Expansion in Pre and Post Pubertal Subjects: A Systematic Review

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Keywords: Rapid maxillary expansion; Rapid palatal expansion; Pre-pubertal; Post-pubertal; Skeletal effects

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Abstract

OBJECTIVE: The aim of this systematic review was to assess the transverse skeletal effects of rapid maxillary expansion (RME) in pre and post-pubertal subjects.

MATERIAL AND METHODS: Five databases were searched till May 2018; Pubmed, Cochrane, Scopus, Lilacs and Web of science in addition to the manual search of other sources. There were no language restrictions. Methodological Index for Non-Randomized Studies MINORS was used to assess the quality and risk of bias of the trials included.

RESULTS: Six studies were finally included in the qualitative analysis. A meta-analysis wasn't performed due to the heterogeneity of methodologies and outcomes. All of the included studies showed drawbacks in their structure yielding weak evidence. On the short term, RME caused an increase in the maxillary and lateral-nasal widths in pre-pubertal subjects by 3.4 mm and 3.3 mm, and by 2.8 and 2.2 mm respectively in post-pubertal subjects. Although statistically insignificant, the maxillary width increase was more than that of the post-pubertal subjects by 0.6 mm. Over the long term, expansion produced permanent increases in the transverse dimensions of both the dento-alveolar and skeletal components of the maxilla and circum-maxillary structures in pre-pubertal subjects. The post-pubertal subjects presented with a statistically significant increase only in the later-nasal width by 1.3 mm than the untreated controls with no permanent increase in the skeletal maxillary width.

CONCLUSION: The literature is very deficient regarding the use of skeletal age as a reference in the treatment of skeletal crossbites using RME. Only weak evidence exists supporting the increased maxillary and lateral-nasal widths after tooth-tissue borne RME in pre-pubertal subjects, with these effects being less in the post-pubertal ones.

Introduction

Transverse maxillary deficiency is a common finding among populations, mostly presented with a unilateral or bilateral posterior crossbite. The posterior crossbite is reported to be the most prevalent type of malocclusion occurring between 8% and 22% [1]. Whether skeletal or dental, crossbites should be treated once diagnosed, as it is believed that skeletal crossbites affect TMJ functions, chewing patterns, breathing habits and tongue posture. The main goal of skeletal crossbite correction resulting from a maxillary deficiency is achieving transverse skeletal expansion of the maxilla with the least dental effects allowing optimum coordination of the maxillary and mandibular dental arches.

In 1975, Melsen [2] divided the morphological development of the median palatine suture (MPS) into 3 stages, proposing that opening of the suture through maxillary expansion is best done before the age of 15 years as, after that age, the growth of the suture was observed to be ceased. This conclusion was also highlighted in 1977 when Person and Thilander [3] demonstrated in a histological study that the degree of suture obliteration increases from the juvenile period

to adulthood, yet complete suture closure was rarely found until the third decade of life. Accordingly, practitioners for decades have been using the chronological age to decide the treatment of choice for the transverse maxillary skeletal deficiency: slow or rapid maxillary expansion (SME/RME) for that who are under 15 years of age and surgically assisted rapid palatal expansion (SARPE) for those who are above that age [4].

Since categorising subjects by chronological age has many limitations compared to measuring the developmental status of individuals about specific stages of skeletal maturation, Fishman [5] in 1994 correlated between his famous eleven skeletal maturational stages and the percentage of the MPS closure using occlusal radiographs. Surprisingly, the study found that at the maturational age of skeletal maturational index (SMI) 11, only 50% of the MPS was approximated. Since then, authors [6], [7], [8], [9], [10] started to investigate the validity of the chronological age in decision making when it comes to expansion, and a paradigm shift has occurred with an ample of researches [11], [12], [13], [14], [15], [16], [17], [18] aiming at suture separation in young adults through non-surgical RME. These studies tried to make use of the partially fused MPS and performed non-surgical RME in postpubertal subjects. A debate has aroused since then around the success of non-surgical RME in adults when compared to RME in pre-pubertal subjects.

To our knowledge, a well-designed systematic review evaluating the effects of RME in pre- and post-pubertal subjects; according to the skeletal age, hasn't been conducted yet. Such review would help to deliver the status of evidence to solve this debate and provide a guide to future clinical practice, aiming for the optimum treatment option customised to each patient according to his/her skeletal age.

Hence, the aim of the current systematic review was to provide the answer to the following question: in Pre- and Postpubertal subjects with skeletal maxillary constriction, does rapid maxillary expansion (RME) cause widening in the mid-palatal suture (MPS) and/or an increase/change in any skeletal transverse maxillary measurement?

Material and Methods

This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement and the Cochrane handbook for systematic reviews of intervention [19]. The protocol was registered at the Evidence-Based Center, Faculty of Dentistry, Cairo University (online registration was not performed).

Information sources, search strategy, and study selection Pubmed, Cochrane Central Register of Controlled Trials (CENTRAL, The Cochrane Library), Lilacs, Web of Science and Scopus were electronically searched till May 2018 with no language restrictions. Details of the PubMed search are shown in table [1]. Manual Search was performed in the following journals *American Journal of Orthodontics & Dentofacial Orthopedics, The Angle Orthodontist, European Journal of Orthodontics, Progress in Orthodontics, Orthodontics & Craniofacial Research* and *Seminars in* Orthodontics (Figure 1).

Table 1: PubMed search strategy

#1	Crossbite OR crossbite OR skeletal crossbite OR skeletal cross bite OR posterior crossbite OR posterior crossbite OR bilateral crossbite OR unilateral crossbite OR constrict* maxilla OR narrow maxilla
#2	Rapid palatal expat* OR rapid maxillary expan* OR palatal expan* OR maxillary expan* OR bone borne maxillary expan* OR tooth borne maxillary expan* OR miniscrews assisted rapid maxillary expan* OR miniscrews assisted rapid palatal expan* OR maxilla* wide* OR Haas OR hyrax
#3 (#1 AND #2)	Cross bite OR crossbite OR skeletal crossbite OR skeletal cross bite OR posterior crossbite OR posterior cross bite OR bilateral crossbite OR unilateral crossbite OR constrict* maxilla OR narrow maxilla AND Rapid palatal expan* OR rapid maxillary expan* OR palatal expan* OR maxillary expan* OR bone borne maxillary expan* OR tooth borne maxillary expan* OR miniscrews assisted rapid maxillary expan* OR miniscrews assisted rapid palatal expan* OR maxilla* wide* OR Haas OR hyrax

At the same time, grey literature and unpublished data were electronically searched at the *Central Library of Cairo University, Egyptian Universities Libraries, Clinical Trials.gov* and *Edinbrugh Research Archive* using the following keywords: "posterior crossbite", "hyrax", "rapid maxillary expansion", "rapid palatal expansion" an "maxillary transverse deficiency".

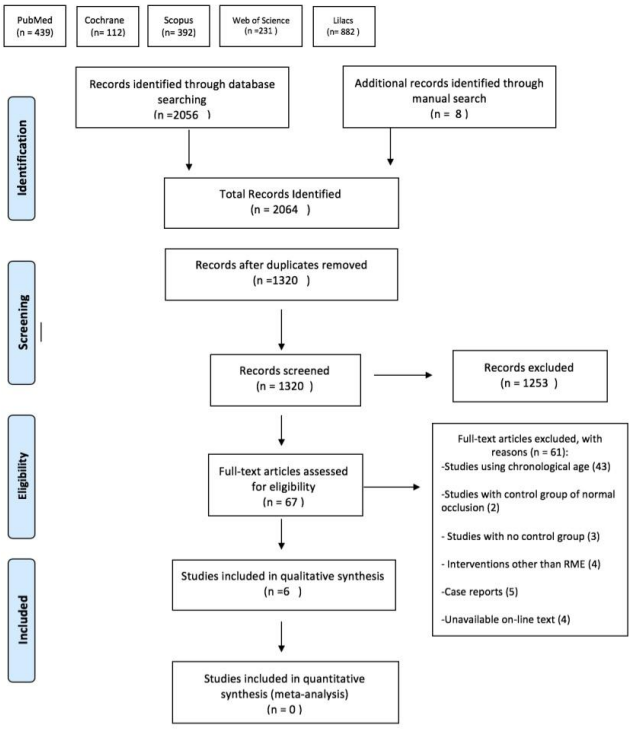

Figure 1: PRISMA diagram of article retrieval

Two investigators (N.S) and (S.K) independently performed the search and any points of disagreements or conflicts were finalized through discussion with the third author (M.F), and the final inclusion of the study was after the agreement.

Eligibility Criteria

The search was conducted upon certain inclusion and exclusion criteria shown in Table 2. An article was considered eligible if it included at least two treatment groups; a study group with rapid palatal expander and an untreated control group. After removal of the internal and external duplicates, the articles obtained from all the databases and manual search were screened by title, abstract and full texts. The full text was obtained when titles and abstracts were insufficient to decide.

Data Items and Collection

The investigators used the identified articles to develop data extraction sheets to, and data were extracted by each investigator independently. The following data were collected: study design, demographic data including participants' maturational status, number, gender and number of groups, an appliance for RME, its anchorage and design, the frequency of activation, retention protocol and timing and method of records collection.

Quality Assessment of Individual Studies

Evaluation of the methodological quality was intended to be performed using the Cochrane Risk of Bias Tool [19] to analyse the risk of bias in RCT's. An overall assessment of the risk of bias (high, unclear, low) was made according to seven criteria for assessment: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of assessors, incomplete outcome data, selective reporting of outcomes, and other potential sources of bias.

MINORS [20] (Methodological Index for Non-Randomized Studies) was the tool of choice for non-randomized controlled and retrospective studies due to its simplicity and reliability. For each article, 12 points were to be assessed: a clearly stated aim, inclusion of consecutive patients, prospective collection of data, endpoints appropriate to the aim of the study, unbiased assessment of the study endpoints, follow up period appropriate to the aim of the study, loss to follow up less than 5%, prospective calculation of the study size, adequate control group, contemporary groups, baseline equivalence of groups and adequate statistical analyses.

The items are scored 0 if not reported, 1 if reported but inadequate or 2 if reported and adequate. The global ideal score is 16 for non-comparative studies and 24 for comparative studies.

Table 2: Inclusion and exclusion criteria of the studies

	Inclusion Criteria	Exclusion Criteria
1. Population	- Any age group concerning the maturational status. - Both sexes - Transverse Skeletal maxillary constriction	- Not referring to the maturational status index - Dental & functional cross bites - Skeletal mandibular asymmetry - Cleft lip and palate - Syndromic patients
2. Intervention	Any rapid palatal expander (tooth-borne or bone-borne)	- SARPE - Corticotomy or any other surgical intervention/ Distractors - Receiving fixed orthodontic treatment or any conjunctive treatment as headgear, face mask, chin cup…etc
3. Comparator	Presence of untreated control groups	If a control group is absent/ with normal occlusion/ receiving any orthodontic treatment
4. Outcomes	The primary outcome of the search was to detect any change in maxillary transverse skeletal measurements following RME. Secondary outcomes included detecting changes in: 1. Nasal airway 2. Maxillary and mandibular dental arches 3. Facial and nasal soft tissues.	Any irrelevant outcome
5. Study Design	1. Randomised clinical trials (RCT's) 2. Quasi-randomised clinical trials. 3. Prospective controlled clinical trials (CCT's) 4. Retrospective controlled trials.	- Abstracts - Comments - Case Reports - Narrative Reviews - Case series - Expert opinion - Systematic reviews and meta-analyses - In vitro studies.

Summary Measures and Approach to Synthesis

The final studies included in this review measured different aspects of the effects RME that made the collected data not combinable. Thus, a meta-analysis was not performed due to the great clinical heterogeneity and variation among the measured outcomes, and only a qualitative analysis was performed.

Results

Study Selection and Characteristics: Two thousand and sixty-four articles were obtained from the electronic search of the five databases and eight articles obtained from the manual search. By applying the inclusion and exclusion criteria, 67 articles were found to be eligible and were read as full text.

Most of the excluded studies [11], [21], [22], [23], [24], [25], [26], [27], [28], [29], [30], [31], [32], [33], [34], [35], [36], [37], [38], [39], [40], [41], [42], [43], [44], [45], [46], [47], [48], [49], [50], [51], [52], [53], [54], [55], [56], [57], [58], [59], [60], [61], [62], [63] divided the samples into treated and untreated control groups according to their chronological age and not according to their skeletal age. Seventeen studies [11], [21], [22], [26], [27], [36], [37], [38], [45], [46], [47], [51], [52], [54], [60], [63], [64], [65] had an untreated control group with normal occlusion or with other types of malocclusions other than posterior

crossbites. Other reasons for exclusion included, subjecting the control groups to treatment [11], [32], [44], case reports [66], [67], [68], [69], using interventions other than RME [50], [51], [53], [54], [56], [64], [65], [70] or absence of a control group [31], [71], [72]. Articles which couldn't be retrieved also were excluded [73], [74], [75], [76], [77], [78]. Finally, six trials [12], [79], [80], [81], [82], [83] were included in the qualitative analysis; four prospective controlled studies [79], [80], [82], [83], one retrospective study[12] and one prospective with a retrospective control group [81] that evaluated the long term changes of RME. Two researchers studied the effect of RME in pre and post pubertal subjects [12], [79] while the remaining four articles evaluated the effects in pre-pubertal subjects only [80], [81], [82], [83].

Description of intervention

In the previously mentioned studies, bonded acrylic splints, banded Haas, bonded and banded hyrax was used for expansion without using any bone borne appliances. The studies reported that expansion was found sufficient when observing the palatal cusps of the upper molars occluding with the buccal cusps of the lower ones [79], [80], [81], [83] or when the amount of screw expansion reached 10.5mm [12]. One study[82] didn't mention the reference for stopping the activation. Retention protocol varied among the six studies from leaving the appliance in situ for an average of 5 months [81], [83], 65 days [12] and one week then replacement by a removable appliance [79]. The method of retention wasn't mentioned in two studies [80], [82] and only the immediate RME effects were evaluated. The characteristics of the included studies are shown in Table 3.

Risk of Bias within the Studies

As mentioned previously, no RCTs were included in the qualitative assessment, and so the MINORS tool was used for quality assessment. Upon applying the Minors methodological index for grading of the 6 included articles, the resulting scores were found to be below the ideal global score 24 [20]. The highest score recorded was 21[83], and the last one was 13 [79]. All the studies had lack of blinding in measurements. The studies didn't report any dropouts or change in follow up sample ratio and lacked proper sample size calculations Table 4.

Results of Individual Studies

Treatment effects of the RME were analyzed according to each study's main outcome: postero-anterior radiographs [12], digital dental casts [81], [83], acoustic Rhinometry [79], 3 dimensional stereo-photogrammetry [80] and direct measurements of the nasal soft tissues were used [82] and shown in Table 5.

Table 3: Design, patients' demographic characteristics, treatment duration and retention, interventions, and outcomes of studies included in the qualitative synthesis.

	Study Design	Eligibility Criteria		Total Sample *n*=	Grouping	Appliance Used / Anchorage	Average Tx Time	Retention Protocol	Records Taken, Timing	Reference of Maturation	Outcomes
		Inclusion Criteria	Exclusion Criteria								
Baccetti et al (2001) [13]	Retrospective Controlled Trial	NA	NA	42	ETG= 29 LTG=13 ECG=11 LCG=9	Haas -2 turns/day till the expansion screw reaches 10.25mm	About 21 days	Haas kept in place for an average of 65 days (42-75 days)	Postero-anterior X-rays T0,T1, T6	CVM ETG:CVS1-3 LTG: CVS 4-6 ECG:CVS 1-3 LCG:CVS4-6	-Transverse skeletal measurements. -Dental measurements.
Bicakci et al. (2005) [16]	Prospective Controlled Trial	-Transverse maxillary deficiency -Bilateral CB -No history of nasal disease -Presence of adequate nasal cavity space.	NA	58	ETG=16 LTG=13 ECG= 16 LCG= 13	Hyrax (fully tooth& tissue borne) -2turns/ day	23-27 days	Hyrax kept in place for 1w, then a new removable app used for retention for 3m	Acoustic Rhinometry At T0, T1, T2 (T0 &T3 only for controls)	CVM ETG: CVS1-3 LTG: CVS4-6 ETC: CVS-3 LTC: CVS4-6	Minimal cross-sectional area (MCA) of the nasal cavity.
Dindaroglu et al. (2016) [17]	Prospective Controlled Trial	-Skeletal Maxillary Transverse Deficiency -Skeletal development not exceeding MP3 cap stage -No history of orthodontic treatment.	NA	50	S.G= 25 C.G= 25	Bonded type expansion appliance (Extended onto occlusal surface of post teeth) -2turns/ day	14-19 days	NA	3D Stereo-photogrammtric images in NHP (natural head position) At T0,T1	MP3 cap stage	Facial soft tissue changes
Geran,et al (2006) [18]	Prospective Controlled Trial with a retrospective control	-Crowding -Lingual CB -Esthetics -The tendency towards a CI.II malocclusion	NA	77	S.G=51 C.G= 26	Bonded acrylic splint appliances (Bonded acrylic splints covered upper D, E,6) -1turn/day	NA	Appliance left in place for 5m, then stabilisation with a palatal plate full time for 12m	Digital dental casts T0, T6 (after completion of phase II treatment), T7	CVM (CVS 1-3)	Transverse dental changes, molar angulation
Santariello et al. (2014) [19]	Prospective Controlled Clinical Trial	NA	NA	102	S.G= 61 C.G= 41	Banded Hyrax on upper 6 -Protocol of activation NA	NA	NA	Nasal Soft Tissue Width Measurement. T0, T1, T3	CVM (CVS 1-3)	Changes in nasal soft tissue dimensions
Ugolini et al. (2016) [20]	Prospective Controlled Clinical Trial	-Unilateral posterior CB -Early or mid-mixed dentition stage -Angle Cl.I or Cl.II - CVS 1-3 -No orthodontic treatment in maxilla or mandible	-Angle Cl.III -Previous orthodontic treatment. -Hormonal imbalances -TMD, Arthritis -Craniofacial Abnormalities	48	S.G= 33 C.G= 15	-Banded Haas, tooth tissue supported -2turns/ day	3w	Haas was left in situ 5-9 m	Digital dental casts At T0, T4	CVM (CVS 1-3)	Transverse mandibular dental changes.

CB; crossbite, CVS; cervical vertebral stage, ETG; early treated group, ETC: early treated control, LTG; late treated group, LTC; late treated control, SG; study group, CG; control group, T0; pre-expansion, T1; immediate post-expansion, T2; 3 months post-expansion, T3; 6 months post expansion, T4; 1 year post- expansion, T5; 3 years post- expansion, T6; 5 years post-expansion, T7: 10 years post expansion.

Bacetti et al., [12], assessed the short and long term skeletal and dental effects of RME in both pre and post-pubertal subjects. On the short term, RME was found to cause more skeletal effects in the pre-pubertal than the post pubertal subjects, causing a statistically significant increase in the latero-nasal width (1.1 mm more than the post-pubertal group) and a statistically insignificant increase in maxillary width (0.6 mm more than the post-pubertal subjects). In both groups, increments for maxillary intermolar width were about 9 mm. Regarding the long-term effects, in the pre and post-pubertal groups, RME therapy produced a significant net gain over the controls of 2.7 mm and 3.5 mm in maxillary intermolar width, respectively. This increase was associated with a significant skeletal maxillary widening and an increase in the laternonasal width only in the early-treated group by 3mm and 2.3 mm respectively. Bicakci et al., [79] evaluated the overall changes produced by RME in both pre and post-pubertal subjects.

Table 4: Applying the MINORS tool on the 6 included studies

	Accetti et al (2001) [13]	Icakci et al (2005) [16]	Indaroglu et al (2016) [17]	Eran et al (2006) [18]	Antariello et al (2014) [19]	Golini et al (2016) [20]
Clearly stated aim	2	2	2	1	2	2
Inclusion of consecutive patients	1	2	2	2	2	2
Prospective Collection of Data	0	1	2	1	2	2
Endpoints appropriate to the aim of the Study	2	2	2	1	2	1
Unbiased assessment of study endpoint	0	0	0	0	0	0
Follow up period appropriate to the aim of Study	2	2	2	2	2	2
Loss to follow up less than 5%	2	0	0	2	0	2
Prospective calculation of the study size	0	0	2	0	0	2
Adequate control group	2	2	1	2	1	2
Contemporary Groups	0	0	2	0	2	2
Baseline Equivalence of Groups	1	0	1	2	1	2
Adequate Statistical Analyses	2	2	2	2	2	2
TOTAL SCORE	**4**	**3**	**8**	**5**	**5**	**1**

They revealed that there was a statistically significant increase in the nasal minimum cross-sectional area (MCA) by 0.34 cm^2 and 0.19 cm^2, and a significant gain over the controls of 0.26 cm^2 and 0.17 cm^2, in both groups respectively. Although the increase in MCA was greater in the pre-pubertal group, the difference was not statistically significantly greater than the post-pubertal group.

Table 5: Main outcomes of the studies included in the search.

	Skeletal Measurements (median)							Nasal Measurements				Facial Soft tissue					Dental Measurements						
		T1-T0		T6-T0				MCA (T2-T0)				Nasal Soft Tissue Width					T1-T0 (median)			T6-0			
		ETG	LTG	ETG	ETC	LTG	LTC											ETG	LTG	ETG	ETC	LTG	LTC
Baccetti et al (2001) [13]	Bizygomatic width	0.4	0.3	10.2	11.6	3.2	2.2										U6 width	9.3	8.1	3.2	0.5	3.5	0.0
	Maxillary Width	3.4	2.8	4.3	1.3	1.8	0.9										L6 width	0.7	0.2	0.7	0.4	1.4	-0.9
	Lateronasal width	3.3	2.2	4.5	2.2	2.2	0.7																
Bicakci et al. (2005 [16]								ETG	ETC	LTG	LTC												
								0.34± 0.26	0.08± 0.10	0.19±0.16	0.02± 0.03												
Santariello et al. (2014) [17]												SG				CG							
													T1-T0	T3-T0	T3-T1	T3-T0							
												AB	0.42	0.36	-0.06	-0.09							
												GAC	0.88	0.79	-0.09	-0.14							
Dindaroglu et al. (2016) [18]													T1-T0										
													U Face	L Face	U Lip	L Lip	Nose						
												SG	0.42 ± 0.17	0.69 ± 0.30	0.62 ± 0.28	0.60 ± 0.34	0.41 ± 0.21						
												CG	0.16± 0.08	0.18 ± 0.07	0.13 ± 0.09	0.17 ± 0.11	0.12 ± 0.07						
Geran et al. (2006) [19]																		T6-T0	SG		CG		
																		IMW	U	4.3 ± 2.0	0.9 ±1.1		
																			L	1.7 ± 1.5	1.9±1.8		
																		IPW 2nd	U	5.3 ± 2.0	1.5 ± 1.4		
																			L	3.2 ± 1.5	2.5±1,8		
																		IPW 1st	U	5.3 ±2.0	1.6 ±1.3		
																			L	4.1 ±1.8	3.4± 2.2		
																		ICW	U	4 ± 1.6	1.4 ±1.5		
																			L	1.5±1.6	1.0±1.7		
																		Arch	U	0.9 ± 3.2	-1.8 ±2.3		
																		Perimeter	L	-2.4 ±3.4	-4.4 ±2.5		
																		Molar	U	6.2 ±5.6	3.2 ±4.1		
																		angulation	L	-5.4 ±6.7	-3.3 ±5.7		
Ugolini et al. (2016) [20]																		T4-T0		SG	CG		
																		L IMW		1.1 ± 1.5	-0.8 ± 0.8		
																		L ICW		0.4 ± 1.6	-0.6 ±0.8		
																		Molar angulation		5.2 ±6.3	-3.5 ±5.5		
																		Canine angulation		0.7 ±6.4	-4.4 ±5.8		
																		Incisor angulation		1.9 ±3.6	-2.4 ±3.7		

AB; distance bet the widest points of the nose insertion into the soft tissues of the face, GAC; distance bet right and left alae, IMW; intermolar width, IPW 2ND: interpremolar width at 2nd premolar, IPW 1st; interpremolar width at 1st premolar, ICW; intercanine width, U; upper, L; lower, ETG; early treated group, ETC: early treated control, LTG; late treated group, LTC; late treated control, SG; study group, CG; control group, T0; pre-expansion, T1; immediate post-expansion, T2; 3 months post- expansion, T3; 6 months post expansion, T4; 1 year post-expansion, T5; 3 years post- expansion, T6; 5 years post- expansion, T7: 10 years post expansion.

Long term treatment changes with RME followed by fixed appliances were assessed by Geran et al., [81] in pre-pubertal subjects.

Their results showed that RME produced greater increments in all variables for maxillary and mandibular arch widths when compared with the controls. Maxillary arch perimeter increased in the study group by (0.9 mm) and decreased in the control group by (1.8 mm). On the other hand, the mandibular arch perimeter decreased less in the study group by (–2.4 mm) versus (– 4.4 mm) in the control group. Long term treatment changes with RME followed by fixed appliances were assessed by Geran et al., [81] in pre-pubertal subjects. Their results showed that RME produced greater increments in all variables for maxillary and mandibular arch widths when compared with the controls. Maxillary arch perimeter increased in the study group by (0.9 mm) and decreased in the control group by (1.8 mm). On the other hand, the mandibular arch perimeter decreased less in the study group by (–2.4 mm) versus (– 4.4 mm) in the control group.

Regarding the effect of RME on soft tissue nasal width in pre-pubertal subjects, Santariello et al., [82] reported an increase in the distance between the widest points of the right and left alae of (0.8 ± 0.2 mm) in the study group with insignificant changes in the distance between the widest points of the insertion of the nose into the soft tissues of the face. When compared to the control group after a retention period of 6 months, a statistically significant increase of only the GAC width was found in the study group.

Spontaneous mandibular response to RME in pre-pubertal subjects was assessed by Ugolini et al., [83] 15 months post-expansion. The study reported a significant increase in the mandibular intermolar width by 1.9 mm. Control subjects showed a tendency towards contraction of the transverse dimensions and a decrease in a molar, canine, and inferior incisor angulation values.

Discussion

Summary of Evidence

Since the debate is still ongoing, and there is no exact recommendation of the best timing or age to perform a successful RME, hence the aim of the present review had emerged. The objective of this systematic review was to search the literature for any valid evidence supporting the effects of RME in pre and post-pubertal subjects.

RME has many effects on the nasomaxillary complex other than transverse maxillary expansion and correction of crossbites. Effects of RME were studied on skeletal transverse, vertical and anteroposterior position of the nasomaxillary structures [84], [85], maxillary and mandibular transverse arch dimensions [86], [87], upper airway dimensions [88], [89], bite force, changes in the masticatory cycle and occlusal force distribution [90], swallowing [25] and condylar response [91], [92], [93], changes in head posture and scapular position, natural head position [94], enamel demineralization and white spot lesion formation [23], [57], hearing loss [95], [96], nocturnal enuresis [21], [97], [98], eruption of 3rd molars [55], Class ll div1 [99], speech and voice function [58], [100], [101], obstructive sleep apnea(102), tongue posture [103] and Holdaway soft tissue analysis [104]. Effects of RME have also been studied on basal bone changes even in the absence of crossbites [105].

For almost four decades, since Melsen [2] revealed the three maturational stages of the MPS, concepts surrounding RME had greatly changed. Great variability of suture fusion among different age groups had been shown [5], [8], [106] Trials that correlate the MPS maturation with different skeletal maturity methods are increasingly aiming to find the eldest adolescent or youngest adult that could benefit from RME treatment [7], [10], [11], [13], [18], [107]. Hence, the time to shift to using the individual's skeletal age rather than the chronological age to gain the maximum effects out of non-surgical RME. Thus, one of the most important inclusion criteria of the studies in this review was dividing the sample according to their skeletal age. Moreover, including studies with untreated control groups was also important to overcome the confounding effect of the craniofacial growth during the study period [35], [43]. The control group had to have a transverse skeletal deficiency as well because various malocclusions are associated with a distinctive craniofacial pattern [108], so it would make more sense to compare subjects with the same malocclusion.

Upon searching the literature, this systematic review included both prospective and retrospective controlled studies that classify their samples according to their skeletal age. Sixty-seven articles were read by full text, and only 6 articles were finally included, four of which were prospective controlled trials, one was a prospective trial with a retrospective control and the final study was a retrospective trial. No well-designed RCTs were found to be eligible for this search, and most of the trials included in this search had methodological problems. None of the studies included assessed the effects of bone- borne RME and instead, tissue and tooth borne RME were studied. Bone borne RME might have yielded different results in this case. The outcomes of the six studies were quite variable as shown in Table 3. Two trials studied the effects of RME on the dental arches: Geran et al., [81] measured the change in both the upper and lower intermolar width, interpremolar width, intercanine width and molar angulation after 5 years of expansion in pre-pubertal patients.

On the other hand, Ugolini et al., [83] measured the changes mentioned above only in the lower arch 15 months following RME, and so the pooling of the results into a quantitative search was impossible as the study endpoints were extremely different. Hence, a meta-analysis wasn't performed. Although non-comparable to one another, the conclusions of Geran et al., [81] resembled that of an earlier systematic review [109] evaluating the long-term dental changes following RME. Two studies [11], [45] included in the mentioned review evaluated the long-term changes on dental casts showing an increase in both the maxillary and mandibular intermolar width with the increase being greater in the maxillary arch.

Since the demand for perfect esthetics is becoming as important as obtaining comfortable, healthy and stable results after orthodontic treatment, the effect of RME on facial soft tissues was considered as an important secondary outcome in this review. These changes were measured by Dindaroglu et al., [80] using 3 D stereophotogrammetric photographs immediately following RME via 3D deviation analyses on 3D stereophotogrammetric images. Being in close relation to the nasomaxillary complex, the nose changes was of great importance and showed a mean maximum deviation limit of 0.77 ± 0.34mm and -0.94 ± 0.41 mm. On the other hand, Santariello et al., [82] targeted the changes in nasal soft tissue widths following RME in pre-pubertal subjects only. Although statistically significant, the increase in the width of the base of the nose was considered clinically insignificant. Nasal measurements were also the primary outcome of interest of Bicakci et al., [79]; the trial measured the change in the MCA of the nasal cavity using acoustic Rhinometry following RME in pre and post-pubertal subjects. It concluded that RME not only expanded the maxilla but decreased the nasal resistance through effectively increasing the nasal MCA, being more effective in pre-pubertal than post-pubertal patients. This finding is emphasised by a recent trial [110] that assessed the short-term nasal changes following RME in mouth breathers using cone beam computed tomography. The study concluded that not only does RME increases the nasal airway volume, but is also capable of changing the nasal form and function, increasing a mouth breather chances to gradually attain normal nasal breathing. In 2016, a systematic review of systematic reviews and meta-analyses [111] was conducted to assess the dental and skeletal effects of palatal expansion techniques among different chronological age groups. It reported that significantly more favourable immediate skeletal changes occurred when RME was performed before the pubertal growth peak, based on low evidence [112], [113]. This systematic review, however, didn't strictly select the articles that used the skeletal age indices in their subject recruitment strategy. Conclusions of that review were mainly based on the study by Bacetti et al., [12] that assessed the transverse skeletal and dental effects of RME in pre and post-pubertal subjects and reported that maxillary adaptations to RME are more likely to be of a dento-alveolar origin rather than a skeletal one when expansion was performed in post-pubertal subjects. Although being the first study to assess the impact of skeletal age on the results of RME, the trail had many limitations. In addition to being a retrospective study, the inclusion and exclusion criteria of the recruited sample were questionable. It wasn't clear if RME was the sole treatment received by the study group or if expansion was performed in conjunction with other treatment modalities as part of a comprehensive orthodontic treatment. Multiple confounders could have been present at baseline, were the only reported baseline similarity between the study and control groups was the skeletal maturational stage without referring to the type of malocclusion of the control group. Comparing changes following RME in transverse skeletal deficiencies to normal skeletal relationships might be misleading. Also, a great variation among the group sizes was observed where 29 subjects were in the early treated group versus 11 controls, and 13 subjects were in the late treated group versus 9 controls. The study had a high detection bias due to the absence of blinding of outcome assessment. Definitive conclusions can't be withdrawn from such a trial owing to the mentioned methodological limitations Correctly choosing the most applicable tool for quality assessment of the non-randomized studies included in the review was of great importance. An ideal index should be simple, yet highly sensitive and reliable, and hence MINORS was used [20], [114]. Since the global ideal score is 24 for comparative studies [20], any article scoring less than 24 points will be considered as of 'poor quality'. In our search, the included articles scored 14 [12], 13 [79], 18 [80], 15 [81], 15 [82] and 21[83] points in the MINORS scale and hence, all studies give poor-quality evidence supporting their outcome. A definitive conclusion cannot, therefore, be drawn neither upon the short- or long-term skeletal effects of RME nor upon its effects on the nasal airway, maxillary and mandibular dental arches, facial and nasal soft tissues in pre and post-pubertal subjects.

Strengths and Limitations: The current review search strategy had strict inclusion and exclusion criteria, had no language restrictions, included multiple electronic database searches and involved a detailed manual searching process. The evidence retrieved from this systematic review has to be interpreted with caution as retrospective studies were included.

Conclusions

According to the existing evidence from this review, the following conclusions could be stated regarding the transverse skeletal effects of RME in

pre and post-pubertal subjects:

1. The quality level of the studies included in this review was not sufficient to draw conclusive evidence regarding the transverse skeletal effects of RME in pre and post-pubertal subjects.

2. Studies considering the skeletal age for a successful RME treatment are very scarce in the literature. The only available weak evidence suggests that patients treated by tooth-tissue borne RME before the pubertal peak exhibit more increase in the skeletal transverse dimension than do post-pubertal ones, with the pre-pubertal sample being more stable in their long-term treatment results than the post-pubertal.

3. Regarding the transverse skeletal effects of RME, as well as its effect on maxillary and mandibular dental arches, nasal airway, facial and nasal soft tissues, weak evidence supports positive changes in those outcomes in pre-pubertal subjects.

4. For post-pubertal subjects, the only available low-quality evidence suggests an increase in the nasal cross-sectional area immediately following RME.

Implications for Research: Carrying out a well-designed randomised controlled clinical trial is strongly recommended to assess the various effects of RME in pre and post-pubertal subjects. Future research should recruit the samples according to their skeletal age and should ensure the presence of a true skeletal maxillary constriction in both the study and control groups.

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Review Article

Gut Microbiome, Probiotics and Bone: An Updated Mini Review

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Abstract

The gut microbiome is now considered as a large organ that has a direct effect on gastrointestinal tract, immune and endocrine system. There is no evidence that gut microbiota regulates the immune system and is responsible for bone formation and destruction. Probiotics have been shown through the gastrointestinal tract to have a positive effect on the management of the healthy bone. This article discusses the latest data available from PubMed and Scopus databases regarding gut microbiome, probiotics and bone briefly.

Introduction

Gut microbiome (GM) is the largest one that includes trillions of bacteria, fungi and viruses that live in the intestinal tract. These *bacteria* have been found to modulate immune responses that are associated with many diseases such as Crohn's, irritable bowel syndrome, celiac, cardiovascular, and rheumatic [1]. GM is a major regulator of bone mineral density via the effects of the immune system [2], [3].

It has been reported by several clinical trials the association of low intestinal bacteria and decreased bone mineral density [4]. There is no convincing evidence of the role of GM in the development of bone formation and destruction [4]. Very recently the importance of microbiome health has become now clear; many consider it as a new large organ, while specific strains have been identified to affect immune cells [5].

Review of Text

Osteoporosis is a major public health burden in our ageing population [6]. Bone undergoes turnover continuously, and the immune system is regulating this process since 1980 [7]. This chronic progressive bone disease is mainly due to the natural cessation of endogenous estrogen marked by the onset of menopause and characterised by decreased bone mineral density (BMD) and negative changes in bone microarchitecture [8]. Estrogen cessation gives rise to two stages of bone loss: an early rapid loss of trabecular and cortical bone due to increased osteoclast activity and decreased osteoclast apoptosis, and a second slower prolonged loss due to decreased osteoblast activity [9]. This notable imbalance between bone formation and bone resorption increases the risk of fractures among postmenopausal women [10], [11]. Although estrogen therapy (ET) was shown effective in the prevention and treatment of estrogen-deficient osteoporosis in

postmenopausal women, its use remains controversial due to its association with increased risk of breast, endometrium and ovaries cancer [12].

Recently, alternative and complementary therapies such as dietary supplements have become the preferred prophylactic treatment of clinicians and patients in the prevention and management of osteopenia [13]. Meta-analyses have shown that daily intake of 1200 mg Calcium (Ca) and 20–25 mg vitamin D (D) can reduce total- and hip-fracture risk by 15% and 30%, respectively, [14], hence, it seems that Ca and D supplementation alone is not sufficient to fully prevent the menopausal bone loss.

In the past decade, a growing body of evidence suggests that probiotics may have favourable effects on bone health. Probiotics are living microorganisms that could influence the GM composition and exert positive effects that have been attributed to several complex mechanisms, including enhanced mineral absorption, beneficial anti-inflammatory pathways [15] and many more. Probiotic strains differ significantly in genotype and phenotype, and they may show different metabolic functions, particularly with regards to immune function [16].

In earlier reports, various strains of Lactobacillus and Bifidobacterium were shown to prevent and even restore bone loss related to estrogen deficiency [17], they were also shown to cause a 45% increase in femoral and vertebral trabecular bone volume fraction in mice [18]. More recently, a randomized, double-blind, placebo-controlled study [19] revealed that multispecies probiotic supplementation (7 specific strains: *Lactobacillus casei, Bifidobacterium longum, Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus bulgaricus, Bifidobacterium breve* and *Streptococcus thermophiles*) among postmenopausal osteopenic women diminished bone resorption through significant effects on serum concentrations of bone biomarkers, such as serum bone-specific alkaline phosphatase (*s*-BALP) and serum C-terminal telopeptide of type I collagen (CTX). Decreased serum levels of BALP, the marker of bone turnover and CTX-produced by osteoclasts during the bone resorption process- compared to the placebo group, suggest a protective effect of this multispecies probiotic against bone resorption. These results are consistent with earlier studies using *Bifidobacterium subtilis* (CH201) [20], *Bifidobacterium longum* [21] and *Lactobacillus reuteri* [22].

The beneficial effects of probiotics on nutrients absorption were highlighted in several recent reports. Various strains of Lactobacillus and Bifidobacteria were shown to influence the pH of the gut and the metabolism of bile acids [23], important factors in the control of nutrients absorption, especially calcium. Lactobacillus helveticus fermented milk exhibited an acute positive effect on calcium metabolism. Thus, in addition to the well-established benefits of calcium and vitamin D content of milk, it is possible that some types of probiotics may aid in the breakdown of proteins contained in milk to biologically active peptides [24]. Furthermore, elevated concentration of Lactobacillus reuteri and *Bifidobacterium longum* in the gut may be involved in promoting mineral (calcium, magnesium, and phosphate) absorption resulting in an increased BMD. Probiotics were also shown to play an essential role in the synthesis of vitamin B and vitamin K, which are critical for the regulation of bone health [25].

On another note, various strains of probiotics were shown to affect bone health via the production of short-chain fatty acids (SCFA). SCFA are byproducts produced by the microbiota during fermentation of dietary fibre and were reported to have direct effects on osteoclasts and osteoblasts. For example, butyrate is an SCFA known to reduce osteoclastogenesis by suppressing the receptor activator for nuclear factor κB ligand signalling pathway [26]. More recently, SCFA were shown to have indirect effects on endocrine factors such as peptide YY and glucagon-like peptide 1 Peptide YY is a gastrointestinal hormone secreted from the endocrine L cells and is negatively associated with the total body and hip BMD in premenopausal women [27]. Glucagon-like peptide 1, an amino acid hormone that is also secreted from the endocrine L cells, has been shown to act as a regulator of bone metabolism by altering the balance between osteoblast and adipocyte differentiation from bone mesenchymal stem cells [28].

When it comes to the immune-modulatory properties, probiotic administration was shown to reduce the expression of several pro-inflammatory and osteolytic cytokines such as Tumor Necrosis Factor-α (TNF -α) and Interleukin-1b (IL-1b) [29]. *Lactobacillus reuteri, Lactobacillus rhamnosus* and *Lactobacillus paracasei* [30] were shown to decrease osteoclastogenesis and bone resorption significantly. *Lactobacillus reuteri* enhanced the suppression of pro-inflammatory cytokines TNF-mediated bone resorption in mice [19] and enhanced the reduction of the percentage of CD4C T cells in bone marrow [22]. The treatment was able to improve bone health in healthy male mice [19]. Similarly, decreased TNF-α and IL-1b, along with increased anti-inflammatory cytokine IL-10 also resulted from oral administration of Saccharomyces cerevisiae [31].

One of the recent applications of probiotics is the incorporation of *Bacillus spp.* in birds' feeds to promote growth, as an alternative to the harmful antibiotic growth promoters (AGP). Several studies show that certain strains of Bacillus subtilis also promote the growth of chickens to a greater extent than AGP [32].

On the other hand, it is important to note that, in contrast with the reported benefits of probiotics on bone health, a recent study [6]

showed that dietary enrichment with powdered whole grape and probiotics (composed of equal parts *Bifidobacterium bifidum, B. breve, Lactobacillus casei, L. plantarum*, and *L. bulgaricus*) exerted either no effect on bone microarchitecture in a mouse model of age-related osteoporosis. However, this negative effect was attributed to possible differences in probiotic strains, the small sample size and the duration of the supplementation.

Conclusions

Current research efforts, although varied, mostly indicate favourable effects of probiotics on bone metabolism. Therefore, long-term investigations with different strains of probiotics are needed to dissect the mechanisms and effects on bone formation and resorption, especially in humans.

This relationship is a promising area of investigation, which potential outcomes could lead to physicians directing their therapeutic efforts to probiotics, among dietary supplements, for most effective treatments for bone-related ailments.

Future directions are focused now on the major role of the gut microbiome in rheumatic disease, and a lot of interest is growing in the gut microbiome manipulation as a therapeutic tool for bone diseases. Preclinical models may also be a future promise for the treatment of bone disease.

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Review Article

Triage Systems in Mass Casualty Incidents and Disasters: A Review Study with A Worldwide Approach

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Abstract

BACKGROUND: Injuries caused by emergencies and accidents are increasing in the world. To prioritise patients to provide them with proper services and to optimally use the resources and facilities of the medical centres during accidents, the use of triage systems, which are one of the key principles of accident management, seems essential.

AIM: This study is an attempt to identify available triage systems and compare the differences and similarities of the standards of these systems during emergencies and disasters through a review study.

METHODS: This study was conducted through a review of the triage systems used in emergencies and disasters throughout the world. Accordingly, all articles published between 1990 and 2018 in both English and Persian journals were searched based on several keywords including Triage, Disaster, Mass Casualty Incidents, in the Medlib, Scopus, Web of Science, PubMed, Cochrane Library, Science Direct, Google scholar, Irandoc, Magiran, Iranmedex, and SID databases in isolation and in combination using both and/ or conjunctions.

RESULTS: Based on the search done in these databases, twenty different systems were identified in the primary adult triage field including START, Homebush triage Standard, Sieve, CareFlight, STM, Military, CESIRA Protocol, MASS, Revers, CBRN Triage, Burn Triage, META Triage, Mass Gathering Triage, SwiFT Triage, MPTT, TEWS Triage, Medical Triage, SALT, mSTART and ASAV. There were two primary triage systems including Jump START and PTT for children, and also two secondary triage systems encompassing SAVE and Sort identified in this respect. ESI and CRAMS were two other cases distinguished for hospital triage systems.

CONCLUSION: There are divergent triage systems in the world, but there is no general and universal agreement on how patients and injured people should be triaged. Accordingly, these systems may be designed based on such criteria as vital signs, patient's major problems, or the resources and facilities needed to respond to patients' needs. To date, no triage system has been known as superior, specifically about the patients' clinical outcomes, improvement of the scene management or allocation of the resources compared to other systems. Thus, it is recommended that different countries such as Iran design their triage model for emergencies and disasters by their native conditions, resources and relief forces.

Introduction

One of the symptoms of disasters is that the immediate needs of the community affected exceed the available resources, so the question is how these resources should be used to have the best outcome for the people. Triage is the allocation of limited resources during a disaster. Although the concept of triage applies to all resources, "patient care" is the most commonly discussed field for which the notion of triage is used [1]. Triage is one of the key principles of the effective management of major emergencies [2]. Triage is derived from the French word "trier", which means separating, categorising or classifying, and refers to the categorization, classification, and prioritization of patients and injured people, based on their urgent need for treatment [1], [3]. The process of triage allows the respondents of disaster, who do not have enough resources to treat everyone, to prioritize care services, so that most services are provided to the greatest number of injured people, and this is essentially the philosophy of doing triage in disasters and mass casualty incidents [1], [3], [4]. Triage is usually performed at three stages: the primary triage that is carried out at the scene of the incident by an emergency technician aims at the prompt assessment of the injured person and rapid transfer to the treatment center. Secondary triage which is used when, due to the large extent of the incident and lack of resources in the pre-hospital, the transmission of the injured person has been prolonged in the scene.

In these cases, triage will be done by an emergency doctor or surgeon as soon as the injured person arrives at the hospital. The third triage is performed to prioritise and decide on receiving care services, including transferring to the operating room or the intensive care unit. This step will be done by a surgeon or a critical care specialist [1], [5].

The triage system is used by individuals to determine which groups of the patients should receive treatment and care services based on their clinical status, the prognosis of disease and available resources [6].

Methods

The present study was conducted through a review of triage in disasters and mass casualty incidents, with the aim of identifying triage systems, relevant criteria, and the order of these criteria all over the world. Accordingly, all articles published in English and Persian language journals between 1990 and 2018 were searched based on several keywords including Triage, Disaster, Mass Casualty Incidents in the Medlib, Scopus, Web of Science, PubMed, Cochrane Library, Science Direct, Google scholar, Irandoc, Magiran, Iranmedex, and SID databases in separation and combination using *and*/*or* conjunctions. Based on this, all English and Persian articles conducted in the world, which discussed triage systems and their algorithms and also were of desirable quality, were included in the study. Accordingly, poor quality studies, those who discussed triage, but did not provide the information needed for triage algorithms and their criteria, or studies that merely discussed the accuracy and the testing of triage systems were excluded from the study.

Results

Based on the search done, triage systems were grouped into three classes including primary triage systems (adults and children), secondary and hospital triage systems. In this study, twenty primary adult triage systems, two primary children triage systems and two secondary triage systems were identified. Primary triage systems that have been identified include START, Homebush triage Standard, Sieve, CareFlight, STM, Military, CESIRA Protocol, MASS, Revers, CBRN Triage, Burn Triage, META Triage, Mass Gathering Triage, SwiFT Triage, MPTT, TEWS Triage, Medical Triage, SALT, mSTART, ASAV. The triage systems identified for children were Jump START and PTT. Moreover, SAVE and Sort triage systems were identified as far as the secondary triage is taken into consideration. In the hospital triage systems, the ESI triage model amongst five-level triage systems, which has a higher level of validity and reliability, and the CRAMS triage system used to triage patients in the emergency units of the hospitals were identified. These systems are described according to the following algorithm.

START triage system

This system is the most commonly used triage system in the United States. This system is also used in Canada and parts of Australia and the Israeli-occupied territories. It was created by the Newport Beach Fire Department and Hoag Hospital in California in 1980 [1]. In this system, all injured adults older than 8 years are evaluated, based on the algorithm of the system in 60 seconds or less (preferably 30 seconds). In this system, the criteria including the ability to walk, respiratory rate, capillary filling, radial pulse and obeying the commands are used. By examining each criterion, the patient will be marked by one of the red, yellow, green and black tags (Figure 1) [7].

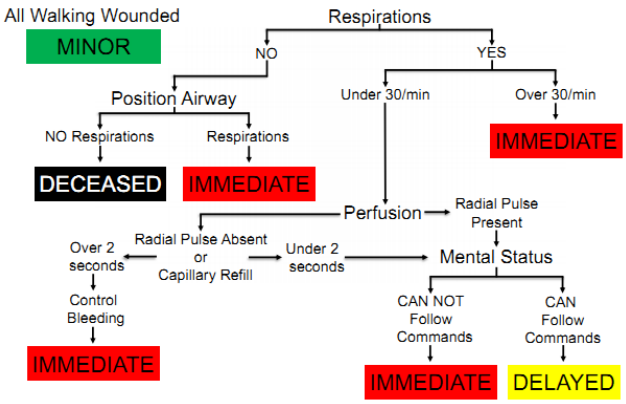


Figure 1: START Triage Algorithm (Bhalla, 2015) [7]

Since the capillary filling criterion in the dark and cold environments in emergencies and disasters is not an appropriate reflection of the circulatory system, this criterion has been omitted in the modified model of the triage system (MSTART) (Figure 2). The only therapeutic measures allowed in this method are opening the airway of the patient and controlling the bleeding by direct pressure on the site of the bleeding.

Reverse Triage

Reverse triage is a method that is commonly used during emergencies and disasters. In reverse triage, injured people with fewer damages and minor injuries are at the priority of receiving services. This is also used in cases, where the treatment team or soldiers, during the war, are injured.

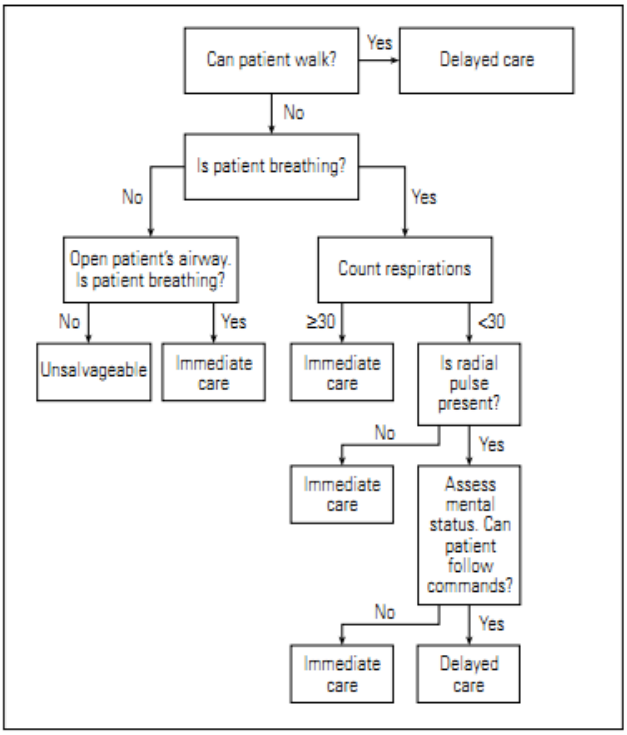


Figure 2: M START Triage Algorithm (Garner, 2001) [14]

Moreover, this kind of triage system is used in the disaster and emergencies, where medical resources are limited, with the aim of returning people as quickly as possible and helping other people [3]. Reverse triage is also a way to increase the capacity of the emergency unit of the hospital during disasters. Accordingly, those patients with mild injuries and those supposed to be without any medical complications for at least 96 hours after discharge are at the top of the discharge list [8].

Military Triage

The main goal of the military triage is to treat and return more injured soldiers to the battlefield. In this method, immediate and rapid classification of the injured people is based on the type and severity of the injury, the probability of survival, as well as the priority of treatment in order to provide the best health care services for the largest number of people [1], [9], [10]. Most military triage systems use T (Treatment) codes including T1, T2, T3, T4 and dead to classify the injured individuals, while others use P (Priority) codes including P1, P2, P3 and P-hold [11].

***MASS triage** (Move, Assess, Sort, Send)*

This system is a disaster triage system used in the United States. Although this system is based on the START triage system, it does classify the injured people before individual examination [1]. This includes four stages of moving, evaluating, classifying and transferring. This system, whose algorithm is very similar to the SALT triage method, has four tags: red, yellow, green and black (Figure 3). Allowed therapeutic measures in this model include opening the airway, controlling bleeding, Antidote injections and chest decompression. After performing the actions for this red group, then the yellow and green groups are considered, respectively [1], [12].

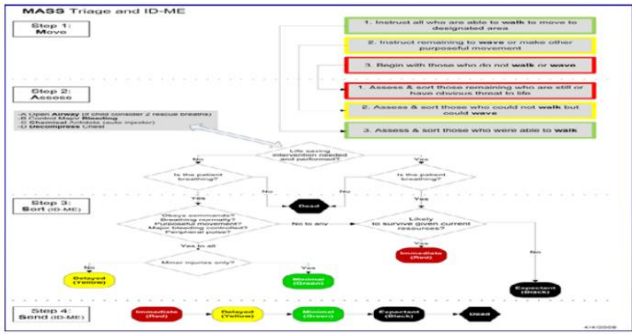


Figure 3: MASS Triage Algorithm (Coule, 2007) [12]

Sieve Triage

Similar to the START method, this method, which is used in parts of Europe, Australia, and the United Kingdom, first uses the walking filter to examine the injured individual, and uses four tags encompassing red, yellow, green and black tags to classify the injured patients (Figure 4) [13], [14], [15].

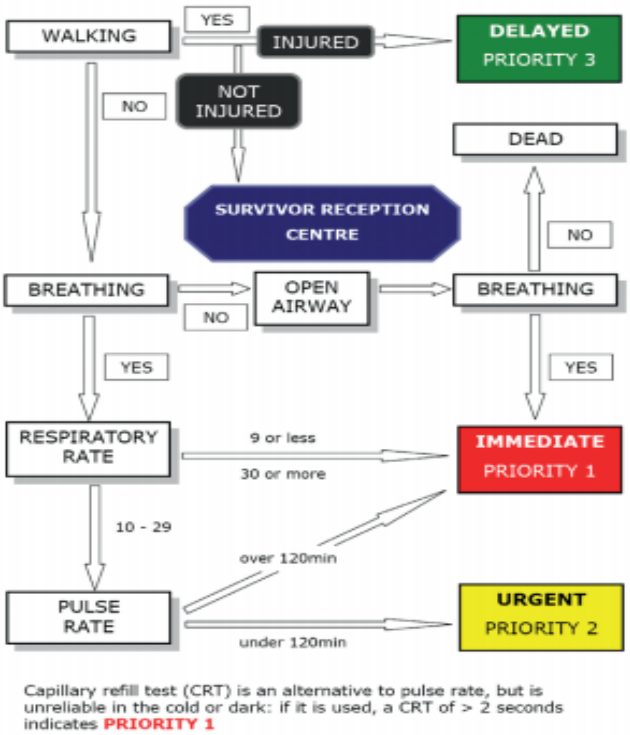


Figure 4: Sieve Triage Algorithm (Smith, 2012) [15]

CESIRA Protocol

This method was designed in 1990. In this method, the injured people fall into three red, yellow

and green classes. The red class includes people, who are unconscious and in shock, have bleeding, and ineffective breathing. The yellow class involves patients with fractures of the bones and other injuries, and the green class includes injured people, who can walk [1], [4].

Homebush Triage

This method was designed in 1999 in Australia, which attempts to integrate the triage protocols in that country [16]. This method is based on START and SAVE triage systems [17] and includes 5 classes of triage (Table 1). Although the application of this system was documented in 2002, there are no data on its accuracy and its impact on specific consequences like other triage systems [18].

Table 1: Classification of the injured people according to the Homebush Triage Standard

Homebush Triage Standard		
RED Immediate	ALPHA	Any of the following: Respirations more than 30 breaths/min. No palpable radial pulse. Not able to follow commands.
YELLOW Urgent	BRAVO	Non-ambulatory patients who do not meet black, white, or red criteria.
GREEN Non-urgent	CHARLIE	Able to walk to a designated safe area for further assessment.
WHITE Dying	DELTA	Dying patients: may have a pulse, but no spontaneous respirations.
BLACK Dead	ECHO	I am not breathing despite one attempt to open the airway.

Triage in special circumstances of the CBRN (Chemical, Biological, Radiological, Nuclear)

Although up to now, damages are often caused by explosion, collision or collapse of buildings in most disasters, there are also other probable scenarios, where damages are caused by chemical, biological, radiation, nuclear, and hazardous materials, which have occurred so far all over the world. It is very difficult to design a comprehensive triage system, which is easy to use and scientifically valid for all hazards. In some resources, it is recommended that, under certain circumstances such as incidents of weapons of mass destruction or hazardous materials, in case of occurring mass casualty incidents, a START-based triage algorithm, with a consideration of a series of special measures based on the type of the incident, such as decontamination, use of personal protective equipment and some special clinical considerations should be used. The SALT triage system is proposed with the aim of establishing a comprehensive method for the triage of injured patients at all hazards, but there is little evidence of its effectiveness in CBRN conditions [19].

CareFlight Triage

This method is a tool for rapid triage in mass casualty incidents, in which such criteria as walking ability, obeying the commands, palpable radial pulses, and airway respiration are evaluated (Figure 5). The injured people are placed in four urgent (red), emergency (yellow), delayed (green) and non-salvageable (black) classes.

The noteworthy point is that in this method the criterion of obeying the commands is examined before the evaluation of breathing and pulse rate. This method is one of the fastest triage methods, which takes only 15 seconds to test each patient [3], [14].

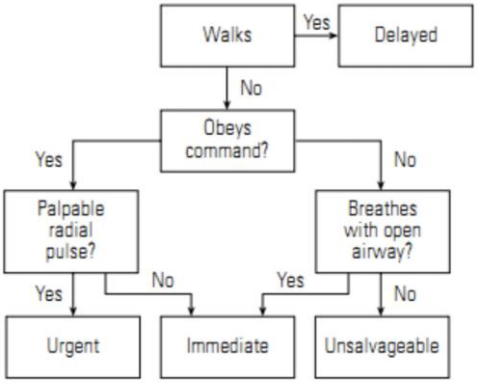


Figure 5: Careflight Triage Algorithm (Garner, 2001) [14]

SALT triage (Sort, Assess, lifesaving intervention, Treatment/Transport)

This is one of the latest triage systems, which was introduced and registered by the CDC in 2008 as a national standard for mass casualty incidents. This process begins by categorising the patients into three groups based on simple voice commands.

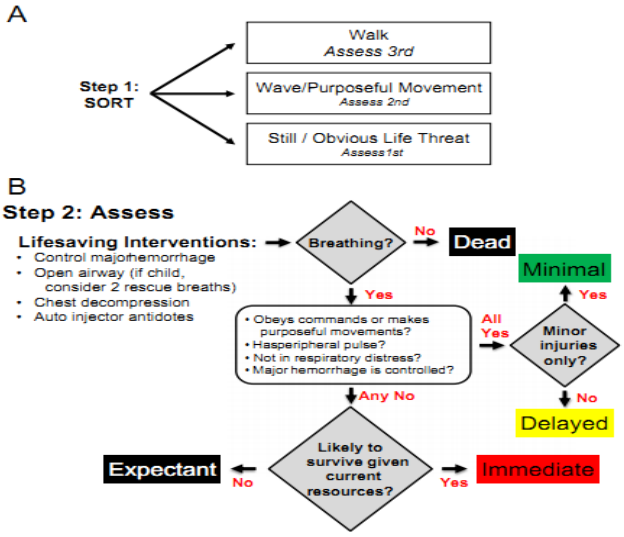


Figure 6: SALT Triage Algorithm (Bhalla, 2015) [7]

The first includes the group of the injured people, who can walk to the area requested by the

person performing the triage. The second group is the injured people, who only can shake their hands or feet, and the third group consists of the injured patients, who have no movement or show life-threatening conditions. This third group will be the first group of individual evaluations. The actions recommended in this kind of triage include airway opening, external bleeding control, Antidote injections for some poisonings, and needle thoracostomy for pneumothorax (Figure 6) [4], [7].

STM (Sacco Triage Method)

This method, which is designed based on a mathematical model and is a numerical triage method, considers the resources, based on time and facilities, in addition to the triage of the injured people. In this method, based on the physiological criteria including respiration, pulse and motor response, the injured people are scored, and by the acquired score, the probability of the survival of the injured person or his death is calculated. The first group of the injured people, with a score of 0-4, is tagged with a black label. The injured people of the second group, who have a score ranging from 5 to 8 are likely to survive through interventions. And the patients of the third group with a score of 9 to 12, have a survival probability rate of more than 90 per cent. After rating the injured people, their situation is announced to the incident command centre and subsequently, hospital resources are considered for the treatment (Figure 7) [3], [20], [21].

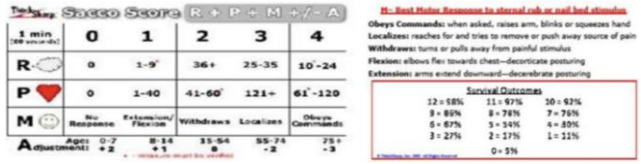


Figure 7: STM Triage Algorithm (Jenkins, 2008) [3]

Burn Triage

In this method, which is used to prioritise injured persons in burn events, the classification of the injured people is based on the severity and level of the burn (Table 2) [22], [23].

Table 2: Classification of the injured people in the Burn triage

Category	Profile
Green group	First- degree and superficial burns
Yellow group	Burns above 30% in people over 5 and under 60 years old
Red group	Second- degree burns in head and neck, genital area and joints Third- degree burns in an anatomical region of the body Burn in people under 5 years of age and over 60 years of age Burn in pregnant women, people with underlying conditions with second- degree burns more than 10%, people with second-degree burns above 30%

META Triage

This method has 4 steps, in which the first and second steps are called Stabilization Triage, and the third and fourth steps are named Evacuation Triage.

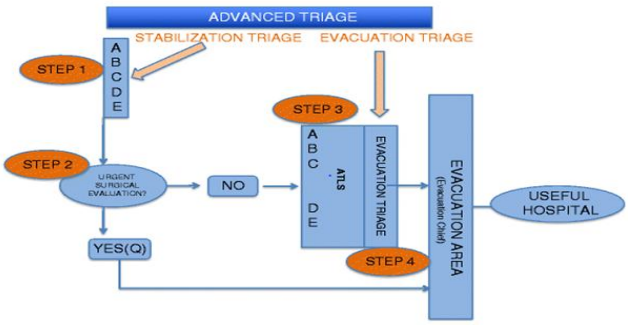


Figure 8: META Triage Algorithm (González, 2016) [24]

In each step, certain actions must be performed according to the algorithm. In the first step, the injured people are placed on the red, yellow, and green classes according to the A, B, C, D and E criteria, and at the next step, the injured individuals are classified based on the evaluation of the surgery and injuries (Figure 8, and 9) [24].

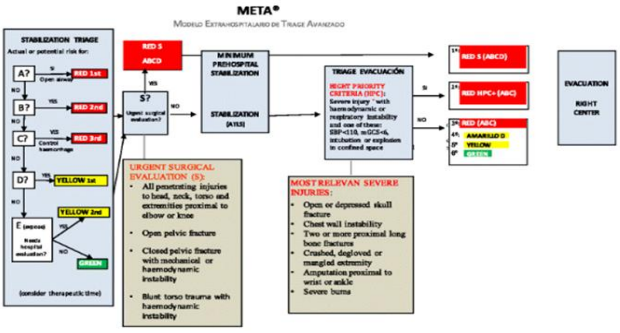


Figure 9: Continuation of the META triage Algorithm (González, 2016) [24]

MASS Gathering Triage

This method is a proposed triage tool for the Australian context in mass casualty incidents that can be used for first responders (Table 3) [25].

Table 3: Classification of the patients in the Mass Gathering Triage (Cannon, 2017) [25]

Category	Description	Vital Signs	Example	Time to see clinician	Rx area
RESUSCITATION	Clinically unstable Requiring active resuscitation Risk of death or severe morbidity without emergent intervention	*Any red BTF criteria RR: <5 or >30 Spo2: <90% HR: <40 or >140 BP: <90 or >200mmHg LOC: responding to pain only, or sudden decrease of >2 points on GCS Pain: severe, uncontrolled pain Temp: <34.5 or >40.0C BGL: <2.0mmol.	• Respiratory or Cardiac arrest • Airway obstruction / choking • Severe respiratory distress • Severe anaphylaxis • Life-threatening bleeding • Major trauma	Immediate	Resus / Acute +MERT
URGENT	Clinically stable At risk of deterioration Needs urgent intervention Potentially life-threatening presentation	*Any yellow BTF criteria RR: 5 – 10 or 25 – 30 Spo2: 90 – 95% HR: 40 – 50 or 120 – 140 BP: 90 – 100mmHg or 180 – 200mmHg LOC: Decrease in LOC from alert to responding only to voice, or new onset confusion Temp: <35.5 or >38.5C BGL: 2.0 – 3.9mmol.	• Shortness of breath • Chest pain • Asthma (moderate) • Systemic allergy • Abdominal pain • Hypoglycaemia	<5 minutes	Acute +HCP review
MINOR	Minor injury or illness requiring assessment or treatment only	Normal vital signs	• Wound (minor) • Soft-tissue injury • ?fractured limb (distal)	<10 minutes	Fast-track +/- HCP
SELF-HELP	Patient could have self-helped if at home	Not required to measure vital signs unless suspicious presentation	Request for: • Band-Aids	<60 minutes	First Aid

Modifiers:
1. Mechanism of injury, i.e. fall >3metres
2. Effects of alcohol and drugs needs to be considered. Abnormal behaviour, especially where there is a serious risk to the patient or others should be up-triaged.
3. Time sensitive medical condition e.g. (i) 12-lead ECG shows ST elevation myocardial infarction; (ii) motor findings suggestive of stroke; (iii) septic shock.
4. Acute effects of exercise may increase RR and HR. This should resolve shortly after cessation of exercise, usually within 5 minutes in healthy individuals

SWiFT Triage (Senior, Without, Families, Team)

This method is a triage tool for disadvantaged older adults during disasters designed to quickly identify the needs of this specific group [26]. This method is designed at three levels and at each level specific actions are taken as shown in Figure 10.

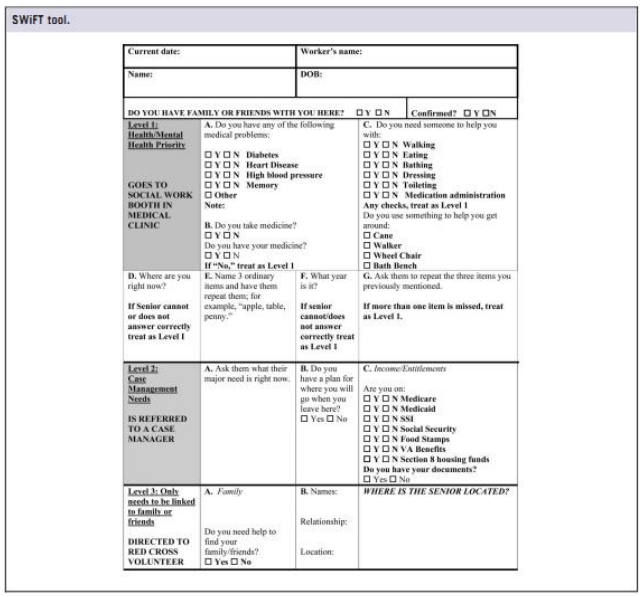


Figure 10: SWiFT Triage Tool (Dyer, 2008) [26]

Medical Triage Protocol

In this protocol, the walking ability criterion is initially controlled, and those who can walk are classified in the green group. Then, other criteria such as the level of consciousness, arterial bleeding, shock, breathlessness, fractures and injuries of the head and spine, and ultimately pathologies such as myocardial infarction, poisoning, burns, hypothermia, and chest pain are checked and the patient is tagged as red or yellow according to the following algorithm (Figure 11) [27].

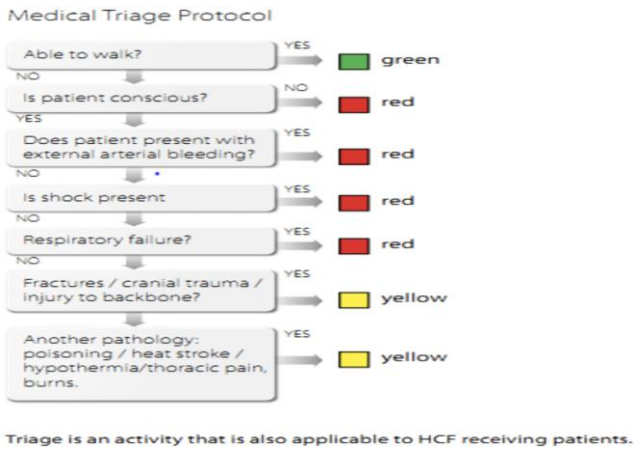


Figure 11: Medical Triage Algorithm (Alexander, 2013) [27]

TEWS triage (Triage Early Warning Score)

This method of triage is a numerical 5- level method, which was designed according to the experts' opinion for the injured people over 12 years of age and above the height of 150 centimetres (Figure 12).

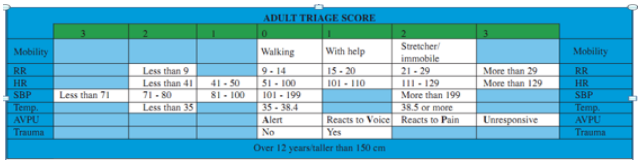
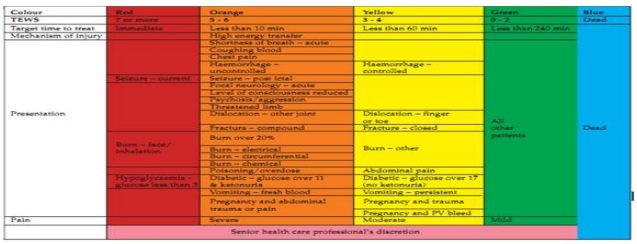


Figure 12: TEWS triage(Wallis, 2006) [28]

The injured person is placed in one of the five classes of red, orange, yellow, green and blue by the final score (Table 4) [28], [29].

Table 4: Classification of injuries in the TEWS triage (Wallis, 2006) [29]



MPTT Triage (Modified Physiological Triage Tool)

The method has four tags including red, yellow, green and black, and the injured patients are assessed based on the ability to walk, respiration, pulse and GCS criteria (Figure 13) [2].

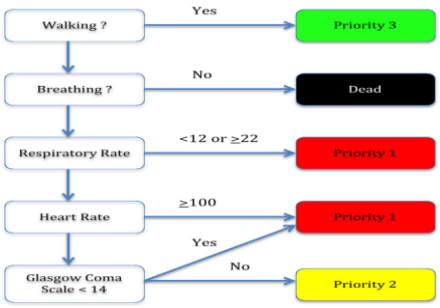


Figure 13: MPTT Triage Algorithm (Vassallo, 2017) [2]

ASAV triage system

Amberg-Schwandorf Algorithm for Primary Triage

In this method, which is considered a primary triage system, the injured individuals are placed in four different classes encompassing red, yellow, green, and black. Accordingly, the injured patient is placed in the black class, when he suffers fatal

injuries. In this method, no respiratory rate is considered for breathing. Instead, some criteria for respiratory distress, such as airway obstruction, bradypnea, apnea, dyspnea, tachypnea and cyanosis, are controlled (Figure 14) [30].

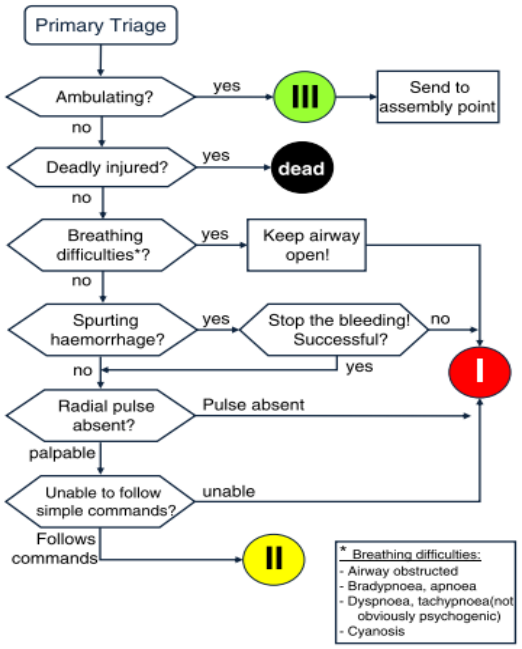


Figure 14: ASAV Triage Algorithm (Wolf, 2014) [30]

Smart Triage System

This method of triage is similar to the START triage system. In this system, it is highlighted that if it is not possible to examine the capillary filling criterion, the radial pulse should be controlled. The injured people are also classified into four categories: red, yellow, green, and black according to the algorithm that sown in Figure 15 [31].

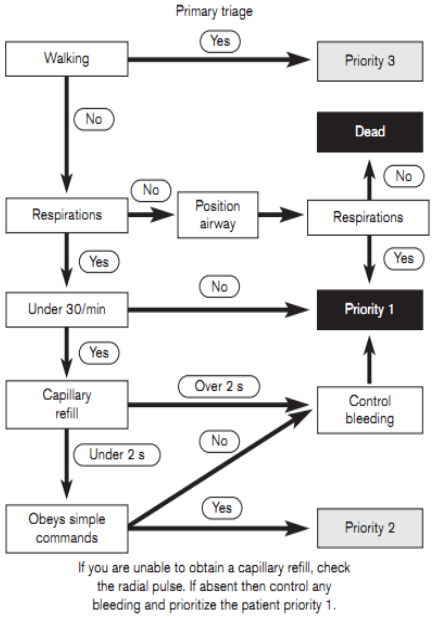


Figure 15: Smart Triage Algorithm (Cone, 2011) [31]

Tactical Triage

In this method of triage, the injured individuals are placed into four classes of green, red, yellow and black (Figure 16). The green group consists of patients, who can walk or have mild damages. The delayed or yellow group includes those patients, who may need surgery, but their general condition allows them to receive any medical or surgical operation with delay and without threatening their life. The immediate or red group includes people, who need immediate medical intervention, including rescue and surgical procedures. The key to the success of the triage is the rapid identification of people with a red tag [32].

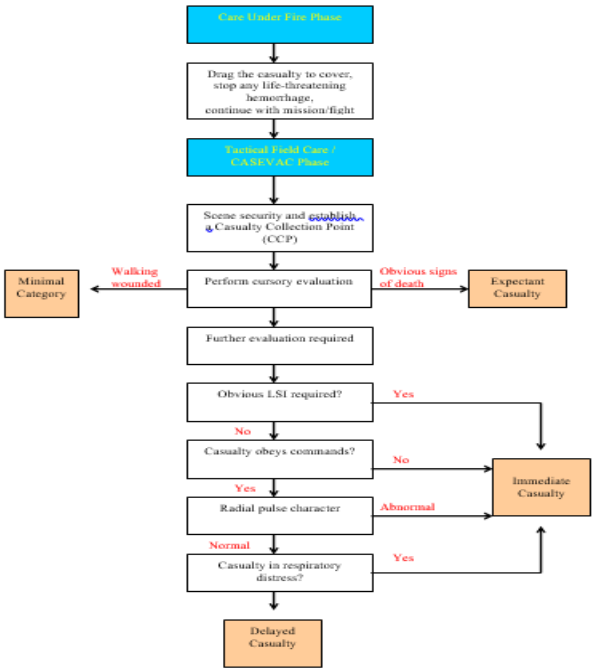


Figure 16: Tactical Triage Algorithm (De Lorenzo, 1991) [32]

Children's triage systems

Why do we need children's triage systems?

There are important and significant physiological and anatomical differences between children and adults, which highlights the need for children's triage systems. Children are more susceptible to head injury, airway obstruction and hypothermia than adults.

Moreover, in children, the respiratory tract is preceded by heart failure. Children have fewer blood counts than adults, and younger children may not have the ability to walk, communicate verbally, and collaborate properly [1]. Two types of these systems have been identified for children, which include Jump START and Pediatric Triage Tape (PTT) [4].

The Jump START triage system

This technique was designed by Dr Romig in 1995 as a tool for the triage of the children under the age of 8, and in 2001, some modifications were made to it, based on the principles of the START triage system [33]. These changes were based on three main differences between adults and children, namely the higher probability of the respiratory failure in children than adults, the number of different breath rates in children, and the inability of the young children to follow verbal commands. In this system, the AVPU was used to assess the level of the children's consciousness, instead of the obeying the commands criterion used in the START triage system (Figure 17) [21].

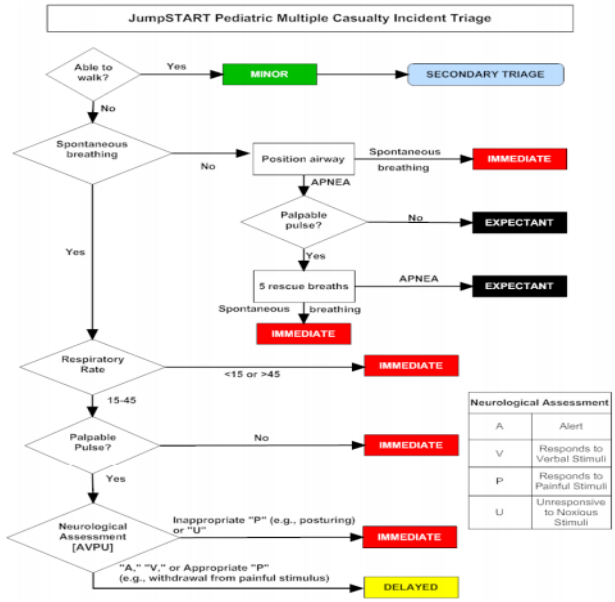


Figure 17: Jump START Triage Algorithm (Romig, 2002) [21]

PTT triage system (Pediatric Triage Tape)

There are three guidelines for this method, based on the height and weight of the infants and children. The first instruction is for the babies with a height of 50 to 80 cm (weighing 3 to 10 kg) (Figure 18). If the child cries and moves his body purposefully, he will be placed in the third priority (delayed). It is necessary to open the baby's airway, in case he does not cry, move and breathe, and if respiration starts after this action, he will be placed at the priority (emergency). Otherwise he would be placed at the last priority (dead). In this guideline, the normal ranges of breathing and pulse are between 20-50 and 90-180 times per minute, respectively. By examining these criteria, the baby is placed in either red, yellow, green or black classes.

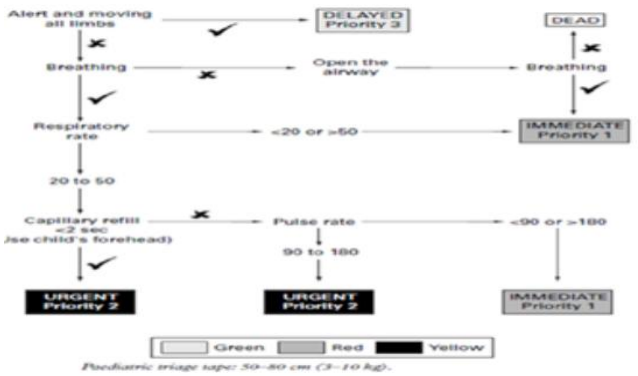


Figure 18: PTT Triage Algorithm in infants with 50-80 cm height [(3 to 10 Kg weight) Hodgetts, 1998] [11]

The second guideline of the PTT triage in a baby with a height of 80 to 100 centimetres and a weight of 11 to 18 kilograms is similar to the first instruction. At this stage, the normal range of respiration and heart rate of the child is 15 to 40 and 80 to 160 times per minute, respectively. In the third instruction, the triage of the child with 100 to 140 cm height (19 to 32 kg weight) is similar to the previous steps. At this stage, the normal number of respiration and pulse rate is 10 to 30 and 70 to 140 times per minute, respectively. In these two stages, it is also necessary to press the child's forehead with a finger to control the capillary filling status [4], [11], [34].

Secondary triage systems

In cases, where the number of the injured people is high, and it is not possible to transfer all the patients to medical centres or hospitals or because of the large extent of the incident and lack of resources in the pre-hospital, the process of transferring all patients from the scene would be prolonged, it is probable that a group of the injured people remains at the disaster scene for a long time. Secondary triage systems are used in these cases as well as at the arrival of the injured patients to the emergency unit of the hospital. The two methods of secondary triage include the SAVE and the Sort triage systems [1].

SAVE Triage

The SAVE method (Secondary Assessment Victim Endpoint) is used to diagnose the patients, who take the most out of the existing care services.

Table5: Criteria in SAVE triage

Criteria in SAVE Triage: Burn Injury, GCS and MESS		
1.Burn Injury: less than 50% chance of survival	2.Head Injury (Adult): Use The Glasgow Coma Score(GCS)	3.Crush Injury to Lower Extremity: Use The MESS Score
70% TBSA Burn	Score 8 or above: Treat better than 50%Chance of a normal or good neurologic recovery	A score of 7 or more: amputate
Age over than 60 with Inhalational injury	Score 7 or less: comfort care only	Score less than 7: attempt limb salvage
Age less than 2 with 50% TBSA Burn		
Age more than 60 with 35% TBSA Burn		

To determine the survival chances and patient classifications, predictive tools of the patient clinical conditions such as limb rescue score, Glasgow Coma Scale (GCS), and survival rate data after burns are used (Table 5, and 6). The injured people, who cannot survive and cannot be treated at the disaster scene, but can be saved if they reach the hospital, will be tagged with a red label. Those patients, who take the most from the available therapeutic interventions, are marked with a yellow tag. Those injured individuals, who can survive even without medical intervention, are tagged with green labels, and finally, the deceased people are labelled with black colour [1].

Table 6: MESS score in SAVE triage

Mangled Extremity Severity Score (MESS)

Type	Characteristics	Injury	Points
1	Low energy	stab wound, simple closed fx, small-caliber GSW	1
2	Medium energy	Open/multilevel fx, dislocation, moderate crush	2
3	High energy	shotgun, high-velocity GSW	3
4	Massive crush	Logging, railroad, oil rig accidents	4
Shock Group			
1	Normotensive	BP stable	0
2	Transiently hypotensive	BP unstable in field but responsive to fluid	1
3	Prolonged hypotension	SBP <90mmHg in field and responsive to IV fluids in OR	2
Ischemia Group			
1	None	Pulsatile, no signs of ischemia	1
2	Mild	Diminished pulses without signs of ischemia	2
3	Moderate	No dopplerable pulse, sluggish cap refill, paresthesia, diminished motor activity	3
4	Advanced	Pulseless, cool, paralyzed, numb without cap refill	4
Age Group			
1	<30y/o		0
2	>30 <50		1

MESS score: six or less consistent with a salvageable limb. Seven or greater amputation generally the eventual result.

From Helfet DL, Clin Orthop 1990 256:80

Sort Triage

This method, which is a kind of secondary triage, has four stages and a numerical system (Figure 19). In this method of triage, patients are tagged according to the score obtained. If the number is 10 or less, the injured individual is placed at the red class, and if the number is equal to 11, he will be placed in the yellow class. A patient with 12 scores will be categorised in the green class [15].

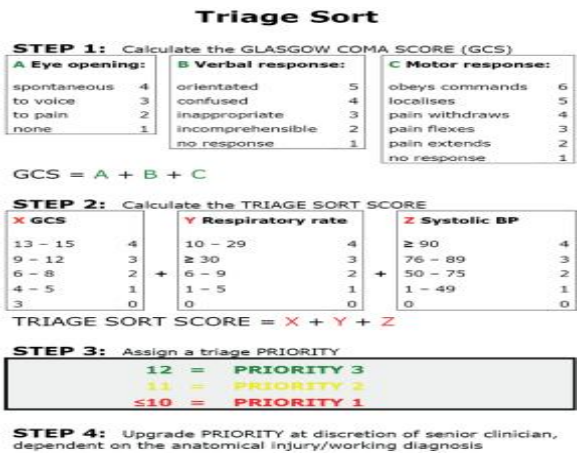


Figure 19: Sort Triage Algorithm (Smith, 2012) [15]

Hospital Triage

The aim of the hospital triage in the emergency department is to place patients in a suitable clinical setting at the right time to receive the appropriate level of health care. There are two, three, four, and five level systems for hospital triage proposed in the world, among which five-level systems including Manchester Triage System (MTS), Canadian Triage and Acuity Scale (CTAS), Australia Triage System (ATS), and and Emergency Severity Index (ESI) have currently shown more validity and reliability scores according to the findings of the previous research [35]. All hospitals should design and develop a program for hospital triage in disaster situations and mass casualty incidents as part of the hospital emergency plan [36].

CRAMS Triage

Circulation, Respiration, Abdominal and Thorax Exam, Motor Response, Speech

This numerical method of triage, as a part of the hospital triage models, is used in some European and American countries (Figure 20). In this method, each criterion is scored from 0 to 2 points. Then, based on the score obtained, the patient with a score of less than 6 will be placed at the immediate class. An injured patient with a score of 7 is placed in the emergency class, and with a score of 8 to 10, he would be categorised in the delayed class [37].

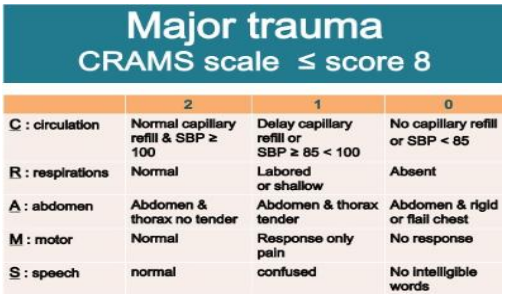


Figure 20: CRAMS triage (Emerman, 1991) [37]

Emergency Severity Index (ESI) Triage

The system was designed in late 1990 in the United States by two emergency medical experts named Richard Weurz and David Eitel [38], [39]. This system, not only determines which patient should be checked first but also indicates which levels of facilities and resources are needed to meet the patient's needs (Figure 21).

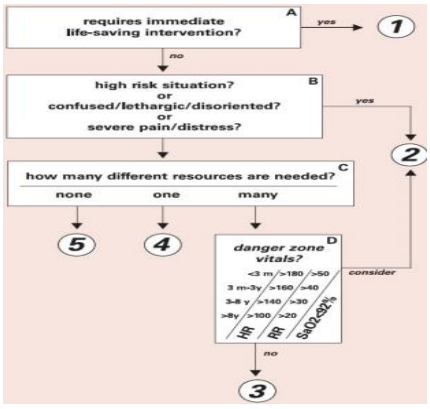


Figure 21: ESI triage algorithm (Eitel, 2003) [38], [39]

Table 7: Comparison of the criteria, their priority and range in different triage systems worldwide

Model	Components and the criteria of the model according to priority - Descriptions required							Model approach
START	1. Ability to walk	2. Respiration	3. Capillary filling	4. Pulse	5. Obeying the commands	The threshold for respiration is 30 times per minute. The pulse has no range or even boundaries, and only its existence or its absence is evaluated.		Algorithmic
Jump START	1. Ability to walk	2. Respiration	3. Capillary filling	4. Pulse	AVPU.5	Breathing between 15 and 45 is normal. The pulse lacks any range. The AVPU criterion is used instead of obeying the commands criterion.		Algorithmic
MSTART	1. Ability to walk	2. Respiration	3. Pulse	4. Obeying the commands	Capillary filling criterion has been eliminated in this model. Breathing below 30 times is considered normal, but there is no range for the pulse criterion and only its presence or absence is controlled.			Algorithmic
Medical	1. Ability to walk	2. Consciousness	3. Arterial bleeding	4. Shock	5. Respiration	6. Traumatic evaluation	The breathing criterion lacks limits and boundaries	Algorithmic
Sieve	1. Ability to walk	2. Respiration	3. Capillary filling	4. Pulse	The respiratory range between 10 and 29 is normal, moreover, the normal range for the pulse is 120 times per minute			Algorithmic
Careflight	1. Ability to walk	2. Obeying the commands	3. Respiration	4. Pulse	In this model, the obeying the commands criterion is controlled prior to the respiration criterion. Respiration and pulse lack any limits or boundaries.			Algorithmic
Mass Gathering	1. Respiration	2. SPO2	3. Pulse	4. Systolic blood pressure	5. Consciousness	6. Temperature and pain	For respiration the range from 10 to 25 and for the pulse criterion the range from 51 to 120, for blood pressure the range of 100 to 180 mm and for the temperature, the range from 35.5 to 38.5 degrees are normal.	Algorithmic

Model	Components and the criteria of the model according to priority - Descriptions required						Model Approach
STM	1. Respiration	2. Pulse	3. Mental status	Walking criterion is not controlled. Breathing ranging from 10 to 24 and a pulse ranging from 61 to 120 are considered natural.			Numerical
MASS	1. Ability to walk	2. Respiration	3. Pulse	4. Obeying the commands	There is no boundary or limit for respiration and pulse. The injured people are evaluated based on the ability or inability to walk in three groups.		Algorithmic
SALT	1. Ability to walk	2. Respiration	3. Pulse	4. Obeying the commands	There is no limit and boundary for respiration and pulse. The injured patients are assessed in three groups based on the ability or inability to walk.		Algorithmic
SAVE	1. Organ rescue scale	GCS.2	3. Burn survival	In the injured people with GCS above 8, and in burns under 50%, young people can hope to survive.			Numerical
Sort	1. Respiration	2. Systolic blood pressure	GCS.3	For respiration, the range of 10 to 29 and for blood pressure the range higher than 90 mm Hg and for GCS the range above 13 are normal.			Numerical
Smart	1. Ability to walk	2. Respiration	3. Capillary filling	4. Pulse	5. Obeying the commands	Breathing below 30 times per minute is normal, but there is no range specified for the pulse, and only its presence or absence is controlled.	Algorithmic
META	1. Respiration	2. Pulse	3. Traumatic evaluation	Criteria A, B, C and D are controlled but the range for respiration and normal pulse is not specified.			Algorithmic

Model	Components and the criteria of the model according to priority - Descriptions required								Model Approach
Homebush	1. Ability to walk	2. Respiration	3. Pulse	4. Obeying the commands	For the respiration criterion, the rate less than 30 times per minute is normal, and there is no specific range for the pulse, and only its presence or absence is controlled.				Algorithmic
CESIRA	1. Ability to walk	2. Awareness control	3. Bleeding	4. Shock	5. Respiration	6. Traumatic evaluation	The respiration criterion has no specific range. Only its quality as well as its presence or absence is controlled.		Algorithmic
PTT	1. Ability to walk	2. Respiration	3. Pulse	4. Obeying the commands	Based on the age and weight, the three ranges including 20 to 50, 15 to 40 and 10 to 30 are normal for respiration. The normal ranges for the pulse criteria are also 90 to 180, 80 to 160, and 70 to 140 times per minute.				Algorithmic
TEWS	1. Ability to walk	2. Respiration	3. Pulse	4. Systolic blood pressure	5. Temperature	6. AVPU	7. Traumatic evaluation	Normal breathing range is 9 to 14 times per minute. The normal range for the pulse criterion is 51 to 100. The normal range for the systolic pressure and temperature is 101 to 199 mmhg and 35 to 38.4, respectively.	Numerical
CRAMS	1. Respiration	2. Systolic blood pressure	3. Motor response	4. Verbal response	5. Abdominal assessment	There is no specific range for breathing, and only the presence or absence of stomach is controlled. The normal systolic pressure is also higher than 100 mm.			Numerical
ASAV	1. Ability to walk	2. Fatal injuries	3. Respiration	4. Control of bleeding	5. Pulse	6. Obeying the commands		Breathing and pulse lack any specific range.	Algorithmic
MPTT	1. Ability to walk	2. Respiration	3. Pulse	GCS.4	The respiration rate is considered to be normal from 12 to 22 times per minute, and for pulse criterion, the range of 100 times per minute is normal. For GCS, 14 and higher is the normal range.				Algorithmic
ESI	1. Respiration	2. Pulse	3.SPO2	There are specific ranges considered for the respiratory and pulse criteria, based on the age range. Moreover, there is a specific range of 92% for the SPO2 criterion.					Algorithmic

The fourth version of the Emergency Severity Index had some modifications and was adopted by the Ministry of Health of Iran as the standard and acceptable method of triage in the emergency department [35]. This system is a useful tool, that can be used in all urban and rural emergency units and general and academic hospitals [38], [39], [40].

Components and approaches of Triage models

According to the results, there are two main numerical and algorithmic approaches for triage in the world. In the algorithmic approach, the injured person is placed in a particular class through examining and controlling each criterion and, if that criterion is normal, the next criterion will be evaluated. But in the

numerical approach, the person performing the triage must first control and evaluate all the criteria in the model. Then, based on the score of each criterion, the final score of the injured person condition, which is based on the total score of all the criteria in the model, is specified. According to the final score, the injured individual is placed in one of the triage classes, which are marked with a specific tag. As indicated in this study, each model of triage consists of several criteria and components. Various ranges are considered for similar criteria of different models of triage. Nevertheless the variety of these criteria is also quite obvious, and even in some of these triage models, the same criteria have different prioritisation. For example, although there are similar criteria in the START and CareFlight models, in the former, unlike START, the criterion of the ability to obey the commands is evaluated before the controlling of the airway and respiratory tract. The following table shows the comparative characteristics of the triage systems in terms of the relevant criteria, their priority and the general approach of the model (Table 7).

Discussion

There are many types of triage systems in the world; however, there is no general or universal consensus on how triage should be performed. As triage is a dynamic procedure, there is no fixed rule for it. Accordingly, these systems may be designed based on such criteria as vital signs, patient's major problems, or the resources and facilities needed to respond to the patient needs. One of the most important features of a standard triage system is its simplicity in performing and reliability [41], [42]. In other words, the most effective triage is a method that is easy for staffs to perform, does not need to classify patients and injured people by complex criteria and at the same time determine the prognosis of the patients at an optimal level.

Because of the specific circumstances of disasters and the constraints for conducting high-quality studies, including randomised, controlled trials in real-world conditions, there are little evidence and information concerning the best method for performing triage and the effectiveness of various types of triage methods [1]. The fact that triage categories should not be considered permanent is of particular importance. After prioritizing, patients may not remain in that particular category during the incident. Therefore, considering that the patient's condition is changing, the re-evaluation of the patient should be done. Given the current congestion of the present-day emergency units, a rapid system for the diagnosis and separation of the acute and injured patients seems to be necessary. Hay in a study, conducted on the improvement of the quality of the emergency services and establishing an emergency triage at the Center of Gambia, showed that the implementation of the plan over the past three years has resulted in the improvement of the services, so that the patients have been classified safely and effectively and checked with the least error [43]. To date, no triage system has been superior specifically in relation to the patient clinical outcomes, improvement in the scene management, or allocation of the resources compared to other systems. But it seems that the use of a standardized and uniform system in one area can result in a better interoperability and mutual understanding between health system staff, when responding to disasters and mass casualty incidents [1]. Triage is an important tool in health management during emergencies and disasters. The absence of a common national and international guideline has led to the confusion of the health system staff [44]. In this regard, different countries have designed their own triage systems, according to their local conditions, their resources and their relief forces. Therefore, considering the conditions and characteristics of Iran, the need to design the national triage method is felt [21]. The selection of precise criteria in triage models can reduce the mortality rate, through placing the injured people in the correct class. Moreover, it helps to allocate the limited resources of the medical centers to be given to the injured patients, who really need these resources.

Conclusion

Considering the diversity of the triage models and the criteria defined for each system in the world, it is recommended that each country, considering the specific circumstances of the region, the diversity of the emergencies and disasters, and the facilities and resources of its centres, choose or propose a model with accurate and appropriate criteria, and test the accuracy of that model in case of scenarios or on actually injured people. Considering the lack of an appropriate triage system in its hospitals, Iran also needs to national a triage system, which can be effectively used during emergencies and disasters.

Acknowledgement

We wish to thank all the researchers whose studies were used in this comprehensive review.

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Review Article

Phytoestrogens and Their Health Effect

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Abstract

Phytoestrogens have structures that are very similar to endogen estradiol derived from plants. Phytoestrogens sources are widespread in Asian regions including in Indonesia. Its have health benefits including reproductive health, heart health, a role in weight loss, hormone-dependent tumours, bone and skin health, and the immune system. This review aimed to provide information on phytoestrogens' effect on human health.

Introduction

Phytoestrogens are estrogen-like compounds derived from plants, which are structurally similar to 17β-estradiol. Four phenolic compounds classified as phytoestrogens are isoflavones, stilbene, coumestan, and lignan [1], [2], [3], [4].

Isoflavones are found in soybeans and other legumes, including in red clover. The main phytoestrogens in the form of isoflavones are genistein, daidzein, glycitein, formononetin, and biochanin A contained in soybeans. Phytoestrogens that are classified as isoflavones are the most widely studied. The amount of isoflavones needed to give health effects is around 40-70 mg/day or an average of 50 mg/day. The average consumption of isoflavones in Asian society is 15-50 mg per day, while in Western countries only about 2 mg per day [5], [6].

The most common stilbene group is resveratrol, which is found in grapes and peanuts. Resveratrol consists of two isomers namely cis and trans. Trans has a higher estrogenic activity. Not all groups of comrades who have estrogenic activity. Contestants that have estrogenic activity come from nuts, broccoli, cabbage and spinach. The main sources of lignans are flaxseed, and also in wheat flour, peanuts, fruits, berries, vegetables, tea and coffee. One example of lignans is matairesinol (non-estrogenic dimer) which can be converted by intestinal microbes into enterolactone which is estrogenic and easily absorbed [1], [2], [3], [4].

The source of phytoestrogens are nuts, seeds, fruits and vegetables. Food sources include soybeans, garlic, celery, carrots, potatoes, rice, wheat, red clover, sweet potatoes, fruits (apples, pomegranates and chaste berries) and coffee [3], [4]. Isoflavones are found in legumes, especially soybeans. Flaxseeds are the main source of lignans, and many contestants are found in clover, alfalfa and soybean sprouts [1], [3]. Phytoestrogens in the diet are digested, then metabolised by bacteria in intestinal, absorbed in the intestinal and conjugated in the liver. Furthermore, the phytoestrogens circulate in the plasma until they are finally excreted in the urine [3].

The most important source of phytoestrogens is isoflavones found in soybeans and their processed products. Phytoestrogen levels in pure soybeans are higher than those that have been processed because of differences in bioavailability. Asian people consume

more soy than Europeans and Americans [1], [7]. The average daily intake of community phytoestrogens in East and Southeast Asia is estimated to be between 20-50 mg per day. While the average intake of phytoestrogens in adults in the United States is only 0.15-3 mg per day, and in Europe, it is much lower at around 0.49 to 0.66 mg per day [1].

Bioavailability and pharmacokinetics of isoflavones are influenced by the texture of food ingredients the source or form of food consumed. Liquid food is absorbed faster and has a higher concentration in plasma than solid food. Isoflavones in the form of aglycones are absorbed more quickly than in the form of glucoside conjugates [7].

The isoflavones contained in soybeans are in the form of a complex mixture of glucoside conjugates. Then it is hydrolysed in the digestive tract by the intestinal mucosa with the help of bacteria β-glucosidase to be aglycon, genistein and daidzein. Anglican can be absorbed directly or metabolised by microflora in the large intestine into active metabolites in the form of equol and inactive metabolites in the form of O-desmethylangolensin (ODMA) 3. Lignan is converted to secoisolaricisinol-diglucoside (SDG), and the constant is converted to coumestrol. The level of absorption and metabolism in the body [5], [7], [8]. The ability to convert phytoestrogens to equol is influenced by race. Japanese, Chinese and Korean can convert equol higher than the Western population which is estimated to produce only around 30-40%. This is related to the role of genetics, the composition of intestinal microbiota and diet [9].

Phytoestrogens have structures that are very similar to endogen estradiol and be able to bind with alpha and beta receptors of estrogen [6], [10]. Alpha and beta estrogen receptors have different functions. Alpha estrogen receptors act in cell proliferation, whereas beta receptors are responsible for cell apoptosis [6]. After the receptor binds to the ligand, then moves from the cytoplasm to the cell nucleus, binding and influencing the area that controls the DNA transcription process or small RNA, which in turn affects the expression of certain genes [3], [6]. Therefore, phytoestrogens have the potential effects to regulated all process that influence by estrogen including the induction of sex hormones that bind to globulin and inhibit aromatase [3].

Endogenous estrogen levels also affect the activity of phytoestrogens. In women of reproductive age, endogenous estrogen levels in high blood pressure. In this condition, lignans will compete with endogenous estrogen to bind to estrogen receptors so that they can inhibit estrogen activity. But at menopause, endogenous estrogen levels are low in estrogen production by the ovaries. When estrogen levels are low, lignans are weak estrogen [4]. Isoflavones, lignans and proteins also function as aromatase inhibitors by inhibiting the action of cytochrome P450 enzymes that convert androgens to estrogen. Elevated levels of the cytochrome P450 enzyme are associated with breast, adrenal and prostate cancers [4].

Phytoestrogens also have biological effects without going through estrogen receptors. Phytoestrogens can activate serotoninergic receptors, insulin-like growth factor (IGF) receptors 1, free radical binding, induction of DNA methylation, affect tyrosine kinases, cycle adenosine monophosphate (cAMP), phosphatidylinositol-3 kinase/Akt and mitogen-activated protein (MAP) kinases, transcription of nuclear factor-kappa β (NF-Kβ) factor, histone modification and RNA expression and act as an intracellular regulator in the cell cycle and apoptosis [6]. This ability causes phytoestrogens to have an antioxidant, antiproliferative, antimutagenic and antiangiogenic role and can improve health and longevity [3], [6], [11].

Phytoestrogens and reproduction health

Phytoestrogens have different effects on the reproductive process. Genistein can stimulate progesterone stimulation in the ovaries, production of estradiol and cAMP production, oocyte maturation and zygote development in the preimplantation stage. Phytoestrogens derived from the green and Indian turmeric can inhibit proliferation, increase apoptosis and affect steroid hormone release by animal ovarian cells. Isoflavones can also change animal sexual development, including changes in puberty, disruption of the oestrous cycle and ovarian function. Several studies suggest that there is an effect of isoflavones contained in infant formula milk on their reproductive development, although phytoestrogen side effects on reproductive health have not been widely reported [11], [12].

Phytoestrogens can also suppress the clinical symptoms of menopause caused by a decrease in the production of endogenous estrogen. Administration of 100 mg of isoflavones per day in postmenopausal women can reduce vasomotor symptoms. The advantage of this phytoestrogen is that it does not hurt the breast and endometrium [3], [11], [13]. Also, phytoestrogens also do not increase the risk of clotting in postmenopausal women [5]. This causes phytoestrogens to be a safe alternative to hormone replacement therapy [4].

Phytoestrogens and Cardiovascular disease

Several studies have proven the protective effects of phytoestrogens on cardiovascular disease. Phytoestrogens can reduce total cholesterol and improve heart function. Genistein consumption of 200 mg per day can reduce total cholesterol [14]. Consumption of phytoestrogens derived from soy can affect plasma lipoprotein levels (lower LDL cholesterol

and increase HDL cholesterol), reduce plasma triglyceride levels, inhibit oxidised LDL and lower blood pressure and C-reactive protein (CRP) [15]. Increased phytoestrogen intake results in lower serum total cholesterol and LDL levels, and in some cases leads to an increase in HDL levels [16].

Isoflavones can reduce hypertension through vasodilatation effects. Six months of isoflavone administration in postmenopausal women has been shown to increase endothelial vasodilation and reduce the number of cell adhesion molecules such as intracellular adhesion molecule 1, vascular cell adhesion protein 1 and E-selectin. Isoflavones can increase kidney blood flow, excretion of sodium, and inhibit the activity of angiotensin converting enzyme (ACE). However, the effect of decreasing blood pressure by isoflavones is more evident in patients with hypertension than normotension [17].

Phytoestrogens also affect blood sugar regulation. Research on Asian and non-Asian women found that phytoestrogens can reduce fasting blood sugar levels, circulating insulin and homeostatic model assessment for insulin resistance (HOMA-IR), but did not affect post prandial blood sugar levels and HbA1c [14].

It is also proven that the lower incidence of chronic diseases is found in East Asian people who consume soybeans containing isoflavones and their processed products. Contrary to Western populations who experience more chronic disease, it is thought that they consume fewer soy products [17], [18].

Phytoestrogens and weight loss

Another benefit of phytoestrogens is to lose weight. Phytoestrogens can increase fat-free mass and reduce fat accumulation by inhibiting the life cycle of adipocytes. Long-term isoflavone supplementation can also reduce visceral fat tissue and serum leptin concentration. Phytoestrogens also inhibit a moderate degree of inflammation that arises in obesity through its antioxidant effects [12], [14]. Some studies suggest that phytoestrogens, especially those from soy and its products, have a significant effect on weight loss, both in animal and in humans studies. A randomised controlled clinical trial by Allison conducted on 74 obese people stated that there was a significant weight loss after administration of soy-based meal replacement formula. This treatment was carried out in two groups (treatment and control groups) for 12 weeks. At the end of the study, we found a significant difference in weight loss between the two groups (7.00 kg vs 2.90 kg) [19]. Other studies in animals have shown the protective effect of phytoestrogens on the risk of obesity in estrogen-deficient mice. Administration of phytoestrogens in female albino rats which were ovariectomized affected the amount of intake and weight. In the group of mice given a diet high in phytoestrogens there was a decrease in the amount of food intake and increase weight loss significantly compared to the control group. This is caused by appetite suppression which is one of the effects of estrogen. Because phytoestrogens have a structure similar to estrogen and can bind to estrogen receptors, so, phytoestrogens also have appetite suppressing properties. It added again with a decrease in the amount of food intake to accelerate weight loss [20]. Also, the increase in fat loss can be attributed to the capability of phytoestrogens to induce the apoptosis of adipocytes, suggesting that at least part of the weight loss is ablation of fat cells [21].

Phytoestrogens and hormone-dependent tumour

The use of isoflavones since adolescence is associated with a reduced risk of breast cancer compared to hormone replacement therapy that can increase the risk of breast cancer in menopausal women. Phytoestrogens also have a protective effect on the risk of endometrial cancer [5]. This is related to the properties of phytoestrogens that can act as antioxidant, antimutagenic, antiangiogenic, pro-apoptotic and anti-cancer [12].

Estrogen or alpha or beta estrogen receptors depend on their concentration. To bind to the alpha estrogen receptor, estrogen must have a concentration 10 times higher than to bind to the estrogen receptor beta. Conversely, phytoestrogens can bind to beta receptors at lower concentrations than alpha estrogen receptors so that phytoestrogens can bind to both receptors at the same time. This is what causes the protective effects of phytoestrogens on breast cancer and endometrial cancer [6], [12].

A study conducted in mice found that administration of phytoestrogens for 8 weeks did not cause changes in the cause of pituitary precancerous growth or change in estrogen-sensitive organs such as the uterus, ovary, cervix, liver, and kidney. This is in contrast to external estrogen-induced effects (diethylstilbestrol/DES) which can be induced weight increase on the ovary and cervix, as well as gross structural changes that include decreases in tissue cell density, increases in vascularity, and multiple hemorrhagic areas. Although in this study it was found that administration of phytoestrogens has not been able to reduce the effects caused by DES, phytoestrogens can be used as an alternative to hormone replacement therapy [23].

Phytoestrogens and bone health

The results of research on the effects of phytoestrogens on bone health are still not consistent. Some studies say that there is no relationship between phytoestrogen intake and bone health. A meta-analysis of randomised clinical trials in humans found a weak link between increased intake of soy

isoflavones and increased bone mineral density [24]. Other studies also state that there are bone sharing benefits from the consumption of phytoestrogens. The daily consumption of 200 mg/kg phytoestrogens in OVX rats for 8 weeks increases the concentration of femoral calcium but also increases the weight of the uterus. Likewise with the administration of isoflavones-rich soy milk for 2 years can increase 2.4% of bone mineral density in older women [25].

Also, the administration of phytoestrogens combined with vitamin D in aged ovariectomized female rats also increases bone mineral density. Vitamin D was given as 2,400 IU/kg singly or in combination with various types of phytoestrogens (resveratrol, quercetin, and genistein) with multilevel doses. The results of this study prove that the combination of phytoestrogens with vitamin D has synergistic effects and may be effective in reducing bone loss after menopause [26]. This increase in bone mineral density may be caused by the consumption of phytoestrogens which can increase osteoblast synthesis and reduce bone resorption, but this positive effect is obtained from long-term consumption of phytoestrogens, at least six months [5]. So, the effect of phytoestrogens on bone health is influenced by the dose, duration, and age of the subject. For many women, adding soy to an already healthful diet may be an option to help stave off bone loss in midlife [5], [27].

Phytoestrogens and cognitive function

The interaction of phytoestrogens with estrogen receptors is also a positive influence on cognitive function. Phytoestrogens as neuroprotectors and antioxidants can reduce the risk of Alzheimer's disease [28]. Phytoestrogens affect the workings of the nervous system via steroid receptors and 5-hydroxytryptamine receptors or by increasing serotonin reuptake. Also, it also can influence the synthesis and uptake of catecholamines through estrogen receptors in the plasma membrane [12].

Phytoestrogens and skin health

Phytoestrogens can act as antiaging in the skin through estrogen receptors or increased production of hyaluronic acid, collagen, and extracellular protein matrix. Also, phytoestrogens can also increase vascularisation of skin, proliferation, prevent skin from oxidative stress and apoptosis. Ageing of the skin can be inhibited by the administration of phytoestrogens [3].

The protective effects of phytoestrogens on skin health can occur through several mechanisms. It can reduce UV-induced cell death in cultured keratinocytes, improve skin elasticity, increase the depth and increase the production of type 1 procollagen. It offers protection against UV induced senescence by significantly upregulating intracellular SOD activity in a dose-dependent manner. Phytoestrogens also have potent antioxidant effects with strong anti-inflammatory properties [29]. Other studies also proved that the provision of concentrated, isoflavone-rich soy extract during the six consecutive months caused significant increases in epithelial thickness, the number of elastic and collagen fibres, as well as the blood vessels. This study was conducted on 30 postmenopausal women before and immediately after treatment with 100 mg/day of isoflavones-rich, concentrated soy extract for six months [30].

Phytoestrogens and the immune system

Phytoestrogens affect the immune system through its ability to inhibit intracellular signalling pathways associated with NF-kappaB and immune responses. Genistein can suppress specific immune responses and lymphocyte proliferation [12].

Phytoestrogens suppress the immune response in vivo and in vitro. Phytoestrogens can suppress specific immune responses and suppress lymphocyte proliferation. Also, genistein can inhibit an allergic inflammatory response. Genistein can increase cytokine production from T cells and enhance cytotoxic responses mediated by natural killers and cytotoxic T cells [31].

A study in mice that gave 8-80 mg/kg of genistein over 28 days found that there were 46–67% decreases in the delayed-type hypersensitivity response, and these effects were reversible. Its also found that there was a decrease in cell infiltration in genetically treated animals compared with controls, and the numbers of CD4 + and CD8 + T cells in normal lymph nodes were reduced on histopathological examination. This study concluded that Dietary genistein (1000 or 1500 ppm) decreased cell-mediated immunity while producing serum genetic concentrations for humans under certain nutritional conditions [32]. However, the mechanism of action of phytoestrogens for the immune system needs to be studied further.

In conclusion, phytoestrogens have many positive benefits for the health of various organs. Many sources of phytoestrogens are also spread in the world which can be used as an alternative to external estrogen substitutes or hormone replacement therapy.

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The Alcmaeon's School of Croton: Philosophy and Science

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Abstract

Among the first physicians and physiologists at the pre-Hippocratic medicine with contradictions and oscillating doctrines was Alcmaeon from Croton in the 6th century BC. For many, he shared as the father of scientific medicine. Unfortunately, we have only eighteen texts written for him and only five fragments of texts written by him. This saved extracts and testimonies they refer mostly to physiology, epistemology and psychology. Was born in the city of Croton (Κρότων) in Magna Graecia (southern Italy) was a reference point for the activity of physicians and philosophers over the years. Furthermore, Herodotus tells us about the excellent practice quality of the physicians, "compared to all the others known in that period", among which Alcmaeon. But Croton is also famous as the centre of thought and activity of the philosopher Pythagoras of Samos (Πυθαγόρας ὁ Σάμιος, 580-500 BC) through his Pythagorean school around 530 BC.

A multifaceted character: Pythagoras

Pythagoras was one of the most important pre-Socratic philosophers. He was born in the Samos Island (Aegean Sea) and he leaves it around the 530 B.C., and he settles at Croton town. Must mention based on Herodotus Democedes (Δημοκήδης) of Croton as the most knowing physician of his time Croton had a medical school already present at the arrival of Pythagoras [1], [2], [3]. Pythagoras was a dynamic social personality which creates a private culture association where his members acted in the political, religious and philosophical fields, spreading his thinking to society as a whole. As we do with Thales and others pre-Socratics we have nothing written but through Aristotle, Herodotus, Plato and others. His thinking supported a pattern of the orderly entity in the universe (*κόσμος)* that came through a well defined the relationship with the numbers (the true nature of being of things). Famous are the Pythagorean table of opposites (a set of 10 pairs of contrary qualities). So his thought research focused mostly on mathematics and geometry, but also in astronomy and music, less on medicine. Anyway on medicine was the first he understood that good health is maintained through physical exercise for the body. We must not omit that Pythagoras himself came to this concept because it was also one that applied to the athletics world. In fact, through his philosophical, medical school, he proposes a proper physical activity, balanced diet and recreation through music and reading. The balance between body and soul (*ψυχή*) is maintained through these rules and opposes the thought that the illnesses are from the ones. This is also because the Pythagoreans followed a similar religious model or if not the same as the religion of Orphism. They developed the thought that the soul does not die but moves to another living body. However, Pythagoreans differ according to their philosophical ideas. Some prefer the mathematical reasoning of numbers (learners: Μαθηματικοι), but

others teach and study ritual and religious subjects (listeners: Ακουσματικοι) [4], [3].

Alcmaeon lived in the time of Pythagoras culture. Is ambiguous if was Pythagorean or not. That's because his activity in some respects was particularly isolated from Pythagorean thought. According to Aristotle (*Metaphysics: "τὰ μετὰ τὰ φυσικά" A.5)* he lived when Pythagoras was old: *"…καὶ γὰρ ἐγένετο τὴν ἡλικίαν Ἀλκμαίων ἐπὶ γέροντι Πυθαγόρᾳ,ἀπεφήνατο δὲ παραπλησίως τούτοις…" (as for the age Alcmaeon was young when Pythagoras was old ....). H*e appears in the Pythagorean school. He is therefore regarded as an integrated member of the Pythagorean School, however, with the specific competence on medical thought. Also demonstrates that his thinking is influenced by the Pythagorean concept of *harmony* (*αρμονία)* and we know that some of them offered not only numbers and that the first of them that had orientation mostly towards a naturalistic thought and towards medicine, physiology as Menestor and others later. That because society so marked by the dynamic Pythagorean thought it was not possible that it was not influenced. Aristotle notes this ambiguity and writes *"Speaking like that of the Pythagoreans; he said that duplications are mostly things about the man. But unlike the Pythagoreans, he did not define what oppositions were (oppositions), but he named what was happening: white-black, sweet-bitter, good-bad, great-small. However, from the Pythagoreans and Alcmaeon, it can be seen that controversies were for them the principles of things that are"* [5], [6], [7].

As we mentioned Pythagorean theory, according to which only ten elements are ordered according to opposites *(των εναντίων)* are principles of things: limit and unlimited, odd and even, one and many, right and left, male and female, mobile and not mobile, straight and curved, light and dark, good and bad, square and rectangle. Alcmaeon of Croton has similar doctrine: *"είναι δύο τα πολλά* των *ανθρωπίνων"* (*most human things are dual)* but puts the oppositions randomly: big-little, black-white, good-bad, sweet-bitter. Certainly, every one of the Pythagoreans has founded his school of philosophic thought as did Parmenides and others but he did not. If we only consider its empirical research towards medicine and physiology can differentiate it from Pythagoreans. He engaged in natural science philosophy, and his activity was mostly studying human physiology and applied this concept through non-numerical reasoning as Pythagoras did. Alcmaeon, in his only book "*Περί φύσεως" (On Nature),* mentioned the Pythagorean Brotinus: *"Ο Αλκμαίων ο Κροτωνιάτης, γιός του Πειρίθοου, είπε τα ακόλουθα στους Βροτίνο, Λέοντα και Βαθύλλο…" (Alcmaeon of Croton, son of Peirithous, said the following to Brotinus, Leon and Bathyllus…),* and Aristotle despite having written a separate book for him in another his monographic work "*The Pythagoreans"* included him among them. Also, we can recall that beyond some writers such as Diogenes Laertius on *"Βίοι καὶ γνῶμαι τῶν ἐν φιλοσοφίᾳ εὐδοκιμησάντων"* (Lives of Eminent Philosophers, *III BC)* writes: *"Ἄλκμαίων Κροτωνιάτης καὶ οὗτος Πυθαγόρου διήκουσε"* consider as Pythagorean. Later other writers as the philosopher Iamblichus and Aristoxenus (4th century), recognised the Pythagorism of Alcmaeon [8], [9], [10], [1].)

Alcmaeon developed activity mainly in the area of medicine and natural philosophy with Pythagorean affinity. This empiricist epistemology orientation influences Hippocrates and was the beginning separation of medicine from the supranational religion. Consequently, it will lead to the evolution of the healing temples (Asklepieions) as medical schools that physician learn and transmit knowledge through a professional ethics [4], [10].

Alcmaeon, the philosopher

Alcmaeon did not teach any religious or ritual arguments, indeed Diogenes Laertius and Aristotle on *Περί ψυχῆς (On the soul, 1.2, p.405, 350 BC*) describes that he supports the immortality of the soul that was always in motion as celestial objects in the universe and that it is it moves continuously like the sun. Indeed Diogenes Laertius on "*Βίοι καὶ γνῶμαι τῶν ἐν φιλοσοφίᾳ εὐδοκιμησάντων,* (Lives of Eminent Philosophers, book H, VIII§83) he writes on Alcmaeon: *"περὶ τῶν ἀφανέων, περὶ τῶν θνητῶν σαφήνειαν μὲν θεοὶ ἔχοντι, ὡς δ' ἀνθρώποις τεκμαίρεσθαι* καὶ τὰ ἐξῆς ἔφη δὲ καὶ τὴν ψυχὴν ἀθάνατον, καὶ κινεῖσθαι αὐτὴν συνεχὲς ὡς τὸν ἥλιον. - *…Of the invisible things and visible things only the gods have certain knowledge, a human can only deduce…the soul moves continuously like the sun…".* Thus Alcmaeon said that the *"Experience is the foundation of knowledge"* (πειρα *μαθήσεως αρχή*), that is distinguishes between the absolute experience knowledge of gods (*σαφές* = clear understanding) but the human knowledge comes through *proves experiences* (*τεκμήρια)*. For this separates human reasoning (φρονεῖν) and the animals feeling (αἰσθάνονται): *"Man differs from the other living because he only understands, while others feel but do not understand".* Indeed for him, the knowledge and the feeling are two different things (not the same thing as for Empedocles). This will be related to the sense doctrine: *"is heard through the ears ... they feel it smells with the nose ... the flavours are distinguished with the tongue ..."*. All the senses are transmitted and controlled by the brain. The human being perceives with reasoning and welcomes with imagination for his actions. So what distinguishes humans and animals are the intellectual fantasy on the one hand and the other can transmit knowledge to evolve. Thus comes to the concept of separating Anthropos (ἄνθρωπος, human being) and animal as an intellect of perception.

But using the word soul does not intend to give a sovereign religious concept but wants to distinguish the intellect's ability and brain function to control feelings and behaviours. According to to *Theophrastus* (a Greek philosopher of the Peripatetic school), Alcmaeon was the first Greek thinker to distinguish between the sensory perception of intellect [11], [12].

Another topic which comes from the texts there is a coincidence between medical and political analogy on terms, precisely the derivation of medical language from the political language. Alcmaeon *On Nature* refers to the idea of equal distribution of strengths called *isonomia* (ἰσονομία). Alcmaeon will say: *"….health is tough as long as the various elements, wet-dry, cold-hot, bitter-sweet have equal rights and that diseases come when one of the opposites prevails. The prevalence of either element tells you to be the cause of destruction. Health is the harmonious mix of opposite qualities".* That conceptual thought, demonstrate the coincidence between medical terms and political terms, precisely the derivation of the medical from the political thought. Indeed "*So health and isonomy*" (isos + nomos = Democracy: Δημοκρατία) it applies mainly to democratic regimes but also moderate oligarchs. Instead, the dominance (Μοναρχία: monarchy) of one of them or part of them generates sickness (νόσος) because pre-eminence and the predominance of opposite on the other must have a dynamic solidarity balance between the constitutive and opposing powers of the body. With this theory sets the bases of the metabolic process of substances in the body that give the physiological balance. So this keeps the balance of the healthy body in contrast to that the numbers are the principle of harmony. So there is no universe ordered by the essence of the number but tension between opposing forces that tend to balance. As we have said Alcmaeon said "*most of the human things are twofold",* that is human problems appear to be contradictory and heterogeneous. But such an equilibrium cannot be guaranteed indefinitely it can only be helped to persist or be rediscovered because all reality appears to men as ruled by couples of opposites who find a momentary but not indefinite equilibrium [13], [14].

Finally, Alcmaeon about the phenomenon of death advances a double explanation, both physically *"Sleep is produced by pretreatment of blood from the veins…"* and philosophically *"Humans die for this because they cannot reunite the beginning* (Αρχή) *with the end* (*Τέλος*) *of the life".* This concept through a mathematical and astronomical form as the circle (κύκλος)who tends to explain the mortality of the body because the circle is precise and eternal as describes Philo of Alexandria, as the structure and movement as in the planets. It is not clear if he presented a cosmological model regarding opposing forces, but we still have a testimony about his views on Astronomy. His spiritual impact on Greek philosophical tradition has been considerable. Alcmaeon has probably influenced at a cosmological theory by Anaximander. From the testimonies offered by Aetius there is also an astronomical interest in Alcmaeon, but more than original hypotheses would have welcomed some interpretations of his time, for example by Heraclitus and Antiphon of Athens shared that the lunar eclipse comes from the different inclination of its cavity as the stars [5], [13].

Alcmaeon, the physician

As we have mentioned for Alcmaeon, the human being has a thought that could interpret rational sensations. Thus Alcmaeon was first considered the brain (Ἐγκέφαλος) as the vital centre of all this, and any alteration of the brain causes turbidity and an impediment to the sensation and our thoughts, so laying the foundations of neuropsychiatric sciences. Aristotle, Plato and Philolaus adopted his reasoning about the soul and the idea that intelligence is based in the brain. This encephalocentric theory of Alcmeon is in contrast with the cardio-centric equilibrium which supports the conception between the heartbeat, the blood flow thus between the body and the universe. He studies these perceptions and their reasonable interpretation of the brain and how they could be transmitted to the brain through the sensory organs by seeing, hearing, tasting, and smelling. Different sensations are explained based on the duality of the principles. So, for example, we perceive the odours because the nose as an organ of smell dissolves the smells contained in food or air with its heat and absorbs them with its humidity. The ear has an inner void that vibrates with the vibration of the inner air. Then there are two realities the outside world which stimulates perceptions and an interior that processes perceptions to understand and act to have the balance through the brain. Anyway, as we mentioned, human knowledge, both for things that do not directly experience that for things has a direct perception, can only come to hypotheses, approximations. But never to an absolute and truthful knowledge [15], [16].

To confirm his ideas used a method of direct observation and experimental testing dissections on animals (as others later: Aristotle, Diocles, Praxagoras, Erasistratus, Herophilus). He is the first anatomically observing the Eustachian tubes which are the ducts between the middle ear and nasopharynx. Thus he seeks to understand the anatomical paths of the nerves that bring the sensations to the brain that will call poroi *(Πόροι)* and makes a discrete description of the anatomy of the eye to understand the transmission of the image from its external path to internal path. These passages are merely transmitting sensations or other information

from the periphery of the body to the centre of the beard, and so he discovers the concept of the nervous system [17], [18], [19], [20].

Conclusions

Alcmaeon was one of the most important characters of the VI century BC. Multifaceted thinker, he improved the medicine offering a new point of view to understand the mechanisms determining the health status and the disease. He dealt a variety of physiology issues about the sleep, diet, death, and pregnancy. From the testimonies of Aristotle, Aetius and Rufus it seems that Alcmaeon had attention and played a particular role to the development of life in humans and animals not only for the period of puberty but also on the beginning of their life, especially for their nourishment, for example, the embryos in the uterus. Indeed, another singular thesis of Alcmaeon on the fetus is that it nourishes from all over the body like a sponge. Reported by Aetius, it turns out that Alcmaeon also connected the brain to the brain sperm formation.

Censorinus, a Roman grammarian, which refers to Alcmaeon about the explanation for determining the gender difference of the unborn reiterates the equality between male and female seed: the sex of the unborn depends on who has been received seed more abundant, if the father is born male, if the mother is born female [21], [16].

At last, Alcmaeon is also considered an inspirator of the Roman medicine, because of the influence of his thoughts on some medical school of that time [22].

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Letter to the Editor

The One Step Melanoma Surgery (OSMS): A New Chance for More Adequate Surgical Treatment of Melanoma Patients!?

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Abstract

One step melanoma surgery (OSMS) is applicable to all patients with clear clinical and dermatoscopic criteria for thin cutaneous melanomas or melanoma in situ lesions, even without the need for preoperative tumour thickness measurement. Amelanotic melanomas and melanomas with clinical and dermatoscopical features for regression zones could be problematic when applying the OSMS. The methodology could be also applicable to all groups of patients where the tumour thickness could be measured preoperatively/by ultrasound (while in parallel also determining the status of the locoregional lymph nodes). For tumours with a tumour thickness between 2 and 4 mm, but also over 4mm the OSMS seems to be the correct choice.

Dear Editor,

One-step melanoma surgery undoubtedly turned out to be one of the innovations of our time [1], [2]. It applies to groups of patients where the tumour thickness could be measured preoperatively/by ultrasound (while in parallel also determining the status of the locoregional lymph nodes) but also to patients where the ultrasound assessment is not obligatory [1], [2], [3]. It is believed that the preoperative high-resolution ultrasound even with 15-MHz-20 MHz to a very large extent correctly identifies thin MMs and allows appropriate surgical margins to be selected and eventually to save the need for further re-excision [4], [5]. The frequency of ultrasonography can reach up to 75 MHz, considering that the main limitations on its usefulness in the diagnosis of tumour thickness may be associated with certain histological characteristics such as the presence of an inflammatory/lymphocytic infiltrate or naevus remainder, which can overestimate the thickness [4], [6].

Furthermore, the possibilities of sonography are limited in terms of differentiating between malignant and benign tumours from a morphological point of view which is a disadvantage especially in the case of-of tumour regression or amelanotic melanoma [7]. The existing standards for the melanoma treatment require the conduct of the so-called excision biopsy followed by a histopathological assessment of the tumour thickness and, depending on it: performing re-excisions with different fields of additional surgical

security, combined or not with the determination of a draining lymph node [8].

The drawbacks of this methodology are many: 1) the different treatment time of the preparations, which, according to the institution, may not coincide with the two-week period for performing a re-excision, for example, 2) the willingness for a second opinion of the patients themselves, which makes the re-excision pointless if the deadlines for the latter are not met. As well as 3) the refusal of re-excision for various other reasons, which can be both objective and subjective.

Because of these facts, the preoperative assessment of tumour thickness creates several unique opportunities to minimise these problems and to optimise the approach to the patients to a maximal extend, namely:

1) For all lesions that are clinically and dermatoscopically indicative of cutaneous melanomas or melanoma in situ, preoperative ultrasound of the primaries should or could be performed. If the primaries have a tumour thickness of less than or up to 1 mm, then a re-excision with a surgical security field of 1cm in all directions should be planned without removing the draining lymph node (Table 1).

Table 1: One step melanoma surgery (OSMS): Tchernev G et al. recommendations [6]. The crucial step of the procedure remains ultrasound. 20 MHz ultrasound may overestimate tumour thickness due to the lymphocytic peritumoral infiltrate. Therefore, 75 MHz has been suggested to overcome this possible drawback [7].

Breslow thickness	Recommended surgical margins
Melanoma in situ	1.0 cm (clinical/dermatoscopical evaluation obligate/if possibility for echographical examination -from benefit)
<1 mm	1.0 cm (clinical/dermatoscopical evaluation obligate/if possibility for echographical examination -from benefit)
1.01 - 2.0 mm	1.0 cm (with SLND), (echographical tumour thickness measurement preoperatively recommended)
2 mm- 4 mm	2.0 cm (with SLND) ethnographical tumour thickness measurement preoperatively recommended
> 4 mm	2.0 cm a) no enlarged lymph nodes- 2 cm resection is sufficient, b) in the presence of enlarged lymph nodes- to be removed together with the excision of the primary tumorous tissue!

2) If the primaries has a tumour thickness above 1 mm and less than 2 mm, the surgical security field will be 1 cm and will be combined with the simultaneous removal of the draining lymph node within one surgical session (Table 1) [1].

3) In tumours with a thickness between 2 and 4 mm, echography could be used analogously to those already mentioned in items 1 and 2, with the surgical security field being 2 cm in all directions (Table 1).

4) For tumours with a thickness greater than 4 mm, the single-step melanoma surgery also finds an adequate application (Table 1). If the locoregional lymph nodes are affected, the primaries is removed within one surgical session along with them (and a corresponding field of surgical security). If they are not affected (of clinical and ultrasound point of view), the determination of a draining lymph node is not recommended (even in tumours over 4 mm) due to the fact that the metastasis is probably already performed: 1) haematogenous, 2) by accessory lymphatic vessels, or 3) for one reason or another, the draining lymph node has not captured the tumor cells.

In all the three options described, the optimization is due to 1) reducing the number of surgical interventions from two to one, 2) the better control of the disease as a whole: the absence of risk of delaying a potential re-excision or patient's failure to attend for the second surgical intervention, and 3) limiting the costs of the subsequent potential second hospitalization [1], [2].

Recently published data are evidencing that the single-step melanoma surgery is also applicable to patients with clear clinical and dermatoscopic criteria for thin cutaneous melanomas, even without the need for preoperative tumour thickness measurement [3], [9], [10], are also of interest. Re-thinking the approach to patients with skin melanomas is yet to come [10].

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