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The Vascular Endothelium in Patients with Dengue Haemorrhagic Fever

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Abstract

BACKGROUND: Dengue fever is the most serious consequence of mosquito-borne infection worldwide. The pathophysiology of DHF in human is complex, which involve endothelial cell activation and impaired endothelial barrier leading to plasma leakage triggering the activation of the haemostatic system. The increased vascular permeability may lead to hypovolemia, hypotension and shock, which is life-threatening.

AIM: The objective of the study was to determine the effects of dengue haemorrhagic fever on the vascular endothelium.

METHODS: Fifty patients (males 34, females 16), were recruited, Grade 1 (n = 41), Grade 2 (n = 6), Grade 3 (n = 2) and Grade 4 (n = 1) DHF. Blood sampling was performed at the febrile, defervescence and convalescent phases for the determination of haemoglobin, haematocrit, platelets, prothrombin fragment F1 + 2, Von Willebrand Factor (VWF), vascular endothelial growth factor (VEGF) and D-dimer levels. Fifteen normal subjects were recruited to serve as normal controls.

RESULTS: The patients aged between 4 and 54 years old. Grades 1 & 2 DHF showed no significant differences in the parameters studied. However, thrombocytopenia, elevated F1 + 2, VWF, VEGF and D-dimer levels were evident in febrile, defervescence and convalescent phases suggesting endothelial activation and plasma leakage. Pleural effusion was observed only in severe DHF. The three patients with Grades 3 and 4 DHF had similar study results. No mortality was recorded in the study.

CONCLUSION: In dengue haemorrhagic fever, the vascular endothelium is activated, causing plasma leakage triggering the activation of the haemostatic system creating a hypercoagulable and enhanced fibrinolytic state evident by marked fibrinolysis.

Introduction

Dengue fever is the most serious consequence of mosquito-borne infection worldwide. There are more than 2.5 billion persons at risk of infection and occur mainly in the sub-tropical regions of Asia, Africa, and America [1] and the attacks have shifted mainly to adults [2]. The actual numbers of dengue cases are underreported or misclassified [3]. One study estimated that 3.9 billion people in 128 countries are at risk of infection with dengue viruses [4]. In Indonesia, the overall incidence increased significantly from 0.05 / 100,000 in 1968 to 35-40 / 100,000 in 2013 [5]. Clinical manifestations of DF include mild or marked febrile syndromes of abrupt onset with headache, pain behind the eyes muscle and bone pain, nausea, vomiting and rash. There is no specific treatment for dengue fever, but maintaining patients' body fluid volume is critical. Dengue as defined by WHO [6] as dengue with and without warning signs of plasma leakage and defined into four grades (Grades 1 to 4).

The pathophysiology of DHF in human is complex and the clinical symptoms due mainly to immune response, which also involve endothelial cell activation leading to plasma leakage and triggering the activation of the haemostatic system. The endothelium plays an important regulatory role in the circulation as a physical barrier and involved in the control of thrombosis and thrombolysis, vascular tone and growth of blood vessels [7]. It plays a critical role in a variety of human disorders. Endothelial injury is associated with elevated Von Willebrand Factor (VWF) and vascular endothelial growth factor (VEGF) a known potent regulator of vascular permeability and angiogenesis is released by platelets [8], [9]. The

platelets are the main transporter of VEGF [10]. Endothelial activation may be responsible for plasma leakage and shock [11]. D-dimer, the lysis product of cross-linked fibrin indicates hyperfibrinolysis in response to clotting activation and fibrin formation [12] It is also a marker for hypercoagulability and has been used to determine thrombosis in myeloproliferative disease [13], [14]. Thrombocytopenia is commonly observed in both mild and severe dengue syndrome and associated with clinical outcome [6], [15], [16], [17]. This may be due to bone marrow suppression, destruction and lengthening of the platelet life cycle [18], [19]. The level of platelet count correlates with severity of DHF, and high haematocrit with marked thrombocytopenia support the diagnosis of dengue shock syndrome (DSS) [2]. It has been considered as an important factor responsible for bleeding events in DHF [20]. Platelet activation is significantly increased in dengue-patients, especially with thrombocytopenia, which exhibited signs of apoptosis pathway activation [21]. Increased activation of coagulation (prothrombin fragment 1 + 2) was reported in a critical phase of severe dengue infection associated with plasma leakage and thrombocytopenia [2]. In the Brazilian study, it was reported that elevated D-dimer and thrombocytopenia with reduced thrombin generation and excessive fibrinolysis are associated with bleeding complications [23].

The objective of the study was to determine the effects of dengue haemorrhagic fever on the vascular endothelium.

Material and Method

The study received ethical approval from the Health Research Ethical Committee No 418 / TGL / KEPK FK USU-HAM / 2018, Faculty of Medicine, University of North Sumatera, Indonesia. The study was conducted at the Murni Teguh Memorial Hospital, Medan Indonesia.

Subjects

The patients admitted to the hospital were mainly from grade 1 DHF with some grade 2 and a few severe DHF. Fifty patients (males 34, females 16) admitted to the hospital with fever were recruited and diagnosed according to WHO protocol (6) to have Grade 1 (n = 41), Grade 2 (n = 6), Grade 3 (n = 2) and Grade 4 (n = 1) DHF. The Inclusion criteria: patients who met WHO criteria for dengue fever and willing to take part in the study and had one or more dengue serology positive for either IgM/IgG antibodies or NS1antigen, Exclusion criteria: patients with other infections and systemic diseases and not willing to take part in the study.

Normal Controls

Fifteen normal subjects (males n = 14, female n = 1) who are normotensive, had not taken any medication recently and no history of health issues was recruited to serve as normal controls for the DHF study. Their mean age was 22.9 ± 1.1 years and ranged between 18 years and 33 years old.

Blood Sampling and Laboratory Investigation

From a clean venepuncture 3 mL EDTA blood was used for routine determination of haemoglobin (Hb), haematocrit (Hct) and platelets performed in the Siemens high volume haematological analyser (ADVIA 2120 / 1), and plasma for serological tests for IgG / IgM antibodies and NS1 antigen (SD Bioscience, Ingbert, Germany). 10 mL of citrated blood was spun in the refrigerated centrifuge at 2500g for10 minutes and the plasma aliquoted and stored at -80°C. Citrated-plasma was used for Elisa analysis of prothrombin fragment F1 + 2 (F1+2), Von Willebrand Factor (VWF), vascular endothelial growth factor (VEGF) (USCN Life Sciences, Wuhan, China) and D-dimer (Vidas D-dimer Exclusion II, Biomerieux SA France).

Statistical Analysis

The Statistical Package for Social Sciences (SPSS 22 IBM Corp) was used to perform statistical analysis. The independent t-test for differences between groups at different DHF phases was performed together with one-way Analysis of Variance (ANOVA). A *P* value of < 0.05 was considered statistically significant.

Results

Characteristics of patients with dengue haemorrhagic fever

Petechiae or rash, headaches/bone and pain behind eyes are seen in all patients; Epistaxis is seen in grades 2 and 3 patients while the grade 4 patient was unconscious at admission, had bled into the brain at defervescence phase as evident from CT-scan. Pleural effusion was only observed in grades 3 and 4 DHF. The liver enlargement was seen in grades 2, 3 and 4 and 14.6% (6 / 41) in grade 1. The patients were discharged in an afebrile state. The clinical characteristics of DHF patients are shown in Table 1.

Comparison of parameters studied in DHF (Grade 1) between cohorts at age seventeen years and below and above seventeen years.

Table 1: Characteristics of patients with dengue haemorrhagic fever

	Grade 1	Grade 2	Grade 3	Grade 4
N	41	6	2	1
Age mean (SD) years	20.6 (11.8)	30.8 (8.7)	38, 43	54
Sex males/females	28/13	4/2	2/0	1/0
Petechiae/rash	41	6	2	1
Pain: headache/bones/behind eyes	41	6	2	1
Nausea	19	6	2	1
Cough	15	4	1	0
Bleeding: epitaxis	0	6	2	1*
Pleural effusion	0	0	2	1
Liver enlargement	6	6	2	1

*Bleeding to the brain (CT scan).

There were twenty cohorts (males n = 13, females n = 7) at seventeen years and below and twenty-one cohorts (males n = 14, females n = 7) above 17 years old. Except for the significance in age ($P \leq 0.001$) and lower mean trend for platelets in the above 17 years cohorts which did not reach statistical significance ($P = 0.05$), there were no statistical differences in the other parameters studied (not shown). They were therefore combined (Grade 1) for further statistical analysis.

Dengue haemorrhagic fever: Comparison of parameters studied between Grades 1 and 2 at febrile, defervescence and convalescence phases and comparison to febrile phase.

The combined Grade 1 DHF cohorts are significantly younger than the Grade 2 cohorts ($P = 0.03$). There were no significant differences in the other parameters studied between the two groups of cohorts at different phases of DHF.

Platelets had higher mean numbers at a convalescent phase in both grades 1 and 2 DHF, but they did not reach statistical differences even when compared to the febrile phase. Thrombocytopenia with elevated F1 + 2, VWF, VEGF and D-dimer was observed. Moreover, there was also no significant differences in the parameters studied when defervescence and convalescence phases were compared to febrile phase, except for D-dimer (Grade 2 DHF) which showed a significant decrease ($P = 0.01$) at convalescence compared with febrile phase even though it remained elevated (Table 2).

Analysis of Variance (ANOVA), One-way ANOVA analysis for Hb, Hct, platelets, F1 + 2, VWF. VEGF in either Grades 1 or 2 between different DHF phases showed no statistical differences except for D-dimer (Grade 2 DHF) showed a significant decrease (($P = 0.04$) at convalescence (not shown). When combined {Grades 1 & 2}, ANOVA analysis showed no significant differences in the parameters studied.

Comparison between normal controls against combined DHF (Grades 1 & 2) at different phases for F1 + 2, VWF, VEGF and D-dimer.

Grades 1 & 2 DHF were combined to analyse against normal controls. There were significant differences ($P \leq 0.001$) at all phases of DHF for elevated F1 + 2, VWF, VEGF and D-dimer levels compared with normal controls (Table 3).

Table 2: Dengue haemorrhage fever: Comparison of parameters studied (mean ± SD) between Grades 1 & 2 at febrile, defervescence and convalescence phases and compared to febrile phase

	Grade 1	Gr1 – P vs Febrile phase	Grade 2	Gr2- P vs Febrile phase	P Gr1 vs Gr2)
Febrile					
N (male/female)	41 (28/13)	6 (4/2)			
Age years	20.6 (11.8)	30.8 (8.7)		0.03	
Haemoglobin g//L	13.6 (1.8)	14.3 (2.1)		0.46	
Haematocrit %	40.6 (5.6)	42.8 (6.1		0.45	
Platelets x 10^9/L	94.8 (70.9	70.5 (60.4)		0.40	
F1 + 2 pg/mL	293.1 (171.5)	296.0 (157.0)		0.97	
VWF ng/mL	109.7 (29.6)	120.0 (29.1)		0.45	
VEGF pg/mL	270.4 (248.6	253.5 (78.1)		0.74	
D-dimer ng/mL	1770.4 (789.3)	1988.6 (472.1)		0.42	
Defervescence					
Haemoglobin g//L	13.6 (1.7)	0.93	14.2 (2.0)	0.91	0.49
Haematocrit %	41.2 (6.6)	0.64	41.4 (8.3)	0.75	0.96
Platelets x 10^9/L	78.4 (51.3)	0.23	66.5 (413)	0.90	0.54
F1 + 2 pg/mL	350.5 (197.4)	0.93	378.9 (129.7)	0.34	0.66
VWF ng/mL	114.0 (24.9)	0.48	112.3 (26.1)	0.37	0.89
VEGF pg/mL	384.7(430.2)	0.27	312.2 (108.5)	0.31	0.65
D-dimer ng/mL	1829.0 (1499.4)	0.85	1525.2 (617.7)	0.22	0.45
Convalescence					
Haemoglobin g//L	13.2 (1.7)	0.35	13.6 (1.8)	0.65	0.63
Haematocrit %	40.1 (5.3)	0.68	40.4 (5.6)	0.50	0.91
Platelets x 10^9/L	101.8 (58.8)	0.63	97.0 (55.3)	0.45	0.85
F1 + 2 pg/mL	313.8 (264,8)	0.67	336.8 (176.2)	0.68	0.79
VWF ng/mL	113.7 (24.8)	0.51	114.3 (13. 0)	0.68	0.93
VEGF pg/mL	349.0 (433.8	0.32	467.5 (534.0)	0.37	0.62
D-dimer ng/mL	1528.1 (1422.8)	0.47	1085.2 (480.7)	0.01	0.16

This suggests that there is endothelial activation, plasma leakage triggering the activation of coagulation, creating a hypercoagulable and fibrinolytic state in DHF.

Table 3. Comparison between normal controls and combined DHF (Grades 1 & 2) at different phases for F1+2, VWF, VEGF and D-dimer (mean ± SD)

	Normal-Control	DHF-Febrile	DHF-Defervescence	DHF-Convalescence
N	15	47	47	47
Prothrombin Fragment F1+2 pg/mL	ND 293.4 (166.1)	354.1 (189.2)	316.8 (263.4)	
P		< 0.001	< 0.001	< 0.001
VWF ng/mL	1.9 (31.4)*	111.0 (29.6)	113.8 (24.8)	113.8 (23.6)
P		< 0.001	< 0.001	< 0.001
VEGF pg/mL	71.7 (27.9)	268.3 (233.4)	340.5 (323.3)	364.2 (442.9
P		< 0.001	< 0.001	< 0.001
D-dimer ng/mL	< 500	1800.7 (751.9)	1785.6 (1405.1)	1463.8 (1334.8)
P		< 0.001	< 0.001	< 0.001

ND = not detectable (F1 + 2 sensitivity < 28.1 pg/mL); * ND (n = 13), VWF sensitivity < 0.94 ng/mL.

The combined Grades 1 & 2 DHF for VWF, VEGF, F1 + 2 and D-dimer at febrile, defervescence and convalescence phases with ANOVA analysis and normal controls are shown in Figure 1. The results from the three severe DHF patients recruited had elevated VWF, F1 + 2, VEGF and D-dimer with thrombocytopenia similar with grades 1&2 DHF but had lower haemoglobin levels. However, the patient with severe DHF (grade 4) was unconscious when admitted and found to have cerebral bleeding (CT scan) at defervescence phase with pleural effusion, hypervolemic shock. Thrombocytopenia with platelets at 43 x 10^9/L and elevated D-dimer of 1620 ng/mL at admission were given electrolyte and crystalloid infusions. The platelet rose to 88 x 10^9/L, and D-dimer level fell to 809 ng/mL at convalescence. His condition improved and discharged after two weeks in the hospital. The other two patients with grade 3 DHF also had pleural effusion and enlarged livers.

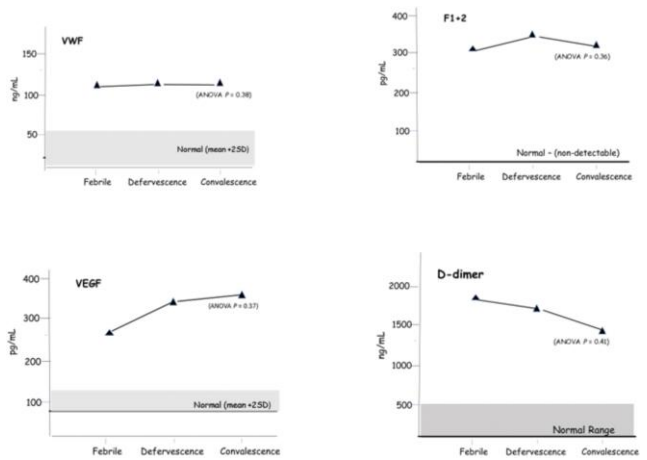

Figure 1: Mean levels of VWF, VEGF, F1 + 2 and D-dimer levels and ANOVA analysis in combined DHF Grades 1 & 2 at febrile, defervescence and convalescence phases

Thrombocytopenia was seen in one patient (platelets 87x 10^9/L) at febrile phase and fell to 11 x 10^9/L at defervescence phase but rose to 141 x 10^9/L at convalescence. The D-dimer levels of 5296.2 ng/mL at the febrile phase fell to 620.4 ng/mL at the convalescence phase. The other patient had normal platelets at admission (173 x 10^9/L), but severe thrombocytopenia was seen in defervescence and convalescence phases (4 x $10^{9/}$L and 37 x 10^9/l) respectively. The D-dimer was 2307.8 ng/mL at admission and 2060 ng/ml at convalescence. They were given electrolyte infusion and other medications and discharged one week later in an afebrile state.

Discussion

Dengue fever is the most serious consequence of mosquito-borne infection worldwide. The pathophysiology of DHF in human is complex as its clinical symptoms are mainly due to an immune response involving the production of cytokine/chemokines as well as endothelial activation, T-lymphocytes, monocytes and platelets. Endothelial damage may also be caused by the virus itself. Thrombocytopenia is responsible for bleeding events in DHF [20], [23] but many factors can contribute to the onset of thrombocytopenia from a reactive immune response against platelets and decreased platelet production [11], [24], platelet activation and apoptosis [21], Dengue virus could bind directly to prothrombin inhibiting the conversion to thrombin [24] causing decreased coagulation activation, reduced thrombin generation and may be associated with bleeding complications in Brazilians with DHF [23] The relationship between dengue and activation of coagulation is controversial [27]. However, activation of coagulation in critical DHF phase was reported in Indonesian patients (22), which was contrary to the Brazilian study [23]. Bleeding manifestations and plasma leakage are complications seen in dengue and bleeding manifestation in adults may occur in the absence of plasma leakage [28].

In our study, petechiae or rash was observed in DHF besides the symptoms of pain in the bones, behind the eyes and headaches. Bleeding episodes like epitaxial were seen in grades 2 and 3 DHF while bleeding to the brain occurred in our grade 4 patient. Pleural effusion was seen only in severe DHF with liver enlargement present in Grades 2, 3 and 4 and about 14.6% (6/41) in Grade 1 DHF. Thrombocytopenia was observed in all phases of DHF even though in the convalescence phase, the mean platelet numbers were higher than in febrile and defervescence phases they did not reach statistical significance between grades 1 & 2 DHF. Normal haemoglobin and no haemo-concentration were observed, but elevated activation of coagulation (F1 + 2), VWF, VEGF and D-dimer suggest endothelial activation, plasma leakage and activation of coagulation in DHF. Activation of coagulation was reported earlier in critical DHF [22] in Indonesian patients, but contrary to this, Orsi and co-workers [23] reported reduced thrombin generation and enhanced fibrinolysis contributing to the bleeding episodes in Brazilian patients. Reduced thrombin generation could result from the dengue virus binding directly to prothrombin inhibiting the conversion to thrombin [26]. Activation of coagulation and elevated D-dimer levels also indicates hypercoagulability and enhanced fibrinolysis. Endothelial activation evident by elevated VWF and VEGF suggests plasma leakage triggering the activation of the coagulation system, creating hypercoagulation and enhanced fibrin-lysis state. Elevated D-dimer was seen in DHF even at convalescence. Normal haemoglobin and no haemoconcentration was observed in DHF grades 1 and 2. No mortality was recorded. Demographic differences and genetic make-up may contribute to these differences. Identifying the mechanisms affecting DHF would improve diagnosis and management therapy limiting morbidity and mortality.

In conclusion, in dengue haemorrhagic fever, the vascular endothelium is activated, causing plasma leakage triggering the activation of the haemostatic system creating a hypercoagulable and enhanced fibrinolytic state evident by marked fibrin-lysis.

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Effect of Nanoherbal Andaliman *(Zanthoxylum acanthopodium)* and Extra Virgin Olive Oil Combination on Preeclamptic Rats Liver Histology

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Abstract

BACKGROUND: Andaliman (*Zanthoxylum acanthopodium*) is a spice traditional Northen Sumatera, Indonesia and these fruits contain alkaloids, steroids and terpenoids. Extra Virgin Olive Oil (EVOO) contains antioxidants. Combination of this plant have activities to reduce preeclampsia.

AIM: To know the safety of the combination of nano herbal andaliman and Extra virgin olive oil (EVOO) on preeclampsia patients' liver.

METHODS: Pregnant rats were made to have preeclampsia with 3 ml of NaCl 6% injections. This research consists of 5 groups: K- (negative control): normal pregnant rats, K+: preeclampsia rats; P1: PE rats were given nano herbal andaliman 1 ml EVOO / day / 20 gBW from the 13th to the 19th day of pregnancy, P2: PE rats were given nano herbal andaliman 100 mg/day / 200 gBW from the 13th to the 19th day of pregnancy, P3: PE was given the combination of 1 ml EVOO/day / 200 gBW and andaliman nano herbal 100 mg/day/200 gBW on the 13th day of pregnancy to the 19th day given orally. Then on the 20th day of pregnancy, the subjects were dissected.

RESULTS: There were significant differences ($p < 0.05$) on the value of SGOT, SGPT, and the average damage of the hepatocyte cells except parenchymatous degeneration after being given the nano herbal andaliman and EVOO. The compared mean of normal hepatocytes cell, hydropic degeneration and necrosis value between all groups were $p < 0.05$ and $p < 0.01$ compared to (K-). The non-significant difference was found in the mean of parenchymatous degeneration between the groups ($p = 0.058$).

CONCLUSION: The combination of nano herbal andaliman (*Zanthoxylum acanthopodium*) and EVOO affected the level of necrosis in hepatocyte cells on preeclampsia rats.

Introduction

Preeclampsia (PE) is a multi-system disorder that is a major cause of maternal morbidity and mortality worldwide. Recent data show that the contribution of preeclampsia is estimated to be about 5 times that of morbidity & maternal-newborn mortality [1]. PE is a life-threatening disease for mother and fetus in Indonesia. According to the Indonesian Demographic and Health Survey in 2007, PE contributed up to 24% of maternal deaths in Indonesia and made it the second leading cause of maternal mortality in Indonesia [2]. The international non-governmental organisation forum on Indonesian Development stated that Indonesia is a country in Southeast Asia with the highest maternal mortality rate of 359/100.000 births [3].

Andaliman (*Zanthoxylum acanthopodium*.) is a spice that is used for traditional Batak cuisine, Norther Sumatera, Indonesia [4]. This plant has been used as a contraceptive for generations as an anti-fertility. Andaliman extract contains chemicals in the form of alkaloids, steroids and terpenoids, which have antioxidant activity and antimicrobial, repellent and kill insects [5], [6], [7]. The content of this plant is thought to have activities to reduce PE because andaliman fruit has also been reported to have anti-inflammatory activity and antioxidant activity [7]. *Extra Virgin Olive Oil* (EVOO) from Olive fruit contains antioxidants, namely Vitamin E, hydroxityrosol and tyrosol [8].

Nanotechnology is a system that has several

advantages, namely being able to modify the characteristics of the surface, small size, high loading capacity so that it can be given in high concentrations [9]. Constraints that often occur in herbal medicines are difficult active substances to penetrate the lipid membrane of body cells because they have a large molecular size and low solubility in water that causes poor absorption and bioavailability [10]. Changes in drug molecules into nanometer scales provide a significant change in physicochemical properties and can improve the efficacy of these drug molecules [11].

The liver is the largest organ in the body that plays a role in detoxifying poisons in the blood, breaking down or changing the nature of toxic substances so that it can be released through urine. The impact of the combination of these two herbs needs to be seen in the liver in terms of their safety effects in preeclamptic patients.

Material and methods

Nanoherbal Andaliman (Zanthoxylum acanthopodium)

Andaliman fruit used comes from the Dairi District of Northen Sumatera. Andaliman is washed thoroughly; then the wind is dried for 3 days at room temperature, then blended until smooth and then sized to nano size using *High energy milling* (HEM). Simplicia as a destructive medium is inserted into the jar container and then inserted a ball with a larger diameter size and continued by inserting a small ball and the sample is placed lastly. The total volume of the ball and the sample inserted do not exceed 2 / 3 of the tube volume. The sample was tightly closed and then placed on a tube inside the HEM device, then HEM was turned on for 2 hours [12].

Phytochemical screening

Alkaloids: 1 g of nano herbal andaliman was put in a test tube then added 18 ml of distilled water and 2 ml of 2 N hydrochloric acids then heated for 2 minutes. The trial was conducted with Meyer reagent. Positive alkaloids if sediment or turbidity occurs.

Glycosides: 3 g of nano herbal andaliman extracted in 30 ml mixture of 7 parts ethanol 96% and 3 parts of distilled water, then added concentrated sulfuric acid and refluxed in 10 minutes. After chilling 20 ml of filtrate added 10 ml of distilled water and 10 ml of lead (II) acetate 0.4 M were shaken and left for 5 minutes. The filtrate that has been filtered in the juice with 20 ml mixture of chloroform and isopropanol (3: 2) to be tested against sugar compounds and non-sugar compounds [13].

Flavonoids: 1 g of nano herbal andaliman mixed in 20 ml of methanol then reflux 10 minutes. After cooling, 10 ml of kerosene ether is added and then shaken and let stand until separation, the methanol layer is taken and then evaporated at 40°C, the remainder is dissolved in ethyl acetate and filtered by filtrate to be tested with 0.5 g zinc powder and 0.1 g powder magnesium [13].

Saponin: 1 g of nano herbal andaliman and 20 ml of hot water have been shaken for 20 seconds. The saponin is positive if there is foam in not less than 10 minutes as high as 1-10 cm after adding 2 drops of hydrochloric acid 2 N foam was not lost.

Steroids/Terpenoids: 2 g nano herbal andaliman were macerated with 40 ml ether for 2 hours. The filtrate was filtered and evaporated. The remaining 4 drops of Lierbermann-Burchard reagent, if there is a red/purple colour changing to blue or blue-green means there is steroid.

Tanin: 0.5 g of nano herbal andaliman mixed in 50 ml of distilled water, then filtered, and then added 1 drop of 1% iron (III) chloride solution. If bluish-green was formed, show tannin compounds.

Antioxidant test with 1.1-diphenyl-2-picrylhydrazyl (DPPH) method

Nanoherbal andaliman was dissolved with methanol so that it becomes 250 µg/mL then homogenised for 10 minutes for 4 times then centrifuged to take the clear solution on top. Two mL of DPPH solution was added with 0.5 mL of nano herbal andaliman solution with 3 repetitions for each extract solution 6,25; 12.5; 25 and 50µg mL, then the absorbance was measured against methanol at a wavelength of 517 nm using a UV-Visible spectrophotometer.

Animal

This study used 25 pregnant *Rattus norvergicus*. Rats were mated at the Biology Laboratory animal house, University of Sumatera Utara. Pregnant rats are made into preeclampsia model by injecting 3 ml of 6% NaCl/day/200 gBW at 6 to 12 days gestation subcutaneously. Preeclampsia rats were evaluated by blood pressure more than 125/80 mmHg, MDA levels and proteunaria values more than 3 g/L [14]. This study consisted of 5 groups: K^- (negative control): Normal pregnant rats; K^+ (positive control): Preeclampsia (PE) pregnant rats, P1: PE rats given 1 ml EVOO/day/200 gBW in pregnancies 13 to 19 pregnancy day orally, P2: PE rats were given nanoherbal andaliman 100 mg/day/200 gBW at 13 to 19 days gestation orally, P3: PE rats were given a combination of 1 ml EVOO/day/200 gBW and nanoherbal andaliman 100 mg/days/200 gBW at 13 to 19 days of gestation orally. Pregnant rats were dissected on the 20th day of pregnancy, for blood and liver to be taken, and then

liver preparations were made with paraffin blocks and *Hematoxylin Eosin* (HE) staining.

Examination of Serum Glutamic Oxaloacetic Transaminase (SGOT) and Glutamic Serum Pyruvate Transaminase (SGPT)

SGOT: Blood was centrifuged for ± 15 minutes at a speed of 5000 rpm. The blood serum then pipetted 200 µL aqua dest into the test tube, added 2000 µL of SGOT reagent 1 and then incubated for 5 minutes at 37°C. 500 µL of reagent 2 SGOT was added after homogeneous absorbance was measured at 365 nm wavelength with a spectrophotometer.

SGPT: Blood is centrifuged for ± 15 minutes at a speed of 5000 rpm. Blood serum piped 200 µL aquadest into the test tube then added 2000 µL of reagent 1 SGPT, then homogenised. After incubation for 5 minutes at 37°C. We have added 500 µL of reagent 2 SGPT then absorbance was measured at a wavelength of 365 nm with a spectrophotometer

Analysis of Data

The data were calculated the average score of liver histopathology changes from five fields of view with the *Manja Roenigk Histopathology Scoring* model. Then data were analysed by Anova test and non-parametric data by *Kruskal Wallis test* in SPSS 22 program.

Results

Phytochemical screening and DPPH test

Based on the research that has been done, andaliman fruit in nanosize has the content of alkaloids, flavonoids, glycosides, steroids and terpenoids. The content of these compounds was similar to the content of andaliman extract in previous studies. DPPH test results on nano herbal andaliman with 3 repetitions for each extract solution 6,25; 12.5; 25 and 50µg / mL is IC50 48.5 µg / mL. Nanoherbal andaliman have very strong antioxidants.

Bodyweight and liver weight

Based on statistical data, ANOVA test on pregnant rat body weight showed that there was no significant difference in each treatment with $p > 0.05$ ($p = 0.060$). However, there were significant differences in liver weight ($p < 0.05$). The highest average weight is in K^+ and the lowest group in the K^- and P1 groups. Based on these data, nano herbal andaliman, EVOO, and a combination of both can affect the weight of pregnant rat hearts. That means the liver here acts in the detoxification of foreign substances that first entered the body of the rat due to the content of secondary metabolites in the nano herbal andaliman and EVOO. The metabolic process in the liver is the process that affects its weight.

SGOT and SGPT

Based on statistical data on SGOT values in pregnant rats, there were significant differences ($p < 0.05$) in each treatment (Figure 1). However, the highest average value was found in the K^+ group and the lowest in the K^- and P3 groups. Nanoherbal andaliman can increase SGOT values higher in pregnant rats than EVOO and a combination of both.

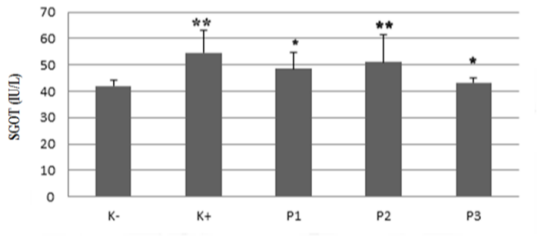

*Figure 1: Data are expressed with Kruskal Wallis test; Mann-Whitney test was applied to compare SGOT value between all groups; **p < 0.01 compared to control (K-); *p < 0.05 compared to K+; K-: Pregnant normal; K+: PE rats; P1: PE rats after given EVOO; P2: PE rats after given nano herbal andaliman; P3: PE rats after given EVOO and nano herbal andaliman*

The SGPT value also has a significant difference with a value of < 0.05. The highest average SGPT value was found in P2, and the lowest was found in the control group (K^-). This means that there is a high level of damage in the PE liver treatment there is P2 so that the value of SGOT and SGPT is higher than other treatments. Based on this data nanoherbal andaliman and EVOO can increase the value of SGOT and SGPT in pregnant rats (Figure 2).

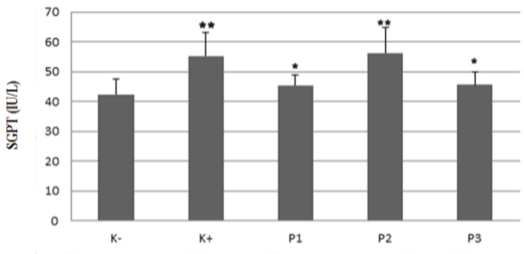

*Figure 2: Data are expressed Kruskal Wallis test; Mann-Whitney test was applied to compare SGPT value between all groups; **p < 0.01 compared to control; *p < 0.05 compared to K+; K-: Pregnant normal; K+: PE rats; P1: PE rats after given EVOO; P2: PE rats after given nano herbal andaliman; P3: PE rats after given EVOO and nano herbal andaliman*

Histology of rat's liver

Based on statistical data on normal hepatocyte cell values, parenchymatous degeneration, hydrophilic degeneration and necrosis in pregnant rats using the cruciferous Wallis test there were no significant differences ($p > 0.05$). But based on the highest average normal hepatocyte cells were found in K^- and P1 while the lowest was in K^+ and P1 (Table 1). However, there was no significant difference in parenchymatic degeneration ($p > 0.05$). The highest parenchymatous degeneration at K^+ and lowest at K^-. The highest hydropic degeneration was also found in P1 and P2 while the lowest was K^-. The data proved significantly with a value of $p < 0.05$. The highest necrosis is at K^+ and lowest at K^- and P3. Based on the statistics of nano herbal andaliman, EVOO and the combination of both causes liver damage with a degree of damage in the form of parenchymatous degeneration, hydrophilic degeneration and necrosis.

Table 1: Average of normal hepatocytes cells and liver damages on preeclamptic rats

Treatments	Normal	Parenchymatous Degeneration	Hydropic Degeneration	Necrosis
K^-	13 ± 1.46	5.52 ± 1.76	5.52 ± 3.20	9.76 ± 3.67
K^+	5.36 ± 1. 49**	8.32 ± 2.69	11 ± 4.72**	27.2 ± 10.00**
P1	12.2 ± 1.31*	5.52 ± 1.94	11.5 ± 2.43	10.88 ± 3.56*
P2	8.56 ± 1.33**	6.72 ± 2.15	11.5 ± 3.43**	16.96 ± 4.21**
P3	11.2 ± 1.48*	7.2 ± 2.45	7.8 ± 3.00*	10.24 ± 4.18*

Kruskal Wallis test and *Post-hock test* were applied to compare mean of normal hepatocytes cell, hydropic degeneration and necrosis value between all groups; *p < 0.05 compared to control (K^-); **p < 0.01 compared to K^-; Non-significant difference was found in the mean of parenchymatous degeneration between the groups (p = 0.058).

Based on the average pattern of hepatocyte cell damage in pregnant rats, the greatest damage was found in the K^+ group and also P2, while the lowest damage was found in K^- and P3 (Figure 3). Based on this data, pregnant women who often want to consume andaliman are better combined with EVOO to prevent liver cell damage.

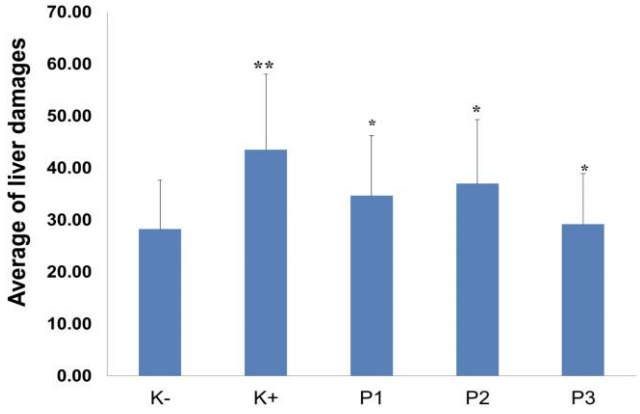

*Figure 3: Data are expressed Kruskal Wallis test; Mann-Whitney test was applied to compare trophoblast cells value between all groups; **p < 0.01 compared to control; *p < 0.05 compared to K+; K-: Pregnant normal; K+: PE rats; P1: PE rats after given EVOO; P2: PE rats after given nano herbal andaliman; P3: PE rats after given EVOO and nano herbal andaliman*

Based on histological observations, it was seen that K^+ (PE) had the greatest damage compared to other treatments. Liver hepatocyte damage was also seen after being given the herbal andaliman EVOO and a combination of both but not as much as K^+ (PE) (Figure 4). This means that the compounds contained in nano herbal andaliman cause liver necrosis. Fat degeneration (parenchymatous degeneration) is also seen in histology such as fat accumulation in the cell cytoplasm where fat in the cytoplasm pushes the cell nucleus to the side, due to interference with hepatocytes so that lipoproteins are not formed. In group P1 there are more parenchymatous degeneration characterised by varied fats and vacuoles but cannot be seen hydrophilic degeneration is also evident in K^+, P1 and P2 where cell forms are like cell swelling (Figure 4). This degeneration is more severe damage; there are vacuoles containing water and cytoplasm that do not contain fat and glycogen.

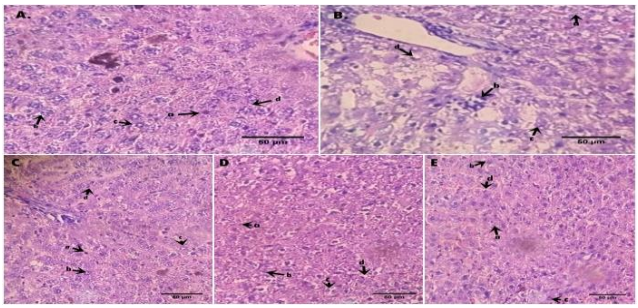

Figure 4: Histology of rats liver; A) K- (Pregnant normal); B) K+(PE rats); C) P1 (PE rats after given EVOO); D) P2 (PE rats after given nano herbal andaliman); E) P3 (PE rats after given EVOO and nano herbal andaliman); A) Normal; B) Parenchymatous degeneration; C) Hydropic degeneration; D) Necrosis (H & E) 40x

Discussion

PE can cause interference with the liver. Liver dysfunction can occur due to vasoconstriction and oedema that shows damage to the liver, muscles, kidneys, pancreas and red blood cells [15]. Liver damage is always associated with necrosis and administration of exogenous antioxidants, may be beneficial in protecting the liver [16]. Natural antioxidants are known to have beneficial effects on hepatitis or liver disorders caused by antitubercular agents [17]. Andaliman possesses unique flavour properties and bioactive compounds. The chemical compound in this herbal was Monoterpenes (46.54%), hydrocarbon monoterpenes (19.75%). The primary volatile compounds in andaliman (relative peak area > 10%) are geranyl acetate (32.04%) and limonene (15.80%) [18]. Andaliman in nanosize also contains alkaloids, flavonoids, glycosides, steroids and terpenoids as well as extracts [5], [6], [7]. Andaliman

fruits and leaves contain terpenoids, alkaloids, flavonoids, and other phenolics, which can function as antioxidants [19]. The ethyl acetate extract of andaliman fruit has antioxidant activity and with an IC50 value of 66.91 ppm and isolating EA.X.6.1 has antioxidant activity and with an IC505.55 ppm value [20]. Andaliman in nanosize has IC50 value of 48.5 µg/mL so that antioxidant properties are very strong compared to extract. Antioxidants are components that can inhibit free radicals and it is estimated that in scala nano can reduce oxidative stress in PE. According to Tensisca *et al.,* [21] andaliman fruit extract with ethanol and hexane has different antioxidant activity which is highest in water systems in emulsion and oily systems though having moderate activity. The content of important compounds is relatively stable during heating, but is heating up to 175°C; it can reduce up to 17%. Andaliman in the form of extract also has different contents and activities when exposed to heat, fluorescent light and ultraviolet [22]. Olive oil is a vegetable oil obtained from olive plants (*Olea europaea*) in the packaging of *Extra Virgin Olive Oil* (EVOO) in low doses can control serum levels of Hsp70, so the process of apoptosis does not occur excessively especially in preeclampsia [23]. The combination of these two plants can reduce hepatocyte cell damage because of the incorporation of antioxidants and vitamin E.

The value of SGPT and SGOT in PE can decrease with the combination of these two herbs (Figure 1 and 2). SGPT and SGOT will come out of liver cells if the liver cells are damaged so that it will cause an increase in SGPT and SGOT levels in blood serum [24], [25]. Increased SGOT treatment is also caused by stress. Hepatocytes are the type of cell that forms most of the liver. These cells are located between sinusoids, which are full of blood, and bile ducts. The liver is often the target organ of toxic substances because most poisons enter the body through the digestive system, then after being absorbed, carried by the portal vein to the liver — the highest parenchymatous degeneration at K^+ and lowest at K^-. Parenchymatous degeneration is the lightest level of category of degeneration. Cells that become parenchymal degeneration are found in granules in the cytoplasm, due to the deposits that cause the cytoplasm to become cloudy and followed by swelling in cells [26]. The highest hydropic degeneration is also in K^+ (Table 1). Hydropic degeneration is a more severe level of damage. These changes are generally a result of metabolic disorders, such as hypoxia or chemical poisoning. This degeneration is also reversible even though it is possible to be irreversible if the cause of the injury persists [26]. Cells that have been injured can cause damage to the plasma membrane and changes in the nucleus. Pregnant women that often consume andaliman should also consume it with EVOO to prevent damage to the liver cells. EVOO contains vitamin E (tocopherol) that is anti-apoptosis [23]. Olives that are converted to *Extra virgin olive oil* (EVOO) have analgesic, anti-inflammatory and anticancer properties [27]. So, using andaliman nano herbal is better along with EVOO to reduce necrosis in the liver.

Between P1, P2 and P3, it appears that more damage is found in P2 (Giving nano herbal andaliman only) means that the compounds contained in andaliman nanoherbal cause liver necrosis. Liver disease in PE have a high risk of pregnancy disorder, although no reports of maternal death but the birth of premature infants [28]. Liver disorders in PE dieaseas may increase liver enzymes, autoimmune, hyperemesis gravidarum, acute fatty liver, and intrahepatic cholestasis [29]. This is in accordance with Emita's study [30]. where there was a change in the color and texture of the liver surface, as well as an increase in hepatocyte damage. Liver disorders in PE can increase liver enzymes, low platelets (HELLP), acute fatty liver, hyperemesis gravidarum, intrahepatic cholestasis, and autoimmune liver [29]. Regulations of damaged liver in the metabolic system can cause gestational hypertension during the first pregnancy and can cause PE, hemolysis, increased liver enzymes, and low platelet syndrome (HELLP) [31]. EVOO contains exogenous antioxidants has anti-inflammatory, anticancer and analgesic properties and neuroprotective activities that can fight oxidative damage to the brain [32]. Thus, andaliman in combination with EVOO is better and safer than just andaliman.

In conclusion, the combination of nanoherbal andaliman (*Zanthoxylum acanthopodium*) and *Extra Virgin Olive Oil* (EVOO) can reduce parenchymatous degeneration, hydrophic degeneration and hepatocyte cell necrosis in preeclampsia rats ($p < 0.05$). Further testing with immunohistochemistry is recommended.

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Basic Science

Red Dragon Fruit (*Hylocereus Polyrhizus*) Extract Decreases Lactic Acid Level and Creatine Kinase Activity in Rats Receiving Heavy Physical Exercise

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Keywords: Lactic acid; Creatine kinase; Red dragon fruit; Exercise

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Abstract

BACKGROUND: Heavy physical exercise causes relative hypoxia. In hypoxic condition, the cell's energy comes from anaerobic metabolism that produces lactic acid. An increment of oxygen need leads to ischemia-reperfusion, triggers free radical formation and damages muscles. Creatine kinase (CK) is a marker of muscle tissue damage. Red dragon fruit (RDF) has potential as antioxidant to reduce free radical formation.

AIM: This study aims to determine RDF extract potential to reduce the lactic acid level and CK activity after heavy physical exercise.

METHODS: A total of 32 male rats (Rattus Norvegicus) were randomly divided into 4 groups: group NORDF, treated heavy physical exercise and distilled water; group RDF100, treated heavy physical exercise and at 100 mg/kg BW RDF extract; group RDF200, treated heavy physical exercise and at 200 mg/kg BW RDF extract and group RDF300, treated heavy physical exercise and at 300 mg/kg BW RDF extract. The rats swam for 20 minutes, 3 times a week for 3 weeks.

RESULTS: RDF300 group showed lower lactic acid level and CK activity as compared to that of NORDF ($p = 0.00$) and RDF100 ($p = 0.00$) groups, but RDF300 are not significantly different for lactic acid ($p = 0.45$) and for CK ($p = 0.68$).

CONCLUSION: Red dragon fruit extract has potential in lowering lactic acid level and CK activity in male rats receiving heavy physical exercise.

Introduction

Regular and measured exercises by the formulation increase physical condition, fitness and performance, and decrease the injury risk [1]. Physical exercises need energy and oxygen supply, especially during the heavy one. Heavy physical exercises result in relative hypoxia, reduction of oxygen supply to tissues. In hypoxic condition, energy source comes from anaerobic metabolism with low energy but high lactic acid level. Lactic acid accumulation in myocytes disturbs muscle performance [2].

Heavy physical exercises increase metabolism and oxygen consumption by 100 – 200 folds [3], [4]. The increase in oxygen need, especially by muscles contraction, results in ischemic reperfusion and free radicals [5]. Free radicals formed during physical exercise induce tissues damage such as blood, liver and other tissues [6], [7].

Creatine kinase (CK) activity increases during heavy physical exercise due to muscle cells damage [8], [9]. Therefore, CK activity has potential as a biomarker for muscle tissues damage, although its activity depends on pathological and physiological conditions [10], [11]. CK activity in athletes who had had different exercises intensity and frequency changes [8], [11], [12].

Curcuma zedoaria extract of 750 mg/d lowers lactic acid level in athletes [13]. Red dragon fruit (RDF) (*Hylocereus polyrhizus*) is a unique and useful fruit for its natural antioxidant potential [14], [15].

This study aims to determine the effect of red dragon fruit on lactic acid level and CK activity of rats after heavy physical exercise.

Methods

This experimental study used post-test and control group design. A total of 32 male rats, 3-4 months old and 180-200 gr, were acclimatised for a week in animal cages. Rats were accessed with food and water *ad libitum* in a room with 12 h light/dark cycle (lights on at 7:00 A.M.). Room temperature and humidity were set at natural condition. Ethical approval was obtained from the ethics committee of the Faculty of Mathematics and Sciences, Universitas Sumatera Utara.

Rats were randomly divided into 4 treatment groups, i.e. (i) group NORDF, treated heavy physical exercise and distilled water, (ii) group RDF100, treated heavy physical exercise and at 100 mg/kg BW RDF extract, (iii) group RDF200, treated heavy physical exercise and at 200 mg/kg BW RDF extract and (iv) group RDF300, treated heavy physical exercise and at 300 mg/kg BW RDF extract. Macerated RDF was extracted using 96% of ethanol.

Before the treatment, the maximum physical activity was carried out by calming the rats until they were almost drowned, and the results showed that the swimming resistance of rats was 30-35 minutes. To determine the length of time the heavy physical exercise of rats is 75% of the maximum endurance swimming of rats is an average of 20 minutes. All rats had heavy physical exercise in form of swimming for 20 min, 3 times a week for 3 weeks. Rats were treated with RDF extract every day for 3 weeks respectively at half an hour before they had heavy physical exercise.

Two days after the rats have completed heavy physical exercise course, all rats had maximal physical activity, i.e. swimming till they almost drown, soon the rat blood sample was taken for CK and lactic acid examination. Blood was examined for lactic acid level and CK activity. Accutrend® device (by Roche Diagnostics USA) with dipstick method measured lactic acid level. CK activity was measured using the enzymatic kinetic method (Randox Laboratories). Data were analysed using Anova test and post hoc Tukey test in SPSS program 23.

Results

The characteristics of the rats are described in Table 1 below. NORDF, RDF100, RDF200 and RDF300 groups were similar in age and weight. Body Weight was measured before treatment.

Table 1: Data of Rats Characteristic (n = 32)

Rats characteristics	NORDF	RDF100	RDF200	RDF300	p
Weight (gr)	197.48 ± 13.59	196.43 ± 15.64	196.71 ± 13.70	198.56 ± 12.82	0.61
Age (week)	12.88 ± 0.84	13.25 ± 0.89	13.25 ± 0.89	12.50 ± 0.76	0.89

Based on Table 1, it shows that NORDF, RDF100, RDF200 and RDF300 groups were similar in age and weight. Bodyweight was measured before treatment.

Table 2: Lactic Acid Level and CK Activity In 4 Treatment Groups of Male Rats Treated with Heavy Exercise and Various Doses of RDF Extract

Variable	NORDF	RDF100	RDF200	RDF300	p
Lactic acid (mmol/l)	6.83 ± 0.43	5.19 ± 0.57	4.49 ± 0.51	4.08 ± 0.21	0.00*
Creatine kinase (IU/l)	578.37±64.01	307.67±70.77	183.85±47.23	142.26±79.53	0.00*

Based on Table 2 and Figure 1, red dragon fruit (Hylocereus polyrhizus) extract decreases lactic acid level in rats receiving heavy physical exercise. The lactic acid level in the RDF 300 group had a lower-trends than the other groups and found the highest lactic acid of the NORDF group. RDF extract in a dose of 300 mg/kg BW can decrease lactic acid significantly different compared at group RDF100 (p = 0.000) and group RDF200 given RDF extract in a dose 100 mg/kg BW (p = 0.000) but group RDF300 which in a dose 200 mg/kg BW of RDF extract are not significantly different (p = 0.476). It indicated that giving in a dose 300 mg/kg BW and 200 mg/kg BW of RDF extract were equally better in decreasing lactic acid than group NORDF, group without giving RDF extract.

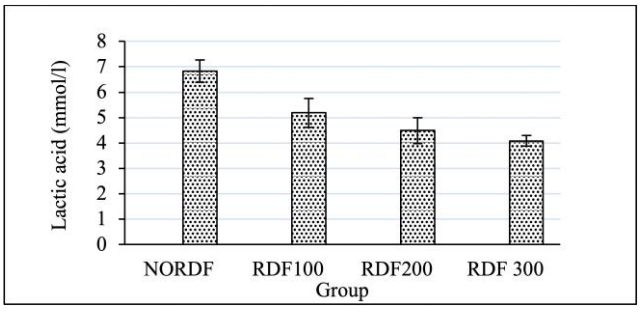

Figure 1: The Relationship Giving RDF and Lactic Acid Between Study Groups

Based on Table 2 and Figure 2, red dragon fruit (*Hylocereus polyrhizus*) extract decreases creatine kinase activity in rats receiving heavy physical exercise. CK activity in the RDF 300 group

had a lower trend than the other groups and found the highest CK activity in the NORDF group Administration of 300 mg/kg BW RDF extract (RDF300 group) decreased CK activity significantly as compared to that of RDF100 group (p = 0.00) and but not to RDF200 group (Table, Figure 4). It showed that administration of 300 mg/kg and 200 mg/kg of RDF extract were better than 100 mg/kg RDF extract in decreasing CK activity, as compared to that of NORDF and RDF100 groups.

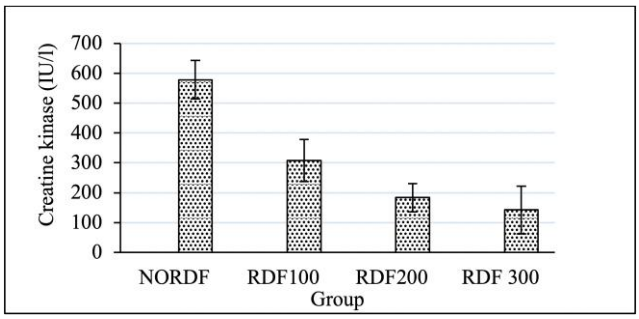

Figure 2: The Relationship Giving RDF and CK Between Study Groups

Discussion

The increment of the lactic acid level after having heavy physical exercise is mainly caused by hypoxia. Hypoxia is the decrease in the amount of oxygen in muscles. In hypoxic condition, energy is obtained from anaerobic metabolism [16], [17]. Rats which were treated for heavy physical activity increased the lactic acid level [18]. The increase in lactic acid level causes purine catabolism into xanthine and indicates acute muscle deoxygenation and ischemic-reperfusion. Purine catabolism and ischemic reperfusion activate xanthene oxidation leading to form free radicals and their accumulation [19]. Free radicals were formed during heavy physical exercise and were inhibited in the presence of adequate amount of antioxidant [20], [21]. The antioxidant in RDF extract indirectly decreased lactic acid level. This present study showed that lactic acid level after heavy physical exercise in NORDF group was 6.83 mmol/L, which is higher than RDF100 group (5.19 mmol/L), RDF 200 group (4.49 mmol/L) and RDF300 group (4.08 mmol/L).

In this study, CK activity after heavy physical exercise in NORDF was 5783.75 U/L, which is higher than that of RDF100 group (3076.75 U/L), RDF200 group (1838.50 U/L) and RDF300 (1422.63 U/L). RDF extract in a dose of 300 mg/kg and 200 mg/kg BW decreased CK activity in rats treated with heavy physical exercise. CK activity increases due to muscle tissues damage [22]. Long and high-intensity exercise results in higher metabolism and physical activity [10]. In mild to moderate physical exercise membrane permeability does not change. However, if the intensity increased to heavy physical exercise, the membrane permeability will surpass the muscle capacity limit and lead the CK to enter circulation [23]. Post-exercise recovery reduces CK activity since low-intensity physical activity lowers CK lymphatic transport and CK release from muscles, the intensity of physical activity relates to serum CK activity [24], [25]. The previous study showed that giving Changbai Mountain after a heavy activity can reduce lactic acid levels and creatine kinase [26]. The study showed an increase in lactic acid and CK after physical exercise [27]. The decrease in lactic acid levels and CK in this study was due to the antioxidant content found in red dragon fruit [28], [29], [30].

In conclusion, red dragon fruit extract decreases lactic acid levels and CK activity in rats treated with heavy physical exercise. A dose of 300 and 200 mg/kg were potential doses in reducing lactic acid and CK activity.

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Clinical Science

The Effect of the Phytocomplex Electrophoresis on the Clinical Symptomatology and Quality of Life of Patients with the Knee Joint Osteoarthritis

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Keywords: Osteoarthritis; Drug electrophoresis; Herbal extract; Knee joint osteoarthritis; Electrotherapy

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Abstract

BACKGROUND: Improving the effectiveness of rehabilitation of patients with osteoarthritis necessitates the use of drug electrophoresis with sinusoidal modulated currents (SMC-electrophoresis) in conjunction with drug therapy. The phytocomplex is proposed for electrophoresis composed of the compared, alfalfa and hops dry extract, containing flavonoids, coumestans, polysaccharides, steroids, essential amino acids, vitamins, mineral components and causing its possible use in osteoarthritis.

AIM: The research aims to study the effect of the phytocomplex SMC-electrophoresis on the clinical symptoms and quality of life of patients with the knee joint osteoarthritis.

METHODS: One hundred and eight patients were randomly distributed into 3 groups (n = 36). The phytocomplex SMC-electrophoresis was assigned to the first group, the amplipulse therapy (SMC) – to the second group, and the "basic" drug therapy – to the third group. The drug therapy of the patients of the third group was comparable with the drug treatment of those in the first two groups. The concentration of phytocomplex in the working solution was 10%. The electrotherapy was carried out in the aligned SMC mode in the first and fourth kind of works. Comparative indicators were as follows: WOMAC index, pain level on a visual analogue scale (VAS), Lequesne index, joint range of motion (JROM), articular and tendon indices, quality of life as per Health Assessment Questionnaire (HAQ).

RESULTS: The use of the phytocomplex SMC-electrophoresis had a more pronounced positive effect on pain, knee joint function and quality of life of the patients compared with the treatment with drugs alone or using amplitude therapy. This was especially pronounced immediately after the rehabilitation. The analgesic effect was consistently maintained in the patients of the first group for up to 6 – 12 months, the second group – up to 3 – 6 months in terms of the level of pain according to the WOMAC and Lequesne indices, VAS, articular and tendon indices. Stable results have been obtained for improving the functions of the knee joint for up to 6 – 12 months using the phytocomplex SMC-electrophoresis as per the WOMAC and Lequesne indices. In the treatment by the phytocomplex SMC-electrophoresis, no side effects were registered.

CONCLUSION: The obtained results give grounds for further research on the evaluation of the effectiveness of using the phytocomplex SMC-electrophoresis in microcirculatory disorders in the affected joint, for correcting connective tissue metabolism and electrolyte metabolism in the patients with the knee joint osteoarthritis.

Introduction

An osteoarthritis is a heterogeneous group of diseases of various etiologies. The basis of osteoarthritis is the damage of all joint components, primarily of cartilage and subchondral bone, synovial membrane, ligaments, and periarticular muscle capsules [1]. The disease has a significant and widespread prevalence in the world [2], [3], [4]. It results from the interaction of multiple genetic and environmental factors, affects mostly people of the older age group, is characterized by a long course with the tendency to recrudescence and progression, deterioration in the quality of life of the patients, and disability [5], [6], [7], [8]. This determines the high social and economic significance of the disease [9], [10].

Traditionally, the treatment of osteoarthritis is comprehensive and includes medical and nonmedical methods, including the physiotherapeutic ones [11], [12], [13]. The low-frequency electrotherapy with SMC, or the amplitude therapy, in osteoarthritis has analgesic, neurostimulation, vasodilator, and trophostimulating effects [14]. To enhance the therapeutic effect, amplipulse therapy is combined with the administration of drugs (SMC-electrophoresis) [15], [16], [17], [18].

The phytocomplex for SMC-electrophoresis offered by the authors is a dry extract from grass and roots of bog strawberry, the alfalfa grass and stems or strobiles of common hop (TU 9375-021-00003938-11 "Bog strawberry, alfalfa and hops dry extract (phytocomplex)") [19]. It contains a set of biologically active substances, including flavonoids, contestants, polysaccharides, steroids, essential oils, tannins, hydroxycinnamic and phenol carboxylic acids, essential amino acids, vitamins, and mineral components, which stipulates its possible use in medicine for inflammatory-degenerative diseases of the locomotor system, including osteoarthritis.

The scope of the work was to study the effect of the phytocomplex SMC-electrophoresis on the clinical symptoms and quality of life of the patients with the knee joint osteoarthritis.

Material and Methods

The study included 108 patients with a verified diagnosis of the knee joint osteoarthritis. Clinical trials were carried out by the ethical principles of the Helsinki Declaration, good clinical practice (GCP), and applicable regulatory requirements. The clinical trials of the phytocomplex SMC-electrophoresis in the rehabilitation of patients with the knee osteoarthritis were allowed by the Interuniversity Ethics Committee under the Association of Medical and Pharmacy Universities of Russia.

The criteria for inclusion of patients in the study were a verified diagnosis of the knee joint osteoarthritis according to the criteria of the American College of Rheumatology (ACR), 1-2 Kellgren-Lawrence grade X-ray stage, without synovitis or with its small manifestations, the intensity of pain in the affected joint on VAS − not less than 40 mm, the administration of Symptomatic Slow Acting Drugs for Osteoarthritis (SYSADOA) as per generally accepted regimens in a stable dosage − at least 3 months before the start of the study, with the possibility of administration of nonsteroidal anti-inflammatory drugs (NSAIDs) in stable, standard daily doses, and the written consent to participate in the test. The exclusion criteria were as follows: secondary knee joint osteoarthritis, intra-articular administration of any drugs within 6 weeks prior to the study, treatment with glucocorticoids during the last month, history of operation on the knee joint tested, pronounced symptoms of synovitis, pregnancy, breastfeeding, contraindications to the use of SMC, individual intolerance to the biologically active substances of the phytocomplex, the presence of other rheumatic diseases, the body mass index above 40 kg/m^2, and the presence of severe comorbidities.

Among the patients included in the study, 73.1% (79 people) were females, and 26.9% (29 people) were males. The ratio of men and women was approximately 1:3. The age range of the surveyed people ranged from 40 to 78 years. *Me* (sample median) and *IQR* (interquartile range, 25th and 75th percentile) of the patients' age, disease duration and body mass index at the time of the survey were 54.4 (50.0 and 61.5) years, 5.3 (3.0 and 7.5) years, and 30.5 (25.6 and 34.7) kg/m2, respectively. The first X-ray stage of the knee joint osteoarthritis was observed in 35.2% of the patients, the second stage – in 64.8%. In the majority of the patients (81%), the pathological process was one-sided, and only in 19%, it was two-sided one. A rapidly progressive course of the disease was observed in 3.7 % of the patients. A number of the patients included in the study were diagnosed with comorbidities, including hypertension (37 people), diabetes (12 people), and metabolic syndrome (4 people), which were combined in some patients.

All patients with knee osteoarthritis included in the study, along with general clinical examination methods, used special research methods.

The clinical condition of the patients with osteoarthritis was assessed by examining the knee joints and quantifying indicators of clinical symptoms.

The range of motion in the affected joint (Mollier flexion) was evaluated using a Goniometer (ISOM 360°, 6″, Baseline, USA). The principle of D.M. Ritchie et al. were taken as the basis of the articular and tendon indices [20]. The level of pain was determined with moderate pressure of about 4 kg/cm^2 (before whitening the nail of the main phalanx) on the joint (along with with the joint space) and the place of attachment of tendons and ligaments. The 4-point scale was used as follows: 0 – no pain, 1 – patient states pain, 2 – patient states pain and frowns, 3 – patient withdraws limb. The duration of morning stiffness was estimated in minutes.

For more detailed clinical characteristics of the patients, the parameters recommended by the Osteoarthritis Research Society International (OARSI) were used, namely: the pain level indicator as per VAS (at rest and in motion); WOMAC index (Western Ontario and McMaster Universities Osteoarthritis Index): pain index (PW), stiffness index (SW), daily activity rate (FW); and Lequesne Index.

The quality of life of the patients with knee osteoarthritis was assessed by HAQ.

The frequency and nature of adverse events, the dynamics of the pulse, blood pressure, electrocardiography (ECG) were used as safety parameters.

All patients were randomly (by random numbers generated using a computer program) divided into 3 groups, comparable in clinical and functional characteristics. Patients of the first group (36 people) were on rehabilitation, including the phytocomplex SMC-electrophoresis. The amplitude

therapy was prescribed to the patients of the second group (36 people) according to the similar procedure with the first group, without the phytocomplex. The observed patients of the first two groups continued to receive drug therapy, which did not change during the physiotherapy course. Patients of the third group (36 people) received only drug treatment: SYSADOA basic – 36 people (100%), NSAIDs – 8 people (22.2%), general tonic agents, and vitamin preparations. The drug therapy of the patients of the third group was comparable with the drug treatment of the patients in the first two groups.

The electrotherapy was performed on the knee joint region using a transverse technique in a straightened SMC mode with the first and fourth type of work, 5 min each. The modulation frequency was equal to 100 Hz, the modulation depth – to 75%, the half-periods' duration was 2 and 3 sec, the current strength – 5 mA, and the duration of exposure – 10 min, with 10 daily procedures per course. The procedures were performed on an Amplipulse-6 apparatus (Elektroapparat, Russia).

A working solution of the electrophoresis phytocomplex was prepared ex tempore by dissolving the dry extract (10 parts) in dimethyl sulfoxide (15 parts) and then adding warm (40°C) distilled water (up to 100 parts). 20 ml of the working solution was applied to pads, which were placed on the medial and lateral surfaces of the affected knee joint. The content of flavonoids in the working solution was 0.7% (in terms of quercetin – the predominant flavonoid of the phytocomplex) or 4% (in terms of the absolute dry residue of flavonoids). The phytocomplex concentration in the working solution was chosen experimentally as a result of the study of transdermal delivery of the phytocomplex biologically active substances under the SMC action in model experiments [21]. It had been previously found that SMC electrophoresis did not destroy the main active substances of the phytocomplex, introduced from two poles.

The results were statistically processed using the SPSS.Statistics.v17.Multilingual-EQUiNOX (SPSS Inc) software.

Results

The main clinical symptoms in the examined patients are presented in Figure 1. Central to the clinical picture was the pain in the joint (100 %), both in the patients with the first and the second disease stage. The overwhelming majority of the patients with the second stage osteoarthritis also had morning stiffness (98 %), limited movement in the joint (79 %), and a crunch in the joint (66 %). It should be noted that all clinical symptoms occurred approximately two times more often in the patients with the second stage of the disease than with the first one and were more pronounced.

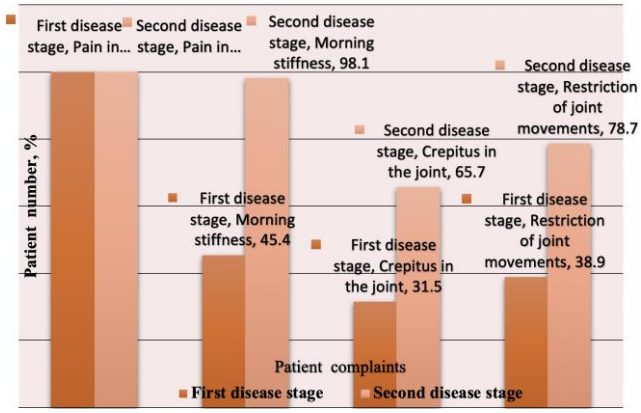

Figure 1: The main complaints of the patients with knee osteoarthritis included in the study

The clinical condition of the patients with the knee joint osteoarthritis was assessed by the JROM, the duration of morning stiffness (MS), the articular (AI) and the tendon (TI) indexes (Table 1). The use of the method of the phytocomplex SMC-electrophoresis (first group) had a beneficial and significant effect on these parameters in the patients with the knee osteoarthritis. Thus, the MS index decreased 2.5 times, palpation pain decreased by 60% (AI) and 55% (TI), and the JROM increased by almost 14%.

A comparative analysis of the investigated rehabilitation methods showed that the phytocomplex SMC-electrophoresis method (first group) was more effective than drug therapy (third group) immediately after the physiotherapy and 3 – 6 months after that according to the values of MS, CI and TI ($P < 0.05$). When analysing two physiotherapeutic rehabilitation methods, it has been established that immediately after the physiotherapy, the phytocomplex SMC-electrophoresis (first group) was significantly better than the amplipulse therapy (second group) in terms of AI and TI ($P < 0.05$). The effect persisted for up to 3 months after the course of treatment in terms of TI ($P = 0.044$). In terms of MS, the phytocomplex SMC-electrophoresis method (first group) was better than the effects of SMC (second group) 3 months after the physiotherapy ($P = 0.020$). The effect persisted for up to 6 months ($P = 0.038$).

Pain in the joint is one of the most important clinical signs of osteoarthritis. Therefore, the pain level, as per VAS is the most significant indicator for assessing the effectiveness of patient rehabilitation. It was found that the studied physiotherapy rehabilitation methods had reliably reduced the VAS indicators by the end of the treatment by 59% (at rest) and 51% (in motion) using the phytocomplex SMC-electrophoresis (first group), 54% (at rest) and 43% (in motion) for the amplitude therapy (second group) (Figure 2).

Table 1: The results of the comparative analysis of the effectiveness of various methods of rehabilitation of the patients with the knee osteoarthritis in terms of JROM, MS, AI and TI

Examination period	Group	Indicator, $M \pm \sigma$* JROM, degree	MS, minute	AI, points	TI, points
Before treatment	1 (*n* = 36)	116.5 ± 19.2	18.2 ± 3.6	1.84 ± 0.36	1.34 ± 0.26
	2 (*n* = 36)	121.5 ± 9.6	15.6 ± 4.0	1.73 ± 0.34	1.37 ± 0.30
	3 (*n* = 36)	124.6 ± 16.8	17.1 ± 3.4	1.79 ± 0.40	1.26 ± 0.22
	R.	P^{1-2} = *Ur* P^1 = *Ur* P^{1-3} = *Ur* P^2 = *Ur* P^{2-3} = *Ur* P^3 = *Ur*	P^{1-2} = *Ur* P^1 = *Ur* P^{1-3} = *Ur* P^2 = *Ur* P^{2-3} = *Ur* P^3 = *Ur*	P^{1-2} = *Ur* P^1 = *Ur* P^{1-3} = *Ur* P^2 = *Ur* P^{2-3} = *Ur* P^3 = *Ur*	P^{1-2} = *Ur* P^1 = *Ur* P^{1-3} = *Ur* P^2 = *Ur* P^{2-3} = *Ur* P^3 = *Ur*
After treatment	1 (*n* = 36)	132.3 ± 25.6	7.3 ± 1.8	0.74 ± 0.30	0,60 ± 0,18
	2 (*n* = 36)	126.6 ± 19.8	7.0 ± 2.4	0.93 ± 0.42	0,79 ± 0,15
	3 (*n* = 36)	125.2 ± 23.6	16.1 ± 3.4	1.68 ± 0.72	1.30 ± 0.32
	P	P^{1-2} = *Ur* P^1 = 0.046 P^{1-3} = *0.049* P^2 = *Ur* P^{2-3} = *Ur* P^3 = *Ur*	P^{1-2} = *Ur* P^1 = 0.001 P^{1-3} = 0.001 P^2 = 0.001 P^{2-3} = 0.001 P^3 = *Ur*	P^{1-2} = 0.040 P^1 = 0.001 P^{1-3} = 0.001 P^2 = 0.002 P^{2-3} = 0.003 P^3 = *Ur*	P^{1-2} = 0,041 P^1 = 0,001 P^{1-3} = 0,001 P^2 = 0,001 P^{2-3} = 0,001 P^3 = *Ur*
3 months after treatment	1 (*n* = 36)	124.2 ± 24.6	8.7 ± 2.4	0.96 ± 0.42	0.91 ± 0.30
	2 (*n* = 36)	127.9 ± 26.4	11.4 ± 3.0	1.15 ± 0.48	0.97 ± 0.18
	3 (*n* = 36)	126.7 ± 33.2	16.8 ± 3.2	1.73 ± 0.54	1.22 ± 0.26
	P	P^{1-2} = *Ur* P^1 = *Ur* P^{1-3} = *Ur* P^2 = *Ur* P^{2-3} = *Ur* P^3 = *Ur*	P^{1-2} = 0.020 P^1 = 0.001 P^{1-3} = 0.001 P^2 = 0.020 P^{2-3} = 0.020 P^3 = *Ur*	P^{1-2} = 0.044 P^1 = 0.002 P^{1-3} = 0.002 P^2 = 0.005 P^{2-3} = 0.010 P^3 = *Ur*	P^{1-2} = *Ur* P^1 = 0.011 P^{1-3} = 0.010 P^2 = 0.020 P^{2-3} = 0.016 P^3 = *Ur*
6 months after treatment	1 (*n* = 36)	120.8 ± 22.4	12.2 ± 4.2	1.44 ± 0.48	1.15 ± 0.24
	2 (*n* = 36)	125.0 ± 31.2	14.2 ± 3.0	1.51 ± 0.48	1.34 ± 0.14
	3 (*n* = 36)	127.2 ± 28.0	17.6 ± 4.0	1.75 ± 0.60	1.25 ± 0.22
	P	P^{1-2} = *Ur* P^1 = *Ur* P^{1-3} = *Ur* P^2 = *Ur* P^{2-3} = *Ur* P^3 = *Ur*	P^{1-2} = 0.038 P^1 = 0.005 P^{1-3} = 0.001 P^2 = *Ur* P^{2-3} = *Ur* P^3 = *Ur*	P^{1-2} = *Ur* P^1 = 0.030 P^{1-3} = 0.030 P^2 = *Ur* P^{2-3} = *Ur* P^3 = *Ur*	P^{1-2} = *Ur* P^1 = 0.042 P^{1-3} = 0.042 P^2 = *Ur* P^{2-3} = *Ur* P^3 = *Ur*
12 months after treatment	1 (*n* = 36)	118.2 ± 27.2	14.9 ± 3.2	1.62 ± 0.48	1.29 ± 0.26
	2 (*n* = 36)	124.1 ± 18.6	14.8 ± 3.8	1.66 ± 0.42	1.30 ± 0.28
	3 (*n* = 36)	126.1 ± 30.6	18.3 ± 4.4	1.78 0 ± .48	1.20 ± 0.34
	P	P^{1-2} = *Ur* P^1 = *Ur* P^{1-3} = *Ur* P^2 = *Ur* P^{2-3} = *Ur* P^3 = *Ur*	P^{1-2} = *Ur* P^1 = *Ur* P^{1-3} = *Ur* P^2 = *Ur* P^{2-3} = *Ur* P^3 = *Ur*	P^{1-2} = *Ur* P^1 = *Ur* P^{1-3} = *Ur* P^2 = *Ur* P^{2-3} = *Ur* P^3 = Ur	P^{1-2} = *Ur* P^1 = *Ur* P^{1-3} = *Ur* P^2 = *Ur* P^{2-3} = *Ur* P^3 = *Ur*

* The experimental, empirical distribution of JROM, MS, AI, TI variables did not differ much from the normal distribution (the Kolmogorov-Smirnov criterion and the schedule of the normal distribution in SPSS); ** The significance of differences between the groups and in the group before and after treatment as per the Student's t-test (numbers indicate the numbers of the groups); Ur is the unreliable.

The phytocomplex electrophoresis method (first group) immediately after the physiotherapy course was not significantly better than the amplipulse therapy method (second group) (*P* > 0.05). When assessing the analgesic action stability after 3 – 12 months, it was assumed that the effect had been stable at the pain level as per VAS of no more than 40 mm. Three months after the rehabilitation, the VAS indicators in the first group of the patients were at a level of less than 40 mm. In the subsequent periods, the analgesic effect persisted and was significantly better than the pain level indicators in the patients of the second and third groups (*P* < 0.05). When exposed to SMC (second group), the analgesic effect persisted for up to 6 months after the physiotherapy; at subsequent follow-up periods, the VAS indicators did not significantly differ from the pain level data in the patients of the third group who had received only drug treatment (*P* > 0.05). The proportion of the patients with VAS ≤ 40 mm during the rehabilitation by the phytocomplex SMC-electrophoresis (first group) after 3 months decreased slightly and amounted to 54%. When using the amplipulse therapy (second group), the proportion of the patients with VAS ≤ 40 mm decreased to 34% 3 months after the physiotherapy, and in the subsequent periods, these figures did not differ from those in the third group.

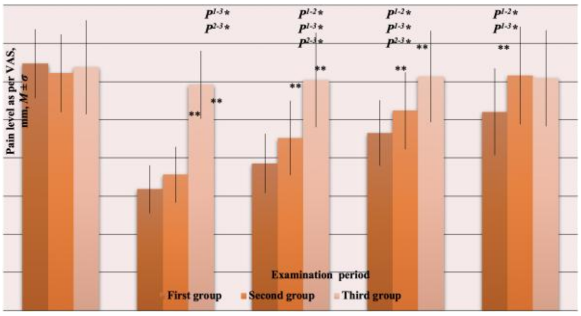

Figure 2: The results of the comparative analysis of the effectiveness of various methods of rehabilitation of the patients with the knee osteoarthritis in terms of pain level as per VAS (in movement). The significance of differences between the groups (*) and in the group (**) before and after treatment as per the Student's t-test (group numbers are indicated in numbers)

The WOMAC index can also be used for a comparative evaluation of the effectiveness of various rehabilitation methods for patients with osteoarthritis. The significant decrease in the PW, SW and FW indicators of the WOMAC index was observed by the end of the physiotherapy course using the phytocomplex SMC-electrophoresis (first group) by 40%, and by the end of the amplipulse therapy (second group) – by 30% (Table 2).

Table 2: The results of the comparative analysis of the effectiveness of various methods of rehabilitation of the patients with the knee osteoarthritis as per the WOMAC index

Examination period	Group	WOMAC index, score, $M \pm \sigma$* PW	SW	FW
Before treatment	First (*n* = 36)	34.3 ± 5.6	13.7 ± 2.4	116.1 ± 20.4
	Second (*n* = 36)	33.3 ± 6.8	13.2 ± 2.7	112.5 ± 22.6
	Third (*n* = 36)	31.9 ± 6.2	12.8 ± 2.0	108.5 ± 20.3
	*P***	P^{1-2} = *Ur* P^1 = *Ur* P^{1-3} = *Ur* P^2 = *Ur* P^{2-3} = *Ur* P^3 = *Ur*	P^{1-2} = *Ur* P^1 = *Ur* P^{1-3} = *Ur* P^2 = *Ur* P^{2-3} = *Ur* P^3 = *Ur*	P^{1-2} = *Ur* P^1 = *Ur* P^{1-3} = *Ur* P^2 = *Ur* P^{2-3} = *Ur* P^3 = *Ur*
After treatment	First (*n* = 36)	20.6 ± 4.2	8.2 ± 1.6	69.9 ± 14.0
	Second (*n* = 36)	24.6 ± 4.0	9.8 ± 2.4	83.1 ± 18.6
	Third (*n* = 36)	30.6 ± 6.4	12.3 ± 3.0	103.9 ± 20.4
	P	P^{1-2} = 0.043 P^1 = 0.001 P^{1-3} = 0.001 P^2 = 0.013 P^{2-3} = 0.027 P^3 = *Ur*	P^{1-2} = 0.040 P^1 = 0.001 P^{1-3} = 0.001 P^2 = 0.009 P^{2-3} = 0.025 P^3 = *Ur*	P^{1-2} = 0.038 P^1 = 0.002 P^{1-3} = 0.002 P^2 = 0.011 P^{2-3} = 0.016 P^3 = *Ur*
3 months after treatment	First (*n* = 36)	23.2 ± 4.6	9.3 ± 1.8	78.6 ± 16.0
	Second (*n* = 36)	27.2 ± 5.2	10.8 ± 2.2	91.8 ± 21.4
	Third (*n* = 36)	31.4 ± 6.8	12.5 ± 2.8	106.6 ± 22.0
	P	P^{1-2} = 0.041 P^1 = 0.005 P^{1-3} = 0.012 P^2 = 0.033 P^{2-3} = 0.040 P^3 = *Ur*	P^{1-2} = 0.037 P^1 = 0.003 P^{1-3} = 0.030 P^2 = 0.028 P^{2-3} = 0.040 P^3 = *Ur*	P^{1-2} = 0.043 P^1 = 0.005 P^{1-3} = 0.035 P^2 = 0.038 P^{2-3} = 0.040 P^3 = *Ur*
6 months after treatment	First (*n* = 36)	24.6 ± 5.0	9.7 ± 2.8	83.6 ± 16.0
	Second (*n* = 36)	28.1 ± 6.2	11.2 ± 2.2	95.7 ± 20.4
	Third (*n* = 36)	29.8 ± 7.1	12.0 ± 2.4	103.9 ± 21.6
	P	P^{1-2} = 0.040 P^1 = 0.010 P^{1-3} = 0.034 P^2 = 0.037 P^{2-3} = *Ur* P^3 = *Ur*	P^{1-2} = 0.042 P^1 = 0.015 P^{1-3} = 0.038 P^2 = 0.040 P^{2-3} = *Ur* P^3 = *Ur*	P^{1-2} = 0.040 P^1 = 0.010 P^{1-3} = 0.033 P^2 = 0.040 P^{2-3} = *Ur* P^3 = *Ur*
12 months after treatment	First (*n* = 36)	27.2 ± 5.8	11.1 ± 2.8	94.3 ± 19.0
	Second (*n* = 36)	31.0 ± 7.0	12.6 ± 2.5	106.9 ± 23.2
	Third (*n* = 36)	31.3 ± 6.2	12.5 ± 2.4	107.0 ± 22.8
	P	P^{1-2} = 0.044 P^1 = 0.030 P^{1-3} = 0.030 P^2 = *Ur* P^{2-3} = *Ur* P^3 = *Ur*	P^{1-2} = 0.042 P^1 = 0.036 P^{1-3} = 0.040 P^2 = *Ur* P^{2-3} = *Ur* P^3 = *Ur*	P^{1-2} = 0.040 P^1 = 0.037 P^{1-3} = 0.040 P^2 = *Ur* P^{2-3} = *Ur* P^3 = *Ur*

* The experimental, empirical distribution of WOMAC variables did not differ much from the normal distribution (the Kolmogorov-Smirnov criterion and the schedule of the normal distribution in SPSS); ** The significance of differences between the groups and in the group before and after treatment as per the Student's t-test (numbers indicate the numbers of the groups).

A comparative analysis of various methods of treating knee osteoarthritis has shown that when using the phytocomplex SMC-electrophoresis (first group) immediately after the rehabilitation, the

WOMAC indicators were significantly better than with the amplipulse therapy (second group) ($P < 0.05$) and with drug treatment (third group) ($P < 0.05$). This picture persisted for 3 – 12 months after the rehabilitation. The WOMAC indices, when exposed to SMC (second group) 6 months after the course of treatment did not significantly differ from the drug treatment data (third group) ($P > 0.05$).

The study of the dynamics of the Lequesne index in the 3 research groups has shown that the largest decrease in this indicator was observed in the first group after using the phytocomplex SMC-electrophoresis (45%) (Figure 3). This method of rehabilitation of the patients with knee osteoarthritis was significantly better by the Lequesne index compared with the amplitude therapy (second group) and drug treatment (third group) immediately after the physiotherapy and 6 months after ($P < 0.05$). The arithmetic means values of the Lequesne index 12 months after the rehabilitation were in the range of 10 – 11 points and did not differ significantly in all research groups ($P > 0.05$).

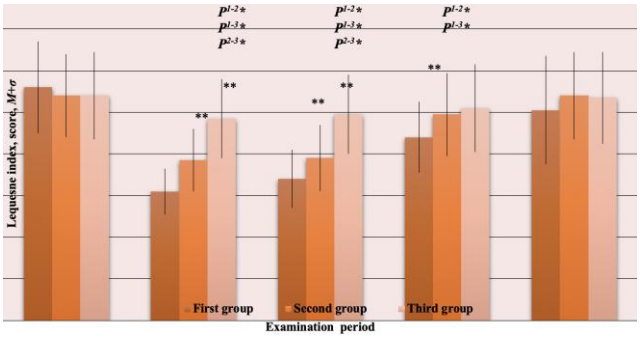

Figure 3: The results of the comparative analysis of the effectiveness of various methods of rehabilitation of the patients with the knee osteoarthritis as per the Lequesne index. The significance of differences between the groups (*) and in the group (**) before and after treatment as per the Student's t-test (group numbers are indicated in numbers)

The quality of life of the patients with knee osteoarthritis was assessed using the HAQ index. A significant decrease in HAQ indices was observed immediately after the rehabilitation using the phytocomplex SMC-electrophoresis (first group − by 34%; $P = 0.003$) and the amplitude therapy (second group − by 28%, $P = 0.015$) (Figure 4). These indicators were significantly better than those of HAQ obtained using only the drug therapy (third group) ($P < 0.05$). After 3 – 12 months of observation, the HAQ values in the first and second groups did not differ significantly ($P > 0.05$). HAQ parameters ≤ 20 points were taken as satisfactory (the functional state of the joint was not impaired). In the groups under study, the predominant proportion of HAQ parameters before the treatment was lower than 20. Therefore, the HAQ values after the rehabilitation and 3 – 12 months after were satisfactory.

Currently, there are no systematic works on the effect of flavonoids on the clinical symptoms and quality of life of patients with osteoarthritis during electrophoresis. The only reliable results concern the relief of clinical manifestations of osteoarthritis of the knee joint according to the WOMAC index (in 64% of cases) when using dexamethasone sodium phosphate electrophoresis [17], pain relief (according to VAS) and the functional disability index decrease immediately after treatment and for the next six weeks when using sodium salicylate electrophoresis [16], increasing the effectiveness of degenerative osteoarthropathy treatment (by 19%) when using electrophoresis of a Chinese drug compared to electric current monotherapy [18], which is consistent with the results of our research.

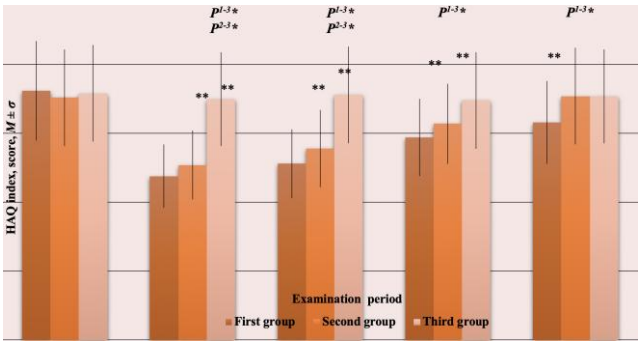

Figure 4: The results of the comparative analysis of the effectiveness of various methods of rehabilitation of the patients with the knee osteoarthritis as per the HAQ index. The significance of differences between the groups (*) and in the group (**) before and after treatment as per the Student's t-test (group numbers are indicated in numbers)

Discussion

The study has shown that the use of the phytocomplex SMC-electrophoresis in conjunction with the drug therapy has a more pronounced effect on the clinical symptoms of the patients with the knee joint osteoarthritis compared with the drug therapy or shared use of drugs with the amplipulse therapy. This was especially pronounced immediately after the rehabilitation. The analgesic effect was consistently maintained using the phytocomplex SMC-electrophoresis for 6 – 12 months, with the amplitude therapy for 3 – 6 months in terms of the pain syndrome (PW) of the WOMAC index, as well as VAS, Lequesne index, articular and tendon indices. Stable results have been obtained in improving the functions of the knee joint during the rehabilitation using the phytocomplex SMC-electrophoresis in terms of stiffness (SW) and daily activity (FW) of the WOMAC index. It should be noted that when using the phytocomplex SMC-electrophoresis, no side effects were recorded.

The obtained results give grounds for further research on the assessment of the effectiveness of using the phytocomplex SMC-electrophoresis in

microcirculatory disorders in the affected joint, to correct the connective tissue metabolism and electrolyte metabolism in the patients with the knee osteoarthritis.

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Clinical Science

Common Practice of Hypospadias Management by Pediatric Urologists in Indonesia: A Multi-center Descriptive Study from Referral Hospitals

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Abstract

BACKGROUND: Hypospadias is the second most common congenital anomalies among human congenital disabilities. There are over 300 surgery techniques being introduced to treat hypospadias. The successful of hypospadias repair is assessed by several outcomes as well as complications following surgery.

AIM: This study aims to show the multicenter hypospadias data in Indonesia descriptively.

METHODS: All the data were compiled based on questionnaires, which were distributed to Indonesian pediatric urologists. The questionnaire includes several questions containing demographic aspect, preferred techniques being used, and complications being found regarding hypospadias repair.

RESULTS: Eighteen Indonesian pediatric urologists from 12 centres involved in this study. The data were collected from June – September 2018 based on the surgeon's experience throughout 2017. From 591 cases based on the returned questionnaire, penile-type hypospadias was the most common type of hypospadias being treated (35.7%) followed by penoscrotal (28.9%) and scrotal-type (12.9%). Moderate severity of chordee was mostly seen among all cases (40.6%). Tubularised incised plate (TIP), + Thiersch Duplay, was the most common technique being used to treat hypospadias (44.3%), followed by onlay island preputial flap (14.9%) and two-stage technique (14%). The incidence of urethrocutaneous fistulae in this study was 13.9%.

CONCLUSION: This study showed how Indonesian pediatric urologists dealt with hypospadias cases. TIP + Thiersch Duplay procedure being the preferred technique used by most participants and the rate of urethrocutaneous fistulae as one of the complications was comparable with previous studies.

Introduction

Several studies reported that hypospadias is the second most common congenital anomalies among human congenital disabilities [1], [2]. The prevalence of hypospadias is around 1 of 250-300 live male births [3]. Hypospadias repair is being performed with some aims such as enable micturition in standing position, good cosmetic appearance, as well as effective insemination [4], [5]. Currently, there are over 300 surgery techniques being introduced as

management of hypospadias, and some newer methods continue to evolve and introduce. However, there are no standard methods to treat all hypospadias. Over the last decades, the surgery is subtle, and it now has a decreased stage of operations as well as postoperative complications [1], [4].

Distal hypospadias has been treated as a one-stage procedure currently as reported by many studies internationally. Transverse incised plate (TIP), Mathieu and Onlay island flap are being some of the techniques most used to treat distal hypospadias. Nevertheless, for proximal hypospadias, the surgical treatment is still debatable into two groups based on the stage of procedures. One group favour one stage procedure such as inlay preputial flap while other groups choose to perform two-stage procedure [6], [7].

The successful of hypospadias repair is assessed by several outcomes, out of which, the complications being the most concerns of all surgeons. Some of the complications after hypospadias repair include urethrocutaneous fistulae, urethral strictures, infections, meatal stenosis and others fewer common ones [8]. Some factors could affect the outcome of hypospadias repairs such as the site of the meatus, the severity of chordee, adequacy of preputial skin and the existence of penoscrotal transposition.

Other factors, including the age of the patients and surgeon's experience, could be counted as well [9], [10]. Some technical factors such as the type of surgery, second layer usage, duration of antibiotic and stenting duration are also notable [2], [11].

Regarding our knowledge, this is the first descriptive multicenter study regarding the hypospadias repair data being performed by Indonesian pediatric urologists and this study aimed to show the multicenter hypospadias data in Indonesia descriptively in terms of demographics, techniques being used, and outcomes in 12 institutions from across Indonesia.

Material and Methods

All the data were compiled from self-constructed questionnaires, which were distributed to Indonesian pediatric urologists who have been completed the pediatric urology trainee to ensure their competence as well as homogeneity handling techniques. The data were collected and analysed from June – September 2018 based on the surgeon's experience throughout the year of 2017.

The questionnaire includes several questions containing demographic aspect, preferred techniques being used, and complications being found regarding hypospadias repair.

As this study was being held, there were 18 pediatric urologists in Indonesia. Ethical approval for this study has been granted by the local Ethics Committee.

Results

Eighteen Indonesian pediatric urologists from 12 centres took part in this study. Of the returned questionnaires and through final analysis, we included data from 591 cases.

However, some questionnaires were returned with incomplete answers. In this case, we were trying to collect data as much and as relevant as we can. The distributions of hypospadias data based on hospital centres are shown in Table 1.

Table 1: The distributions of hypospadias data based on the hospital centres

Hospital Center (City)	n (%)
Sanglah (Denpasar)	83 (14)
Cipto Mangunkusumo (Jakarta)	59 (10)
Hasan Sadikin (Bandung)	44 (7.5)
Saiful Anwar (Malang)	30 (5.1)
Sardjito (Yogyakarta)	28 (4.7)
Wahidin Sudirohusodo (Makassar)	27 (4.6)
Harapan Kita (Jakarta)	48 (8.1)
Fatmawati (Jakarta)	119 (20.1)
M. Djamil (Padang)	41 (6.9)
Soetomo (Surabaya)	56 (9.5)
Adam Malik (Medan)	25 (4.2)
Persahabatan (Jakarta)	31 (5.3)
Total (%)	591 (100)

The distributions of the age group of hypospadias patients about the occurrence of urethrocutaneous fistulae after a repair, the techniques of hypospadias repair in relation to type of hypospadias, and the severity of chordee are shown in Table 2, 3, and 4, respectively.

Table 2: The distributions of age group of hypospadias patients in relation to the occurrence of urethrocutaneous fistulae after hypospadias repair

Age group (years)	Urethrocutaneous fistulae		n (%)
	Yes	No	
0-1	-	15	15 (2.5)
>1-2	3	36	39 (6.6)
>2-3	3	58	61 (10.3)
>3-4	14	101	115 (19.5)
>4	62	299	361 (61.1)
Total (%)	82 (13.9)	509 (86.1)	591 (100)

The length of neo-urethra during hypospadias repair, the placement of percutaneous cytostomy during hypospadias repair, and the size of urethral splint are shown in Table 5, 6, and 7 respectively.

The complication founds after hypospadias repair other than urethral fistulae was 14 cases (4%) from 350 cases.

Table 3: Techniques of hypospadias repair being used about the type of hypospadias

Urethroplasty Technique	Glandular	Subcoronal	Penile	Penoscrotal	Scrotal	Perineal	Chordee only	Failed urethroplasty	Total (%)
Chordectomy and orthoplasty	-	14	-	-	-	-	3	-	17 (2.8)
MAGPI	13	12	-	-	-	-	-	-	25 (4.2)
Mathieu	1	9	6	-	-	-	-	-	16 (2.7)
TIP + Thiersch Duplay	10	29	130	56	25	3	-	8	261 (44.3)
Onlay island preputial flap	-	-	39	35	13	1	-	-	88 (14.9)
Duckett	-	3	10	25	7	1	-	-	46 (7.8)
Dorsal inlay preputial flap or graft	-	-	15	16	2	2	-	-	35 (5.9)
Koyanagi	-	-	5	4	1	-	-	-	10 (1.7)
Two-stage technique	-	1	6	35	28	10	-	3	83 (14)
Fistulae repair	-	-	-	-	-	-	-	10	10 (1.7)
Total (%)	24 (4.1)	68 (11.5)	211 (35.7)	171 (28.9)	76 (12.9)	17 (2.8)	3 (0.5)	21 (3.6)	591 (100)

Discussion

Hypospadias is being one of the common congenital anomalies of the penile [12]. Currently, there have been more than 300 surgery techniques in hypospadias repair [1].

Table 4: The severity of chordee

Severity of chordee	n (%)
Mild	118 (28.7)
Moderate	167 (40.6)
Severe	126 (30.7)
Total (%)	411 (100)

All of those techniques have the same goal, which is to achieve cosmetically appropriate penile with acceptable shaped of penile glans that has a meatus at the tip of the penile.

Table 5: The length of neo-urethra during hypospadias repair

The length of neo-urethra (cm)	n (%)
< 1	28 (8.4)
1-2	79 (23.7)
> 2-3	107 (32.1)
> 3-4	83 (24.9)
> 4	36 (10.9)
Total (%)	333 (100)

Nonetheless, high rates of complications still be an issue in hypospadias repair compared to other reconstructive surgeries. Moreover, until currently, there is no same consensus about the preference procedures to treat any hypospadias.

Table 6: The placement of percutaneous cystostomy during hypospadias repair

Placement of percutaneous cystostomy	n (%)
No	213 (61.1)
Yes	136 (38.9)
Total	349 (100)

The urethrocutaneous fistulae rate as a complication in our study was quite comparable with previous studies. The rate of urethrocutaneous fistulae in our study was 13.9%. Results from other literature were quite varying from 4-60%.

Table 7: The size of the urethral splint during hypospadias repair

The size of urethral splint (Fr)	n (%)
6	127 (32.6)
8	181 (46.4)
10	45 (11.5)
12	27 (6.9)
14	9 (2.3)
16	1 (0.3)
Total	390 (100)

However, the higher rates of complications were commonly in the studies with more severe hypospadias [6], [7], [8], [13], [14], [15], [16], [17], [18], [19], [20], [21], [22]. We have also noticed in this study that higher complication rate was associated with severe chordee as well as proximal hypospadias. The prevalence of proximal hypospadias (penoscrotal, scrotal, and perineal) was very high in this study (44.7%) as compared to other studies [9], [22], [23], [24]. This issue might be due to some of the distal hypospadias patients did not seek medical advice. Also, all Indonesian pediatric urologists work in each hospital centre where lots of severe cases will be referred to them from all around the country.

Hypospadias repair is recommended to perform around age 6 – 18 months. Some studies even showing minimal complication of hypospadias repair in patients with age 4 – 6 months [4], [10], [20]. In our study, most of the cases being treated were older than 4-year-old (61.1%). One of the main reasons for this problem was most of the cases were came to a physician at an older age, and most of the patients were coming from a distant area.

Tabularized incised plate (TIP) procedure has become very popular since Snodgrass introduced his initial technique of TIP for hypospadias repair in 1994. This technique is very popular for the treatment of distal hypospadias [25]. The treatment of proximal hypospadias is more challenging. A surgeon must be ready to use some different techniques to deal with proximal hypospadias. The majority of Indonesian pediatric urologists prefer TIP technique (44.3%) as a management to treat hypospadias which is comparable to another study [6], [22], [23], [24], [25], [26]. Not only for distal hypospadias, but TIP technique was also used for proximal hypospadias which had very mild chordee and only minimal dissection required for correction. Nonetheless, it should be considered that the TIP technique has a greater risk to produce an unsatisfying cosmetic outcome as management of proximal hypospadias [27].

The previous study has shown the correlation of cystostomy placement to a low incident of urethrocutaneous fistulae following hypospadias surgery [28]. Nevertheless, most of the Indonesian pediatric urologists prefer not to use cystostomy in this study. It is understandable that the placement of percutaneous cystostomy in hypospadias surgery

cannot be applied to all cases because it needs a thoughtful decision from one case to another. A large number of surgeons use urethral stenting following hypospadias repair. However, until currently, there is no agreement in terms of its need, size, or material to be used [29]. In this study, all participants use urethral stenting for their patients.

In conclusion, this multi-centre descriptive study showed how Indonesian pediatric urologists dealt with hypospadias cases which were comparable to international level practices. TIP procedure is the preferred technique used by most participants, and the rate of urethrocutaneous fistulae as one of the complications was comparable with previous literature as well.

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Prevalence of Montelukast Use as an Add-On Therapy among Iraqi Asthmatics on Treatment Attending Al-Kindy Teaching Hospital and Al-Zahraa Center of Asthma and Allergy

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Abstract

BACKGROUND: Montelukast (Singulair) is a cysteinyl leukotriene receptor antagonist, used for the maintenance treatment of asthma and to relieve symptoms of seasonal allergic rhinitis and asthma, also used for exercise-induced bronchospasm.

AIM: This study was performed to determine the prevalence of Montelukast use as an add-on therapy among Iraqi asthmatic patients on treatment. Comparing the effectiveness of regimens with and without montelukast.

METHODS: This descriptive cross-sectional study was carried out on 73 Iraqi asthmatic patients on treatment of both sexes with age range (18-60) years old, attending Al-Kindy Teaching Hospital and Al-Zahraa Centre of Asthma and Allergy, Baghdad, for the period between February and March 2017. A questionnaire was specifically prepared to meet the objectives and was used to collect the data of the study.

RESULTS: There was a significant statistical reduction of frequency in asthmatic attacks after Montelukast treatment (p-value < 0.05). Out of 73 patients, 39 were males, and 34 were females, 46 were jobless, 37 were married, 63 were urban residents, 63 were educated. Prevalence of exacerbation factors was as following: infection was found in 60.3% of the patients, exercise in 57.5%, dust in 72.6%, smoking in 60.6%, food in 24.7%, others (stress, perfumes) in 20.5%. The prevalence of Montelukast use in this study was 46% (34 patients). Out of 34 patients using Montelukast, 28 were using inhaled salbutamol, 5 were using oral salbutamol, 15 were using inhaled corticosteroids, 9 were using systematic corticosteroids, 2 were using xanthines, and 6 were using ketotifen.

CONCLUSION: Montelukast was used as add-on therapy with the inhaled corticosteroids to reduce the required dose of inhaled corticosteroids also the use of Montelukast lead to reduced number of exacerbations which will be reflected on the use of inhaled salbutamol and systematic corticosteroids. Also, Montelukast was superior to xanthines and ketotifen as an add-on therapy.

Introduction

Asthma is defined as a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. In susceptible individuals, this inflammation causes recurrent episodes of wheezing, dyspnea, chest tightness, and coughing, especially at night or early morning [1]. An estimated 300 million people worldwide have asthma, with 250,000 annual deaths attributed to the disease [2]. This prevalence of asthma is expected to increase from 300 to 400 million by 2025 [3]. In Iraq, about 200,000 patients per year with asthma are either hospitalised or treated in an emergency room. Prevalence of asthma in the adjacent countries to Iraq includes 5.6 % in Saudi Arabia and 8.5 % in Kuwait [4], [5].

The major etiologic factors of asthma are a genetic predisposition to type 1 hypersensitivity (atopy) and bronchial hyperresponsiveness to a variety of stimuli. In asthma, all cells of the airway are involved and become activated. Included are eosinophils, T cells, mast cells, macrophages, neutrophils, epithelial cells, fibroblasts, and bronchial smooth muscle cells [6]. Activation of these cells leads

to release of proinflammatory cytokines and mediators [7]. The risk factors for development of asthma include genetics, prenatal tobacco smoke (maternal smoking), prenatal diet and nutrition, prenatal antibiotic use (maternal use), mode of delivery, family size and the number and order of siblings, exposure to environmental tobacco smoke, socioeconomic status, viral infections and antibiotic use [8]. Also, there is asthma-related to workplace exposures [9].

Montelukast (Singulair) is a cysteinyl leukotriene receptor antagonist used for the maintenance treatment of asthma and to relieve symptoms of seasonal allergies, also used for exercise-induced bronchospasm. It acts by blocking the action of leukotriene D4 (and secondary ligands, leukotrienes C4 and E4) on the cysteinyl leukotriene receptor 1 (Cys-LTR1) in the lungs and bronchial tubes by binding to it [10]. It is a controller drug that shouldn't be used for immediate bronchodilation. It is usually taken once a day with or without food.

Corticosteroids effect is mediated by their ability to inhibit the production of inflammatory cytokines, potentiation of effects of B-agonists, but their most important action is inhibition of infiltration of asthmatic airways by lymphocytes, eosinophils, and mast cells [11]. The beta-2 adrenergic agonists are potent bronchodilators that are widely used in the management of bronchial asthma. These agents act by binding to the beta-2 adrenergic receptors on the smooth muscle of bronchial tissue, relieving bronchospasm and reducing airway resistance [12].

There are three important methylxanthines (theophylline, theobromine and caffeine). Methylxanthines act by inhibiting several members of phosphodiesterase family enzymes, especially PDE4. Inhibition of PDE4 results in higher concentration of cyclic adenosine monophosphate (CAMP). CAMP is responsible for relaxation of airway smooth muscle [13].

Ketotifen is a controller drug which inhibits the release and activity of mast cell and basophil mediators, including histamine, neutrophil, and eosinophil chemotactic factors, arachidonic acid metabolites (prostaglandins and leukotrienes) [14]. Oral ketotifen has been used in patients with asthma, allergic rhinitis, allergic conjunctivitis, atopic dermatitis.

This study was performed to determine the prevalence of Montelukast use as an add-on therapy among Iraqi asthmatic patients on treatment comparing the effectiveness of regimens with and without montelukast.

Material and Methods

This descriptive cross-sectional study was carried out on 73 Iraqi asthmatic patients on treatment (patients taking Montelukast were included in the study), of both sexes with age range (34.18 ± 14.84), attending Al-Kindy Teaching Hospital and Al-Zahraa Centre of Asthma and Allergy, Baghdad, for the time period between February and March 2017. Patients were excluded if they have the pregnancy, metabolic disease, and psychiatric condition. The diagnosis of facial palsy was a clinical diagnosis done by an internal medicine specialist. After obtaining the formal approval from the Scientific and Ethical Committee in Al-Kindy College of Medicine, University of Baghdad, a questionnaire was specifically prepared to meet the objectives and was used to collect the data of the study. After obtaining verbal consent from each patient, an interview using the questionnaire was conducted. The data were prepared as frequencies, relative frequencies, charts, mean ± Standard deviation, Chi-square test and paired sample t-test were used for statistical analysis using SPSS program version 17. P-value < 0.05 was used as level of significance.

Results

Seventy-three patients participated in the study. Data from all of them were included in the analysis.

Table 1 shows the number and percentage of asthmatic patients in each demographic characteristic like gender, occupation, marital status, address, and educational level.

Males and females were nearly equal in number; jobless patients were more than patients who have a job, urban residents were much more than rural ones, married and unmarried patients are much nearly equal, educated patients were much more than non-educated ones.

Table 1: No. of asthmatic patients in different demographic characteristics

		Frequency	Relative frequency (%)
Gender	Male	39	53.4
	Female	34	46.6
Occupation	Job	27	37
	Jobless	46	63
Address	Rural	10	13.7
	Urban	63	86.3
Marital status	Married	37	50.7
	Unmarried	36	49.3
Educational level	Educated	63	86.3
	Uneducated	10	13.7

Table 2 shows the number and percentage of asthmatic patients in each age group. Age groups were (18-25), (26-40), (41-54), and (55-60) (years). The group which contained the largest number of patients was (18-25) years. The group which contained the least number of patients was (41-54) years.

Table 2: Number of asthmatic patients in different age groups (years)

Age groups (years)	Frequency	Relative frequency (%)
18-25	35	47.9
26-40	15	20.5
41-54	10	13.7
55-60	13	17.8
Total	73	100.0

Table 3 shows the number and percentage of asthmatic patients in each disease duration category. Disease duration categories were, < 15 years, (16-30) years, (31-44) years, (45-58) years. Disease duration category which contained the largest number of asthmatics was (< 15 years). Disease duration category which contained the least number of asthmatics was (45-58) years.

Table 3: Disease duration (years) and no. of asthmatic patients in each category

Disease duration (years)	Frequency	Relative frequency (%)
< 15	33	45.2
16-30	26	35.6
31-44	11	15.1
45-58	3	4.1
Total	73	100.0

Figure 1 shows the percentage of asthmatic patients who have certain exacerbation factors like infection, exercise, dust, smoking, food and others (stress, perfumes). Most prevalent one was dust while the least prevalent factors were other factors (stress, perfumes).

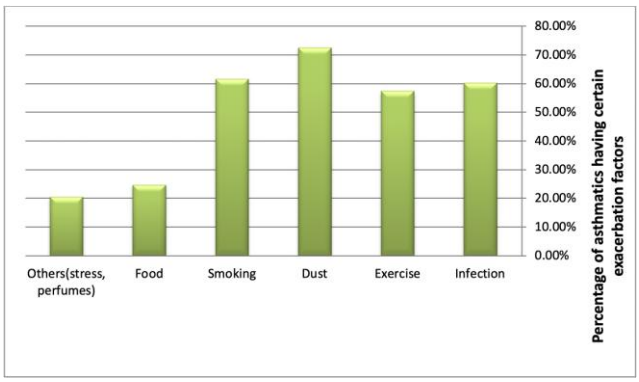

Figure 1: Prevalence of exacerbation factors

Figure 2 shows the percentage of asthmatics who use Montelukast versus those who don't use it.

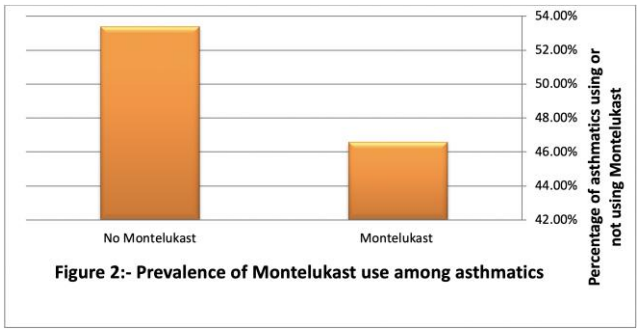

Figure 2: Prevalence of Montelukast use among asthmatics

Table 4 shows the number of asthmatics who use Montelukast in different age groups, versus those who don't use it. The age groups were (18-25), (26-40), (41-54), (55-60) years.

Table 4: Montelukast use in different age groups (years)

Age groups (years)	Montelukast use		Total
	Yes	No	
18-25	10	25	35
26-40	6	4	10
41-54	8	7	15
55-60	10	3	13
Total	34	39	73

Table 5 shows the number and percentage of asthmatic patients who use certain drugs with Montelukast, also shows the number and percentage of asthmatics who don't use these drugs with Montelukast. Most of the patients used inhaled salbutamol with Montelukast. The very small percentage used xanthines with Montelukast.

Table 5: No. of asthmatic patients using other drugs with Montelukast

		Frequency	Relative frequency (%)
Inhaled salbutamol	No	6	17.6
	Yes	28	82.4
Oral salbutamol	No	29	85.3
	Yes	5	14.7
Systematic corticosteroids	No	25	73.5
	Yes	9	26.5
Inhaled corticosteroids (ICS)	No	19	55.9
	Yes	15	44.1
Xanthines	No	32	94.1
	Yes	2	5.9
Ketotifen	No	28	82.4
	Yes	6	17.6

Table 6 shows the mean of asthmatic attacks/month before Montelukast use and the mean of asthmatic attacks/month after Montelukast use. The asthmatic attacks/month were significantly reduced after Montelukast use, as p-value was < 0.05.

Table 6: Comparison between the frequency of asthmatic attacks before and after Montelukast use in terms of means

	mean of attacks/month
Attacks/month before Montelukast use	6.47 ± 2.415
Attacks/month after Montelukast use	2.29 ± 1.169

Discussion

In Table 1, most of this study asthmatics were living in urban areas, urban areas are known to have more industrialization and pollution, and this agrees with a study done by Hirshon J. et al., (2006), in which Maryland showed the highest rates of emergency department visits for asthma in urban areas [15].

In Figure 1, the infection was an exacerbation factor in more than half of the asthmatics in this study, this agrees with a study done by K G Nicholson et al., (1993), in which it was concluded that respiratory infections especially viral infections are commonly

associated with asthmatic exacerbations [16]. Half of this study asthmatics were complaining from exercise as an exacerbation factor. Exercise-induced bronchospasm has been reported present in as few as 40% and as many as 90% of patients with asthma. In current study 34 patients were taking Montelukast, and Montelukast provided significant protection against exercise-induced bronchospasm as soon as 2 hours after a single oral dose, with persistent benefit up to 24 hours [17]. Majority of this study asthmatics were complaining from dust as a triggering factor for asthmatic exacerbations, according to a study conducted by Woong Park, Hee Lim (2005), it was concluded that dust events impact on the respiratory symptoms of subjects with bronchial asthma, and ambient air pollution, particularly elevated particulate matter < 10 um in diameter, might be one of the aggravating factors [18]. Smoking was an exacerbation factor in more than half of the study asthmatics, A finding that agrees with a review conducted by Stapleton, Howard-Thompson (2011)

In Table 5, the majority of asthmatics who use Montelukast were also using inhaled salbutamol, but a quarter of asthmatics who use Montelukast was not using inhaled salbutamol. According to a systematic review done by Zhang, Jia (2014), it was found that in comparison with placebo, adults with chronic asthma receiving Montelukast had significantly reduced number of exacerbations and this would be reflected on the use of inhaled salbutamol [20]. Only a small percentage of asthmatics were using oral salbutamol in addition to Montelukast, the difference in percentages between those using inhaled salbutamol and those using the oral one maybe due to the fact that oral salbutamol is less effective, having slower onset and more side effects than inhaled salbutamol [21]. More than half of the asthmatics using Montelukast, were not using inhaled corticosteroids. According to a study conducted by Andrew McIvor, Kaplan (2009), it was concluded that Montelukast is an effective alternative to inhaled corticosteroids in patients with mild asthma only. Others, who use Montelukast with inhaled corticosteroids, can use lower doses of inhaled corticosteroids in the presence of Montelukast. This agrees with previous studies, such as one performed by AL-Salami, Zaid (2011). Most of this study asthmatics using Montelukast, were not using xanthines, only very small percentage (5.9%) were using xanthines in addition to Montelukast. According to a study conducted by Shah (2004), it was concluded that the use of Montelukast along with inhaled bronchodilators and corticosteroids has a superior effect than addition of an oral sustained – release theophylline (a xanthine), on clinical and pulmonary function parameters in patients of chronic bronchial asthma [24]. Only a few asthmatics were using ketotifen with Montelukast (17.6%). According to a study performed by Al-Hamdani (2010), ketotifen and Montelukast were compared to each other and in conclusion, both ketotifen and Montelukast showed significant changes in asthma symptoms and pulmonary function tests after one month of treatment, but the changes were more significant with Montelukast group compared with ketotifen group and this indicated that Montelukast was more effective than ketotifen in treatment of asthmatic patients [25].

In Table 6, asthmatic attacks/month were significantly reduced after the use of Montelukast, this result agrees with a systematic review done by Miligkos, Bannuru (2015), in which it was found that the administration of Montelukast to adults and adolescents with asthma significantly reduced the risk of an exacerbation (asthmatic attack) and improved asthma control compared with placebo [26].

In conclusion, montelukast can be used as an add-on therapy with the inhaled corticosteroids to reduce the required dose of inhaled corticosteroids to reach the sufficient control, also the use of Montelukast lead to reduced number of exacerbations which will be reflected on the use of inhaled salbutamol and systematic corticosteroids. Also, Montelukast has a superior effect than xanthines and ketotifen as an add-on therapy.

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Clinical Science

Changes of QT Dispersion in Patients Suffering from Aluminium Phosphide Poisoning (Rice Pill)

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Keywords: Aluminum phosphide (ALP); ALP poisoning; Cardiotoxicity; QT dispersion

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Abstract

BACKGROUND: Aluminium phosphide (ALP) or rice pill is a substance used in developing countries due to its low cost as pesticides. The availability of this substance has been lead to an increased rate of the use of this toxic inorganic compound for suicide. Complications are considered to be dose-related toxicity and hospitalisation time, varying from hemodynamic disorder, hypoglycemia, hyperglycemia, shock, cardiotoxicity, pulmonary and renal failures. The consumption of this substance is one of the major causes of mortality due to heart arrhythmia. QT dispersion represents a regional difference in ventricular repolarisation and electrical instability of the heart.

AIM: The purpose of this study was to investigate the effect of ALP poisoning on QT dispersion.

METHODS: In this study, 70 patients with ALP poisoning were enrolled, and 10 patients were excluded due to the exclusion criteria. QT dispersion rate was calculated in 60 patients using the standard electrocardiography at the time of referral. The above data were compared with the control group, which included 40 subjects with normal coronary angiography, and without cardiovascular risk factors.

RESULTS: The findings presented herein indicated a significant correlation between QT dispersion and control group ($P < 0.0.5$). There was a significant relationship between the severity of acidosis and the patient's tablets – taking a number ($P < 0.05$). However, there was no relationship between QT dispersion with the severity of acidosis and mortality in patients.

CONCLUSION: Because there is no CAD risk factor in the population, it can be concluded that increase in QT dispersion in these individuals can be due to ALP poisoning; nevertheless, this is not considered to be a factor in increasing the morbidity of these patients.

Introduction

Aluminium phosphide (ALP) or rice tablet is a substance that is used in developing countries because of its cheapness as pesticides [1]. The availability of this substance has been led to an increased rate of the use of this toxic inorganic compound for suicide [2]. ALP poisoning is considered to be one of the major causes of suicidal deaths, and is sometimes accidental and rarely due to homicide [2], [3]. ALP poisoning has a high degree of mortality (30-100%), and the survival of patients is unlikely when swallowing more than 1.5 g of ALP [4].

Toxicity with this substance is observed in both children and adults. ALP releases phosphine in the gastrointestinal tract, which is cytotoxic and releases free radicals and thus exerts its high toxicity [1]. These effects are not unique to a particular organ and are multisystemic [5], [6]. The effects of phosphine toxicity occur in a minute to 60 minutes after taking it. Phosphine causes cell hypoxia and small vessel damage, which ultimately leads to cardiotoxicity following anoxic myocardial damage and shock [7]. The cardiovascular effects of rice tablet include increased jugular venous pressure, S3, hypotension, shock, arrhythmia, myocarditis, and pericarditis [7]. As a matter of fact, the greatest toxic effect of ALP is myocardial suppression and severe cardiovascular collapse [8]. In various studies, myocardial damage and its effects on the electrocardiogram have been proven to be responsible for the mortality caused by ALP poisoning

[8]. Various studies have reported that 38-91% of patients have electrocardiogram changes as cardiac conduction defects, including RBBB, LBBB, atrioventricular block, and rarely sinus block.

On the other hand, there is arrhythmia, including junctional rhythm, extra supraventricular systole and ventricular fibrillation (V-fib or VF). Eventually, reparative disorders such as ST-segment depression, ST-segment elevation, and T wave inversion occur following ALP poisoning [8]. Therefore, the patient should be monitored for cardiac arrhythmias immediately.

QT dispersion was defined to be the difference between the maximum and minimum QT interval of the 12-standard lead of electrocardiography (ECG), which indicates ventricular repolarisation and electrical instability of the heart. If this difference is at a high level, the risk of arrhythmias can be increased (9). QT dispersion is a phenomenon described by Campell et al. They showed that there are small but consistent differences between the QT intervals of different leads. Evidence suggests that the degree of QT variability in a variety of leads can provide valuable clinical information demonstrating the underlying abnormalities, including ventricular repolarisation [9]. Previous studies have shown that QT dispersion changes during myocardial ischemic episodes. Recent studies indicate that QT dispersion reaches its highest level during the early hours of ischemia and decreases over time as a result of receiving thrombolysis treatments. It also increases in patients who develop ventricular fibrillation (VF). Ischemia at the microvascular level or autonomic changes at the heart rate may be the cause of this phenomenon.

Changes in QT dispersion during myocardial infarction have been interesting but have not yet been clinically proven. Although they were already used in the area of the efficacy and safety of antiarrhythmic drugs [9]. The difference between the QT intervals of different leads and its measurement as a 'QT dispersion' can make challenging our current approach more likely to assess the risk of arrhythmias. QT dispersion provides a simple, inexpensive and non-invasive potential method of measuring the underlying dispersion of improved ventricular irritability. Continued development of QT dispersion can be linked to important clinical benefits, especially the benefits and risks of treatment with antiarrhythmic drugs [9].

Accordingly, this study was conducted to determine the effect of ALP on QT dispersion as a possible risk factor for sudden cardiac death, arrhythmias and a fatal change in ECG. Since this substance is one of the most important causes of mortality and morbidity of heart arrhythmia [10], the aim of this study was to investigate the cardiotoxicity effects of ALP on the cardiovascular system and particularly the QT dispersion in ALP poisoning.

Material and Methods

The medical records of 70 patients diagnosed with ALP poisoning were investigated in Emam Reza Hospital, Mashhad, Iran. ECG was evaluated at the time of admission, and their QT dispersion was calculated. Also, prognostic factors such as the number of hospital days, the need for a ventilator, the severity of acidosis, and the use of drugs such as magnesium sulfate and calcium gluconate were used.

The control group consisted of 40 patients without a cardiovascular risk factor that had normal coronary angiography,

Inclusion criteria: Patients with a definite diagnosis of ALP poisoning that did not have a history of heart disease.

Exclusion criteria: Patients who have a branch block in the ECG and patients with a history of heart disease.

Data analysis

Data are presented using descriptive statistics like frequency and mean ± standard deviation. T-test and Mann-Whitney-U tests were applied to assess the relationship between qualitative and quantitative variables. Also, the Kruskal-Wallis test was used as a non-parametric method for testing groups, and the relationship between qualitative variables was determined using chi-square test. Statistical analysis was performed using SPSS version 22. A p-value of less than 5% was considered statistically significant.

Ethical considerations

A) The patient's secrets were completely preserved until the end of the study.
B) In the case of intervention or diagnosis, common diagnostic and therapeutic methods along with success rate, complications and benefits of common and interventional methods were determined.

Results

In this study, out of 70 patients, 10 patients were excluded according to the exclusion criteria. Then, patients were compared with the control group. The average age of the patients was 25.55 ± 7.53 in the population. The lowest and highest age was determined as 12 and 45 years, respectively. Of the 60 patients, 27 (45%) were female, and 33 (55%) were male. The distribution of smokers and non-smokers showed that 30% of patients were smokers. In the present study, blood pressure, hyperlipidemia

and diabetes were also investigated. Among all 60 patients enrolled in the study, only one person was found to suffer from hypertension, and no diabetic or hyperlipidemic patients were diagnosed (Table 1).

Table 1: Basic characteristics of patients with aluminium phosphide poisoning

	Number of patients with poisoning with aluminium phosphide: 60 patients		
	Number	Per cent	Mean ± SD
Age			25.55 ± 7.53
Female	27	45%	
Male	33	55%	
Cigarette	18	30%	
Blood pressure	1	1.7%	
Diabetes	_	_	
Hyperlipidemia	_	_	

Table 2 shows the frequency of QT dispersion changes in the population under study, with an average QT dispersion of 60.66 ± 22.08.

Table 2: Frequency QT dispersion changes in patients with ALP poisoning

QT dispersion (ms)	Number	Percent
40	30	50%
60	2	3.33%
80	25	41%
100	2	3.33%
120	1	1.6%

Based on T-test, there is a significant relationship between patient group and control group, with QT dispersion was increased in the patients' group (P = 0.027), (Table 3).

Table 3: Comparison of QT dispersion values between the control group and the patient's group

QT dispersion values	Number	Mean	Standard deviation
Group case	60	60.66	22.08
Group control	40	39.25	19.26

Comparison of two variables such as age, number of hospital days, number of pills, duration of hospitalisation, acidosis severity and QT dispersion was performed. The results demonstrated a significant relationship between the number of taking the pills and the severity of acidosis. The increase in the number of pills consumed was highly linked to increased acidosis (P = 0.020), and no significant relationship was found between other variables such as acidosis and QT scattering. There was also a correlation between age and QT dispersion, where QT dispersion increased with age (P = 0.040; Table 4).

Table 4: Correlation test for age variables, hospital admission time, acidosis severity, number of pills, duration of hospital visits in comparison with QT dispersion

	Comparison of QT dispersion value with hospital admission time	Comparison of QT dispersion value with the number of pills	Comparison of QT dispersion value with a duration of a hospital visit	Comparison of QT dispersion value with acidosis severity	Comparison of QT dispersion value with patient age
P value	0.293	0.236	0.692	0.902	0.040

Figure 1 shows the frequency distribution of hospital admission time in patients with ALP poisoning, demonstrating an average referral time of 4.006 ± 3.22.

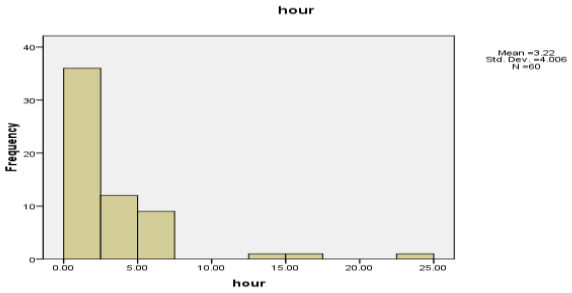

Figure 1: Frequency distribution of hospital visit time in patients with ALP poisoning

The frequency distribution of patients based on the number of pills consumed is shown in Figure 2 that patients consumed an average of 1.09 ± 1.70 pills.

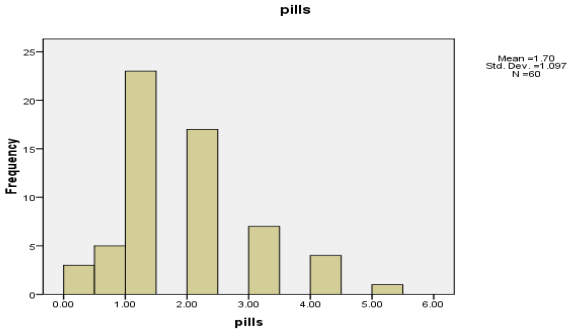

Figure 2: Distribution of patients in terms of the number of pills used

In the current study, the patients' medical records were reviewed, and the type of treatment was recorded, were 4 treatment groups was found (Table 5).

Table 5: Frequency of patients with ALP poisoning based on the type of treatment received

type of treatment received	Number of patients	per cent
HCO3+MgSO4+CaGluconate (First therapeutic group)	24	40%
HCO3+MgSO4 (Second Therapeutic Group)	12	20%
HCO3 (Third Therapeutic Group)	20	33.33%
Conservative Tx (Fourth Therapeutic Group)	4	6.66%

The frequency of patients with ALP poisoning is indicated in Figure 4, based on the number of hospital admissions days, and the average number of admission days was determined as 5.343 ± 3.08.

Frequency of mortality in patients with ALP poisoning by sex revealed that 8 of the patients who died were female and 10 were male, as well as 19 of the surviving patients were female, and 23 were male. Based on the Chi-Square test, mortality of patients with ALP poisoning was not related to their sex (P = 0.955). The findings revealed that 33.3% (20 patients) of the patients needed intubation, and the remaining

did not require intubation. Of the 18 dead patients, 5 were smokers. Among 42 survivors, 13 patients were found to be smokers. Chi-Square test indicated no significant relationship between the mortality rate of the patients and their smoking (P = 0.806).

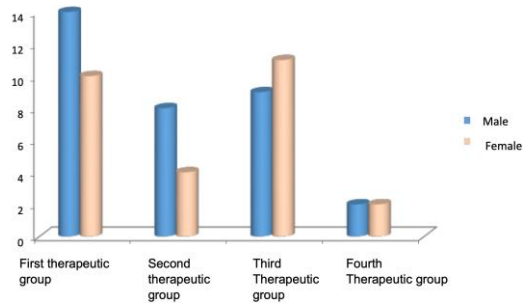

Figure 3: Frequency of patients with ALP poisoning by type of treatment and sex

The Mann-Whitney test revealed that there was no significant correlation between QT dispersion and mortality in patients with ALP (P-value = 0.471).

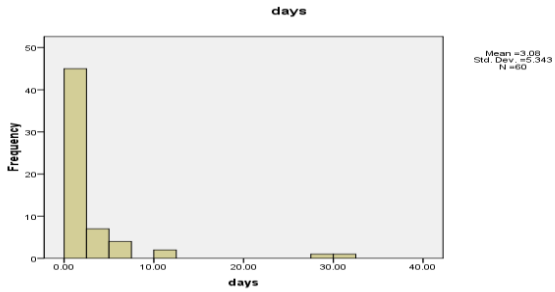

Figure 4: Frequency of patients with ALP poisoning based on the number of hospital admissions days

According to the T-test, a significant correlation was found between the mortality of the patients and the severity of acidosis (P = 0.000).

Based on the Chi-Square and Cross tab tests, out of the 20 cases underwent intubation, 18 of them finally died. In other words, all died patients were under intubation. Also, a direct linear correlation was found between the patients who died and the patients with intubation (P = 0.000). Additionally, no significant correlation was determined between the mortality rate and the type of treatment (P-value: 0.232).

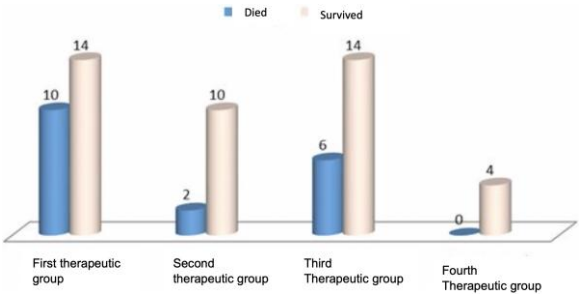

Figure 5: Frequency of mortality in patients with ALP poisoning based on the type of treatment

Mann-Whitney test exhibited that there is a significant relationship between mortality rate and several pills used (P = 0.018), as well as between mortality rate and several hospital days (P = 0.004). Based on the findings presented herein, no relationship was observed between mortality and the age of the patients and the duration of the visit.

Discussion

ALP poisoning has a high toxic effect, where its complications are dose-dependent and hospital-based, and vary from hemodynamic disorder, hypoglycemia, hyperglycemia, shock, cardiotoxicity, pulmonary and renal failures [10], [11], [12], [13].

Direct toxic damage of ALP on the myocardium causes cardiac arrhythmias [14]. Hypotension and shock occur 3 to 6 hours after taking ALP. In patients who survive, cardiotoxicity and hypoxia can be disappeared after 5 to 7 days following phosphorus excretion and the return of normal cell metabolism. This toxic injury causes a variety of lethal changes in the ECG 6 to 24 hours after taking ALP in patients who have died, and non-lethal changes appear in patients who survive, within the first 12 to 24 hours of use, and also disappear 56 to 80 hours later. Death following the use of a rice pill in the first 24 hours is cardiogenic due to ECG abnormalities and shock [7]. In a study by Lall et al., (1997), it was reported that ECG changes were found at all dose in rats, including initial tachycardia and ST-segment elevation progressing to QRS broadening. The cause of cardiotoxicity of ALP, in addition to reducing cellular metabolism of the myocardium, is due to necrosis of the cardiac tissue following the release of reactive oxygen intermediates [15].

In a study by Katira et al., (2005), 90 patients with ALP poisoning were studied over 3 years. According to this study, death was due to poison-induced toxic chemical myocarditis, which was accompanied by electrocardiographic changes [16]. In the study of Chugh and colleagues, arrhythmias, it has been revealed that conduction disturbances and ischaemic pattern were observed in the same frequency. ECG abnormalities, including varied sino-atrial blocks, early repolarisation syndrome bradycardia-tachycardia syndrome that has not been reported before, were seen in this study. Clinical profile of patients was the same regardless of the existence or absence of ECG changes. According to the aforementioned study, ECG abnormalities do not affect motility. Hypoxemia and shock, as well as severity of poisoning, dose of poison consumed, were not known to cause these abnormalities [17].

According to these studies, several ECG changes have been discussed, but QT dispersion

changes have not yet been evaluated. Therefore, the current study was aimed to determine the effect of aluminium phosphide on QT dispersion as a possible risk factor for sudden cardiac death and arrhythmias and a deadly change in ECG. In the study, 70 patients with aluminium phosphide poisoning were enrolled, and the QT dispersion rate was calculated in 60 patients using a standard electrography that was taken at the time of referral. Furthermore, the data were compared with the control group in which 40 subjects included without cardiovascular risk factors, with normal coronary angiogram.

Regarding the findings, the prevalence of ALP poisoning in the young population of Iran was higher. Also, the prevalence of ALP poisoning in the study population was higher in males than females. The mean QT dispersion in the studied population and the control group was calculated as 60.66 ± 22.08 and 39.25 ± 19.26, respectively. There was a significant correlation between QT dispersion as a comparison of both group (P = 0.027), and the QT dispersion rate was found to be higher in the patient's group when compared with the control group. The findings revealed no significant correlation between mortality rate of patients with QT dispersion rate. Also, no significant relationship was found between the mortality rate of patients with the type of treatment received. QT dispersion did not correlate with the severity of acidosis in patients. However, there was a significant correlation between QT dispersion rate and ageing (P = 0.040).

On the other hand, a significant relationship was observed between the severity of acidosis and patient's tablets –taking the number (P = 0.02). Moreover, there was a significant relationship between the mortality of the patients and the severity of acidosis (P = 0.000), but there was no relationship between the severity of acidosis and the hospitalisation time. A significant correlation was also found between the mortality rate of patients and the taking numbers of pills and the number of hospital admissions days (P = 0.004), were taking larger numbers of pills was associated with the more mortality rate in patients (P = 0.018). Also, there was a significant relationship between patients in need of intubation and those who eventually died, as a matter of fact, most intubated patients died (P = 0.000). According to the patient records, the cause of mortality was the appearance of multiple organ failure symptoms, as well as cardiogenic shock, and arrhythmia.

In conclusion, given that there is no risk factor for CAD in the population studied, it can be concluded that increased QT dispersion in these individuals was due to ALP poisoning. But this is not a factor in increasing the morbidity of these patients. However, further studies are recommended in this area to assess the risk of arrhythmias following increased QT dispersion in these patients.

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Clinical Science

Reliability of Contrast CT and Positron Emission Tomography in Post-Surgical Colorectal Cancer and Its Association with Obesity

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Abstract

BACKGROUND: Post-surgical recurrence of cancer colon occurs in one-third of patients within the first two years, so early detection is important. The assessment of the therapeutic response is important to change protocol strategy. Positron emission tomography/computed tomography PET/CT, a valuable tool gives both metabolic and anatomic information for whole-body regions. Obesity is an important risk factor for colorectal cancer.

AIM: To evaluate post-surgical and therapeutic colorectal cancer by PET/CT and study obesity association to its prognosis.

METHODS: This was a prospective study involved 93 patients with, post-surgical colorectal cancer examined by PET/CT, then follow up after 4-6 months.

RESULTS: There was a statistically significant difference between PET/CT and contrast CT. The sensitivity& the specificity were (96.4%-100% & 92.3%-98.2%) for PET/CT and (84.2%-90.2% & 76.5%-85.4%) for contrast CT respectively. Post-therapeutic follow up showed; progressive course (24.5%), stationary course (26.4%), partial regression (28.3%) and complete regression course (20.8%). Obesity is a risk factor for progression with highly statistically significant to treatment response. Obese patients had a progressive or stationary course of the disease. Also, there was a highly statistically significant association between total abdominal fat & visceral abdominal fat areas with good response of treatment.

CONCLUSION: PET/CT is the most appropriate imaging technique to detect any recurrence or metastases in post-surgical colorectal cancer with high sensitivity and specificity comparing to CT. Obesity is a predictor risk factor for prognosis of the disease, as generally and abdominally (total & visceral fat) had an association with therapeutic response.

Introduction

Worldwide more than one million people get colorectal cancer yearly [1]. Also, it is the third most commonly diagnosed cancer in males and the second in females, with 1.8 million new cases and almost 861,000 deaths in 2018, according to GLOBOCAN database. The highest incidence rates are in Europe, North America, Australia, and New Zealand, while the lowest rates are in South-Central Asia and Africa [2]. Colorectal cancer is the 7th commonest cancer in Egypt; it represents 3.5% of male cancers and 3% of female cancers [3]. The estimated numbers of colon cancer patients were more than three thousand in 2015 [3].

Post-surgical recurrence of cancer colon occurs within the first two years. It can recur loco-regionally or at distant sites [4]. In therapy, resection of one metastasis is associated with good survival rate while multifocal metastatic lesions give a less favourable prognosis [5]. Also, the assessment of the therapeutic response (chemo-radiotherapy) is important for change protocol strategy of ineffective and toxic chemotherapy [6]. So, early detection helps design the clinical therapeutic guidelines; secondary operation, radiotherapy or chemotherapy.

Serum carcinoembryonic antigen (CEA) and contrast computed tomography (CT) are conventional methods. As serum CEA levels are used for recurrence monitoring, with its high-level imaging modality will be necessary to localise the site of recurrence and metastases [7]. Regarding changes of

anatomical structures and fibrous tissue in the operative region, contrast CT is likely unable to differentiate postsurgical changes from recurrence and may miss metastatic deposits. While the functional imaging; Fluorine-18 fluoro-D-glucose positron emission tomography / computed tomography (18F-FDG PET/CT) scan can be greatly used to recognise the metabolic characteristics of the lesions and detect any active cells [8]. The integrated PET/CT gives both metabolic and anatomic information with a single device at one diagnostic session for the whole-body regions to detect any recurrence and metastases [9].

Several studies reported that obesity is an important risk factor for colorectal cancer [10], [11], [12], [13], while Scarpa et al., showed the role of obesity in postoperative recurrence and multifocal disease [14].

The aims of this study; to evaluate post-surgical and therapeutic colorectal cancer by PET/CT for proper management, also predict the effect of obesity as a risk factor in prognosis among a sample of Egyptian patients.

Sample size estimation

The sample size was calculated using PASS 11 (USA), regarding the proportional of PET/CT sensitivity at the previous study; 90 subjects were adequate with power 90.0%, α = 0.05, and B = 0.1

Patients and Methods

Design: A prospective study

Ethics: This study was approved by the research ethics committee, faculty of medicine, Helwan University (FMHU 1-2019) and informed written consent were signed by each patient.

Participants: Ninety-four Egyptian's patients with post-surgical cancer colon, examined for follow up after 4-6 months by PET/CT, both genders were included in this study (55 males and 39 females); their ages were ranged from 38 to 75 years. They were referred from clinical oncology and surgery departments due to elevated CEA or follow up to assess the effect of treatment. The inclusion criteria: pathologically proven colorectal carcinoma and underwent appropriate therapy for 4-6 months. Exclusion criteria included those who had a bad general condition, impaired renal function, allergy to intravenous contrast material and a blood glucose level > 200 mg/dl at the time of the study.

The duration of the study: January 2019- June 2019.

Location: Misr Radiology center (MRC), Cairo, Egypt.

Methods

Patient preparation: allow low carbohydrate and high protein diets with liquids (24 hours before), then fasting 6 hours before the examination.

The day of examination: complete history was taken and indication (High tumour marker CEA and previous PET/CT scan for follow up), then measurements were taken before starting the examination: Fasting blood sugar, body height and weight (using a Seca scale balance and anthropometer with light clothes and no shoes, the measure was taken to nearest 0.1 cm and 0.01 kg respectively) [15]. The Body mass index (BMI) was calculated as; the weight (kg)/ height (m^2) and classified into; (18.5 ≥ normal weight < 25), (≥ 25 overweight < 30) and obese (≥ 30) [16].

Examinations: Intravenous injection of 5 – 10 mCi as an average dose of ^{18}F-FDG (0.1 mCi/Kg) one hour before starting the scan. Each patient was examined by PET/CT using Phillips Ingenuity TF, 128 slice machines (Cleveland, OH, USA) as the following A low-dose non-contrast CT for attenuation correction followed by PET scan from the skull to the mid-thigh, then a diagnostic post-contrast CT using nonionic contrast medium. Those PET images were assessed by both visually & semi-quantitatively for the regions with pathologic tracer accumulation using maximum standardized uptake value (SUV_{max}); loco-regional lesion (recurrent) was identified by presence of metabolically active tumour tissue with high FDG accumulation and correlated this activity to its anatomical site in the combined PET/CT images, the lymph nodes and distant metastases (lung, liver, bone, brain, and others) were evaluated as well. The comparison between the recent scan and the previous one in follow up cases was made to evaluate the response of treatment (Fig. 1).

The assessment of therapeutic response evaluated by PET/CT according to RECIST criteria [17]:

- Complete response (CR): The disappearance of FDG uptake at the target tumour lesion.no new FDG avid lesion.

- Partial response (PR): reduction at least a 30% in target measurable tumour FDG uptake, taking the baseline lesion as a reference.

- Progressive disease (PD): at least a 25% increase in tumour SUVmax peak uptake, taking a reference the baseline lesion from starting of treatment or an appearance of a new lesion or more.

- Stationary disease (SD): no sufficient

changes, almost same as reference baseline lesion from starting of treatment; less than 25% increase (not PD) and 30% decrease (not PR). No new lesion.

Regarding abdominal fat assessment, no extra-scan was required, the analysis was processed by special software at an advanced workstation (AW Volumeshare2- version 4.4 Software), assessed total, visceral and subcutaneous abdominal fat compartments at the L4-L5 level by drawing then a calculation of area was done.

Statistical Analysis

SPSS version 22 software was used to analyse the data; mean ± standard deviations (SD) for parametric data, numbers (percentage) for the frequency distribution of non-parametric data, crosstabs for sensitivity and specificity, Chi-square, Pearson´s correlation test, and odds ratio. A significance was set at P = 0.05

Results

This study included 94 patients: 55 male (58.5%) and 39 female (41.5%), their age ranged from 38 to 75 years (mean ± SD: 58.3 ± 4.1 years), weight; 61-109 kg (90.9 ± 5.8 kg), height; 153-169 cm (162.8 ± 3.7 cm), BMI; 22.6-39.8 kg/m2 (34.4 ± 5.4 kg/m2) and fasting blood sugar; 70-197 mg/dl (101.2 ± 2.4 mg/dl). Regarding BMI; 31 (33%) were of normal weight (20 males and 11 females), 12 (12.8 %) were overweight (7 males and 5 females) and 51 (54.3%) were obese (28 males and 23 females), then classified into two groups; the first one included normal weight patients and the second one included both; overweight & obese to involve 63 patients (67%)(35 males and 28 females).

Regarding indications; 41 patients underwent PET-CT post-surgical, while 53 patients follow up post-therapeutic (chemo and radiotherapy) to assess the response of treatment, as well, 62 patients (66%) had elevated tumour marker CEA, and 32 had a negative marker (34%). The CEA was (0.9-116 ng/ml).

The frequency distribution of local recurrence lesions and metastatic deposits detected by contrast CT and PET/CT imaging for a total of 94 patients (Table 1), revealed; lymph nodes metastasis were the most frequent site (36.2% and 46.8%) for CT and PET/CT respectively followed by local recurrence & hepatic deposits (25.5%) by CT, while local recurrence represents (34%) by PET/CT then peritoneal deposits (18.1% and 28.7%), pulmonary deposits (14.9% and 17%) and osseous deposits (11.7% and 23.4%) by CT and PET/CT respectively. Although PET/CT gives additional information about active tumour cell by measuring its avidity to ^{18}F-FDG uptake and measuring the maximum standardised uptake values (SUVmax). Its ranges were; 9-29.4 (mean 17.2 ± 5.4 SD) for local recurrence, 4.5-29.7 (mean 13.3 ± 6.9 SD) for LN metastasis, 5.7-23 (mean 10.7 ± 5.2SD) for hepatic deposits, 7.7-15.3 (mean 11.9 ± 2.5SD) for peritoneal deposits, 7.7-15.3 (mean 10.8 ± 6.1SD) for pulmonary deposits and 4.5-11.8 (mean 9.1 ± 1.7SD) for osseous deposits.

Table 1: Frequency distribution of local recurrence and metastatic lesions detected by Contrast CT and PET/CT

	Contrast CT	PET/CT		p-value
	No. &frequency	No. &frequency	SUVmax value Mean ± SD	
Local Recurrence	24 (25.5%)	32 (34%)	17.2 ± 5.4	0.000
LN Metastasis	34 (36.2%)	44 (46.8%)	13.3 ± 6.9	0.000
Peritoneal Deposits	17 (18.1%)	27 (28.7%)	11.9 ± 2.5	0.000
Pulmonary Deposits	14 (14.9%)	16 (17%)	10.8 ± 6.1	0.000
Hepatic Deposits	24 (25.5%)	24 (25.5%)	10.7 ± 5.2	0.000
Osseous Deposits	11 (11.7%)	22 (23.4%)	9.1 ± 1.7	0.000

There were statistically significant differences between contrast CT and PET/CT (P = 0.000); 8 cases of local recurrence were missed by CT and detected by PET/CT, 10 cases of metastatic LNs and peritoneal deposits detected only PET/CT may be due smaller in size to localize by CT, as well extra 11 osseous lesions were detected by PET/CT (bone marrow affection) compared to CT, while two pulmonary nodules couldn't be detected by CT as it surrounded by consolidation area and pleural effusion.

Then, the sensitivity and the specificity of PET/CT was done related to elevated tumour markers, measuring (96.4%-100% & 92.3%-98.2% respectively) compared to contrast CT (84.2%-90.2% & 76.5%-85.4% respectively), the positive and negative predictive values were 94% and 84% for PET/CT, and 81% and 76.3% for CT.

Regarding obesity, all patients were classified according to BMI categories; normal weight and (overweight & obese) with PET/CT findings to detect frequency of local recurrence and metastatic deposits on each group (Table 2), There was an insignificantly statistical association between obesity and PET/CT findings (no significant differences regarding sex), however, the most frequent local recurrence and metastatic deposits were detected at obese patients (71.9%-81.2%).

Table 2: Comparison between BMI categories (normal weight and overweight &obese) with PET/CT findings

PET/CT	Normal Weight No.&%	Overweight & Obese No.&%	p-value
Local Recurrence	9 (28.1%)	23 (71.9%)	0.312
LN Metastasis	12 (27.3%)	32 (72.7%)	0.074
Peritoneal Deposits	9 (33.3%)	18 (66.7%)	0.865
Pulmonary Deposits	3 (18.8%)	13 (81.2%)	0.410
Hepatic Deposits	5 (20.8%)	19 (79.2%)	0.314
Osseous Deposits	6 (27.3%)	16 (72.7%)	0.786

Then frequency distribution between obesity and response of treatment (post-therapeutic follow up) was done (Table 3). Fifty-three patients were

classified; normal weight and (overweight and obese), The assessment depends on the avidity of the lesion to 18 F-FDG uptakes, quantitative analysis by measuring (SUVmax) value and compared with the previously PET/CT scan from 4-6 months. Thirteen patients (24.5%) had a progressive course of the disease, all were obese, while good response of treatment was recorded at 40 patients (75.5%) as the following; stationary course (26.4%) (57.1% of them were obese), partial regression (28.3%) (60.0% of them were within normal weight) and complete regression course (20.8%) (54.5% of them were within normal weight).

Table 3: Frequency distribution between obesity and response of treatment (Post-therapeutic follow up)

	Total No. &Frequency	Non obese No.&%	Obese No.&%
Progression	13 (24.5%)	0 (42.9%)	13 (100%)
Good response to treatment:	40	20	20
Stationary	14 (26.4%)	6 (42.9%)	8 (57.1%)
Partial Regression	15 (28.3%)	9 (60.0%)	6 (40.0%)
Complete Regression	11 (20.8%)	6 (54.5%)	5 (45.5%)

The odds ratio was done to know the effect of obesity as a risk factor on the progression of cancer colon (Table 4). There was highly statistical significance with a response of treatment (p = 0.001, odd value > 2 and CI = 1.46-2.72), also hepatic and pulmonary deposits had high precision by odd value and 95% confidence interval (CI), followed by LN metastasis and local recurrence, while peritoneal and osseous deposits had a low association with obesity.

Table 4: Odds ratio to predict if obesity a risk factor for the progression of the cancer colon

	Odd Value	95% Confidence Interval	P-Value
Response of treatment	2.0	1.46-2.72	0.001**
Local Recurrence	1.4	0.555- 3.56	0.472
LN Metastasis	1.5	0.622-3.61	0.365
Peritoneal Deposits	0.9	0.352-2.366	0.851
Pulmonary Deposits	2.3	0.601-8.755	0.215
Hepatic Deposits	2.1	0.703-6.341	0.177
Osseous Deposits	1.3	0.463-3.843	0.594

** Highly Significant at P ≤ 0.001.

For more specification of obesity, the abdominal obesity assessed by CT and measured; total abdominal fat, subcutaneous fat and visceral abdominal fat areas (cm^2), their range (100.4-998.7 cm^2), (80-789.6 cm^2) and (16.27-267 cm^2) respectively. Then a comparison between abdominal obesity and response of treatment (post-therapeutic follow up) regarding sex was made (Table 5).

Table 5: Comparison between abdominal obesity and the response of treatment (Post-therapeutic follow up) regarding sex

	Sex	Progressive course No. (13)	Good response to treatment No. (40)	p-value
		Mean ± SD	Mean ± SD	
Total abdominal fat (cm^2)	Male	837.72 ± 60.5	618.20 ± 25.5	0.008
	Female	834.74 ±10.2	463.04 ± 24.9	0.054
Subcutaneous abdominal fat (cm^2)	Male	412.11 ± 36.0	349.16 ± 44.8	0.203
	Female	203.54 ± 53.5	334.70 ± 45.5	0.370
Visceral abdominal fat (cm^2)	Male	217.50 ± 38.3	138.02 ± 35.7	0.000
	Female	229.50 ± 53.5	100.75 ± 42.5	0.004

It was revealed that; 31 males and 9 females had a good response of treatment, while 11 males and 2 females had progressive course after treatment. There was a highly statistically significant difference between total abdominal fat & visceral abdominal fat areas with good response of treatment at both sexes (P ≤ 0.001). However, no statistically significant difference was detected with a subcutaneous fat area.

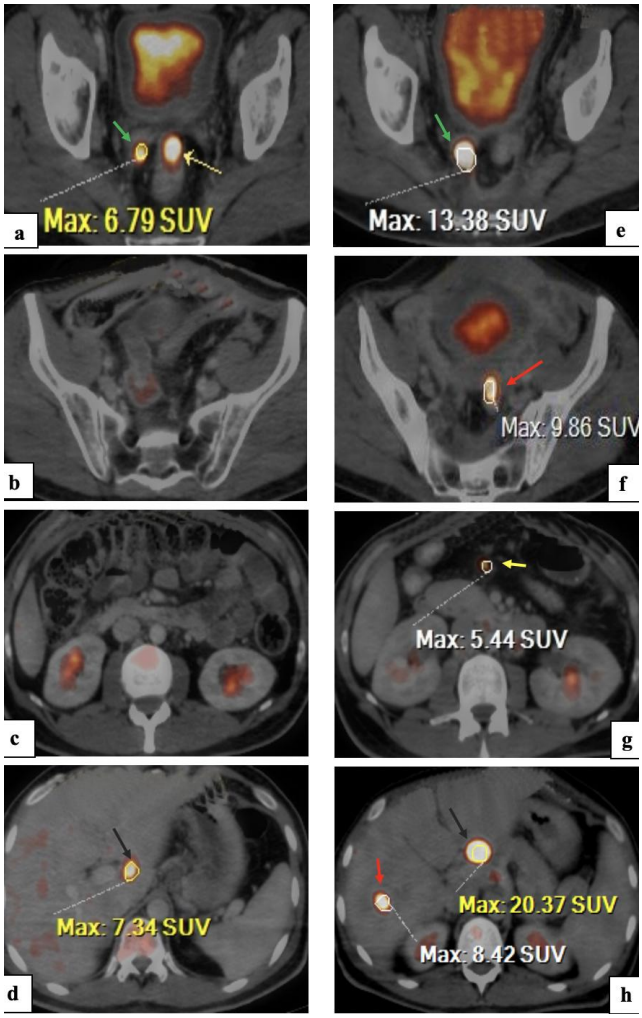

Figure 2: A 72-years old obese male patient, referred after resection of the recto-sigmoid mass and chemo-radiotherapy for follow up. Axial PET/CT images for two examinations; the first (a-d images) and the second examination (e, f, g, h images) after 4 months of treatment for comparison revealed; (a and e) progression of hyper-metabolic peri-rectal soft tissue nodule achieving 13.38 SUVmax (6.79 SUVmax previously) (green arrow), while another lesion (yellow arrow image a) can't be detected in newly one (b and f) a small active hypermetabolic lesion (recurrent) is seen at the distal sigmoid colon, achieving 9.86 SUVmax (red arrow image f) (c and g) Newly developed a small hyper-metabolic peritoneal nodule is noted achieving 5.44 SUVmax (yellow arrow image g) (d and h). Metabolically and morphologically progression of porta-hepatis lymph node, achieves 20.37 SUVmax (7.34 SUVmax previously) (back arrow) and newly developed active right hepatic lobe focal lesion is seen (segment VI) achieves 8.42 SUVmax (red arrow)

Discussion

The most serious problem of colorectal

cancer is a recurrence, as it represents around 10% - 50% within 5 years after the surgery in the form of local or distant. So, the key to diminishing postoperative recurrence is early detection for fast proper management to improve the survive [9].

Postoperative monitoring was done by CEA serum level when elevated suspected of recurrence and imaging modality is necessary to detect any metastasis [18]. Contrast CT could be detected only sizable morphological changes, however, its inability to discriminate inflammatory lesions from recurrence or metastases [7], while ^{18}F-FDG PET/CT shows early metabolic changes to detect any recurrence or metastases for choosing an adequate plan of therapy [8].

Several studies and meta-analysis studies reported a strong positive association between obesity and colorectal cancer. It estimated 30%-50% of new diagnosed colorectal cancer cases [14, [19], [20], [21]. Also, obesity had an effective role in recurrence and prognosis of treatment, as those patients were obese had a higher incidence of recurrence than those had normal or over-weight [13]. Obesity was assessed by BMI, while abdominal obesity was evaluated by CT scan cut at L4 – L5 level [22].

In this Egyptian study, the first purpose was evaluating the role of PET/CT in post-surgical cancer colon comparing to contrast CT, revealed that sensitivity of CT was 84.2%-90.2% and for PET/CT was 96.4%-100%, whereas the specificity of CT was 76.5%-85.4% and for PET/CT was 92.3%-98.2%. These were in agreement with the previous studies; that had approved PET/CT was the technique of choice for postoperative assessment of colorectal cancer to detect recurrence with sensitivity (93%-100%) and specificity (74%-96%) [5], [6]. While, Stuckle et al., reported the sensitivity of CT was 38% – 82% in the detection of the recurrence [23].

In this study, more lesions were detected by PET/CT compared to CT, in spite of the same number of hepatic and almost pulmonary deposits were found in both imaging modalities. This, in agreement with Choi et al. as well had added abdominal LN [24].

Additionally, lymph nodes were the most frequent site of recurrence (46.8%) in the current study by PET/CT, followed by local recurrence (34%), peritoneal deposits (28.7%), hepatic deposits (25.5%), osseous deposits (23.4%) and pulmonary deposits (17%).

Many studies reported that lymph nodes were the most frequent site of recurrence [25], [26]. However, Owen et al. found the liver metastasis was the most frequent site (50%) [27] and Chiewvit et al., reported, the pulmonary metastatic was the second site [28]. Regarding osseous lesions more lesions detected by PET/CT due to bone marrow affection, this is by Bar-Shalom et al., study, as no corresponding CT findings (osteolytic lesions or destruction of bone) at the same detected site by PET/CT [29].

The second purpose of this research was to assess the association between obesity and colorectal cancer recurrence. Our findings revealed that the most frequent local recurrence and metastatic deposits were detected at obese patients (71.9%-81.2%). Several studies concluded the association between obesity and colorectal cancer, as well obese patients had higher recurrence and mortality rates than normal and overweight patients [14], [30], [31], [32], the incidence of obese patients had colorectal cancer was 11.9%-40% in Italian study [33]. The commonest mechanism could be clarified this association; effect of high leptin level at obese persons, which induce pre-neoplastic epithelial cells of the colon [34].

Our results regarding post-therapeutic follow up and prognosis of the disease showed that obesity was highly statistically significant with response of treatment ($p = 0.001$, odds value > 2 and CI = 1.46-2.72), as obese patients had progressive or stationary course (100% and 57.1% respectively), while normal-weight patients had partial and complete regression course (60.0% and 54.5% respectively). Also, there was a highly statistically significant difference between total abdominal fat & visceral abdominal fat areas with good response of treatment at both sexes. This agreement with Jochem and Leitzmann, they found general obesity (BMI) and abdominal obesity had increased risk of colorectal cancer in both sexes [32]. Increased visceral fat area, not subcutaneous or total body fat, was established as the metabolic risk factors for colon cancer, those patients had 1.5 times of the visceral fat area compared to patients without that [35].

Finally, this research has an important recommendation to add at the therapeutic strategy plan of colorectal cancer; reduce body weight and preserve it within normal to improve the response of the treatment.

In conclusion, positron Emission Tomography (PET/CT) is the most appropriate imaging technique to detect any recurrence or metastases in post-surgical& therapeutic follow up colorectal cancer patients with high sensitivity and specificity compared to computed tomography (CT). General obesity is a predictor risk factor for prognosis of the disease, although abdominal obesity (total & visceral fat) had an association with a therapeutic response; as the progressive and stationary courses of the disease were noticed at obese patients with high visceral and total abdominal fat.

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Author Contribution

Safenaz Y. El Sherity (corresponding author): designed the study, statistical analysis, interpretation of the data and wrote the manuscript. Shymaa A. Shalaby: collected the data and shared in manuscript writing. Nayera E. Hassan, Sahar A. El-Masry, and Rokia A. El-Banna: gave conceptual advice and manipulation of the data. All authors share in references collection, drafting the article and approval the final version.

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Clinical Science

The Connection between the Endometrial Thickness and the Risk of Endometrial Malignancy in Postmenopausal Women

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Keywords: Postmenopausal; Endometrial thickness; Endometrial malignancy

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Abstract

BACKGROUND: Postmenopausis is a period that starts one year after the last menstruation. Late menopause, after 70 years, is called senile.

AIM: To examine the correlation between endometrial thickness and the risk of endometrial malignancy in postmenopausal.

MATERIAL AND METHODS: Prospective clinical study involving 120 postmenopausal patients treated at the University Clinic for Gynecology and Obstetrics – Skopje, divided into two groups: control and examination. The control group included 40 postmenopausal patients, hospitalised and operated due to urogenital pathology. The examined group consisted of 80 patients divided into three subgroups according to the ultrasound verified thickness of the endometrium: from 5-8 mm; > 8-11 mm and above 11 mm. A detailed history and intervention were taken in the patients from both groups, and the material was sent for histopathological analysis to determine eventual malignancy.

RESULTS: The probability of endometrial malignancy significantly increased by 1.012 times in the group with a thickness of the endometrium from 5-8 mm, 1.769 times in the endometrial thickness group > 8-11 mm and 4.737-fold in the group over 11 mm compared to the control group.

CONCLUSION: In postmenopausal patients, the likelihood of endometrial cancer significantly increases with the thickness of the endometrium.

Introduction

Postmenopausal is a period that begins one year after the last menstrual period. In this period,a new source of oestrogens is estrone. The average age for menopause in developed countries is 51.4 years [1]. It is divided into early and late menopause. Late menopause, after 70 years, is called senile. In 10-15% of cases, postmenopausal bleeding is caused by endometrial cancer, and usually abnormal uterine bleeding is caused by endometrial polyps or atrophy [2]. The incidence of endometrial cancer in postmenopausal patients is 0.7% but increases in patients with additional risk factors [3]. In this period, abnormal uterine bleeding belongs to polyps, endometrial atrophy, endometrial hyperplasia, endometrial carcinoma, submucosal fibroid, hormone therapy, uterine or uterine infections, use of certain drugs [4], etc.

According to FIGO, the International Federation of Gynecology and Oncology, the stages are subclassified into two pathological types. Type 1-estrogen-dependent [5] in which in 30-80% of cases, the mutation of the PTEN gene is responsible for this type of malignant tumour. It occurs from complex atypical hyperplasia [6]; it is associated with estrogen stimulation and is not aggressive [7]. According to Kurmani's collaborators, this type of cancer is characterized by low malignancy, diagnosed in the early stage, has a superficial invasion of the myometrium and has high sensitivity and good

prognosis, with 85% five-year survival [8], [9]. Type 2-neurosurgeon-dependent [5] endometrial cancer is poorly differentiated, with a deep myometrial invasion, including lymph nodes, low progestin sensitivity and 58% five-year survival [8], [9]. It develops from an atrophic endometrium and is not associated with hormone stimulation [6] metastasizes and grows outside of the uterine hull [7]. Mutations of the P53 gene occur in 50% of cases.

Papillary serous and mesonephrom belongs in this group. This neoplasia is very aggressive.

The purpose of the study was to investigate the predictive role of the thickness of the endometrium in the onset of endometrial malignancy in postmenopausal patients.

Material and Methods

This is a prospective clinical study, including 120 postmenopausal patients treated at the University Clinic for Gynecology and Obstetrics - Skopje. Patients were divided into two groups: a control and examination group. The control group included 40 postmenopausal patients, hospitalised and operated due to urogenital pathology, and ultrasonically detected endometrial thickness less than 5 mm. The examined group included 80 postmenopausal patients hospitalised due to endometrial bleeding with an ultrasound detection of an endometrial thickness greater or equal to 5 mm. According to the thickness of the endometrium, the examinees from the examined group were divided into three subgroups: a) subgroup 1 = 5-8 mm; b) subgroup 2 = 8-11 mm; and c) subgroup 3 => 11 mm. A detailed history and intervention were taken in the patients from both groups, and the received material was sent for histopathological analysis to determine eventual malignancy.

The study excluded patients in generative reproductive age, patients who were not able to do fractional exploratory curettage, patients with a personal history of malignant disease (past or current), patients with a personal anemia for benign or malignant tumors of the ovary , breast cancer patients treated with tamoxifen , patients with any pelvic surgery due to other gynecological pathology.

Statistical analysis

The data during the survey were processed with the statistical package SPSS 20.0. The Pearson Chi-square homogeneity test was used to establish an association between certain attributive dichotomies of the two groups of respondents. The Shapiro-Wilk W test was used to determine the frequency distribution frequency of certain variables. To test the significance of the difference between two and more numerical variables with regular or irregular distribution of frequencies was the Studentov T-test for independent samples, the Mann Whitney U test and the Kruskal-Wallis ANOVA test. A significance level of $p < 0.05$ was used to determine the statistical significance.

Results

Characteristics of the sample

In the investigated group of patients, the average age was 62.3 ± 7.7 years, and in control, it was 64.4 ± 7.5 years (Table 1). The tested difference between the two groups relative to age, $p > 0.05$, did not indicate a significant difference (Mann-Whitney U Test: $Z = -1.3138$; $p = 0.1889$). Patients from the investigated or control group have an average number of years in menopause 11.9 ± 6 v.s. 11.8 ± 4 years difference in significance between groups (Mann-Whitney U Test: $Z = -0.4397$; $p = 0.6601$).

Table 1: Descriptive analysis of the sample according to certain parameters and groups

Group	(Means)	Number	Standard deviation (Std.Dev.)	Minimum (Min)	Maximum (Max)	Median (IQR)
Age						
Examination	62.33	80	7.68	49	84	61 (56-67)
Control	64.37	40	7.51	52	79	65 (57.5-68)
Years in menopause						
Examination	11.97	80	6.01	3	30	10 (7.5-15)
Control	11.85	40	4.03	5	18	11.5 (8-15)
BMI						
Examination	29.46	80	5.42	14.9	42.7	29.7 (26-32.3)
Control	28.66	40	3.85	21.8	41.4	28.2 (26.1-30.3)

In the whole sample, the majority of the respondents were married 115 (95.8%), 1 (0.8%) were single, and 4 (3.3%) divorced (Table 1). For $p > 0.05$, no significant association was found between the group and the marital status of the subjects (Fisher-Freeman-Halton exact test: $p = 0.9999$).

Both the examination and the control group are dominated by the majority of respondents who are non-smokers, and consequently, 52 (65%) v.s. 30 (75%) (Table 1). For $p > 0.05$, there is no statistically significant association between the group to which the examinees belong and the smoking status (Pearson Chi-square test = 0.2323; df = 1; $p = 0.2669$).

Patients in the examined group had an average BMI of 29.5 ± 5.4, and those of the control 28.7 ± 3.8 without a significant difference between the two groups compared to this parameter (Student's t-test for independent sample = 0.8346; df = 118; $p = 0.4056$) (Table 1).

Endometrial analysis

The average thickness of the endometrium in the examined group was 10.8 ± 5.6 mm with a minimum thickness of 6mm and a maximum thickness of 32mm while in the control group it was 2.7 ± 0.8 mm with a minimum thickness of 1mm and a maximum thickness of 4.5 mm (Table 2). According to the median analysis, 50% of the patients in the control or examination group had endometrium thickness greater than the corresponding IQR = 2.8 mm (2-3) v.s. IQR = 9mm (7-12). The analysis, for $p < 0.05$, indicated a significant difference between the examinees of both groups in terms of endometrial thickness (Mann-Whitney U Test: Z = 8.907235 p = 0.00001) in favour of a significantly thicker endometrium in the assay group.

Table 2: Analysis of the thickness of the endometrium (mm) in the control and examination group

Group	N	X ± sd	Minimum (min)	Maximum (max)	Mediana (iqr)
Examination	80	10.8 ± 5.6	6	32	9 (7 – 12)
Control	40	2.7 ± 0.8	1	4.5	2.8 (2 – 3)

Mann-Whitney U Test: Z = 8.907235; p = 0.00001; ** Significant for p < 0.05.

According to the results of the ultrasound-ultrasound measurement of the thickness of the endometrium, the examination group (N = 80 patients) was divided into three subgroups: (a) 5-8 mm with a total of 36 (45%) patients; (b) < 8-11 mm with a total of 17 (21.25%) patients; and (c) < 11 mm with a total of 27 (33.75%) patients (Table 3).

Table 3: Division in subgroups of the examination group by endometrial thickness

Subgroups by the thickness of the endometrium (mm)	Number	%
5-8	36	45
> 8-11	17	21.2
> 11	27	33.7
Total value	80	100%

In the control group, the average thickness of the endometrium was 2.7 ± 0.8 mm, with a minimum thickness of 1mm and a maximum thickness of 4.5 mm. In the first subgroup with thickness of endometrium 5-8mm, the average thickness of the endometrium was 6.9 ± 0.9 mm with a minimum of 6.0 mm and a maximum of 8.0 mm. According to the media analysis, 50% of patients in this subgroup have an endometrial thickness greater than IQR = 7 mm (5-8 mm). In the second subgroup with an endometrial thickness of 8.0-11.0 mm, the average thickness of the endometrium was 9.4 ± 0.5 mm with a minimum of 9mm and a maximum thickness of 10mm. According to the analysis of the media, 50% of patients in this subgroup have an endometrial thickness greater than IQR = 9 mm (9.0-10.0 mm). In the third subgroup with a thickness of endometrium > 11 mm, the average endometrium thickness was 16.8 ± 5.8 mm with a minimum of 11 mm and a maximum of 32 mm. According to the median analysis, 50% of patients in this subgroup had an endometrial thickness greater than IQR = 14 mm (12.0 -22.0 mm) (Table 3).

Table 4: Thickness of endometrium (mm) in subgroups of the examination group

Subgroups	The thickness of the endometrium (mm)	N	X ± *SD*	Minimum	Maximum	Median (IQR)
Subgroup 1	5 – 8	36	6.90 ± 0.88	6	8	7 (5 – 8)
Subgroup 2	> 8 – 11	17	9.40 ± 0.49	9	10	9 (9 – 10)
Subgroup 3	> 11	27	16.82 ± 5.82	11	32	14 (12 – 22)

For $p < 0.05$, a significant difference was found between the three subgroups of the examinated group compared to the thickness of the endometrium (Kruskal-Wallis ANOVA: H = 68.967; p = 0.00001). The individual subgroup analysis, for $p < 0.05$, indicated a significant difference between the first and second , first and third vs. the second and third subunits for consequently Mann-Whitney U Test: Z = -5.831; p = 0.00001 vs. Mann-Whitney U Test: Z = -6,750; p = 0.00001 vs. Mann-Whitney U Test: Z = -5.532; p = 0.00001 (Table 4).

Table 5: Binary logistic regression analysis of the predictive role of endometrial thickness for prediction of endometrial malignancy

Variable	B	S.E.	Wald	Df	Sig.	Exp(B)	95% C.I.for EXP(B)	
							Lower	Upper
The thickness of endometrium about unit thickness of endometrium								
endometrium (mm)	0.164	0.052	10.073	1	0.002*	1.178	1.065	1.304
Endometrium thickness - reference category / < 5mm								
5 mm -8 mm	1.897	1.381	2.864	1	0.049*	1.012	1.014	3.549
>8 mm - 11 mm	2.377	1.164	4.167	1	0.041*	1.769	1.099	5.488
>11 mm	2.690	1.098	6.001	1	0.014*	4.737	1.712	12.840

* Significant for p < 0.05.

The thickness of the endometrium is a significant predictor of endometrial malignancy ($p < 0.05$). With each millimeter an increase in endometrium, the likelihood of endometrial malignancy increases significantly by 1,178 [p = 0.002, 95% CI = 1.065-1.304] times. Compared to patients with a thickness of endometrium < 5 mm, binary logistic regression indicated that the probability of endometrial cancer was for: a) 1.012 [p = 0.049, 95% CI = 1.014-3.549] times greater in endometrial thickness of 5-8 mm; b) 1.769 [p = 0.041, 95% CI = 1.099-5.488] times greater in endometrial thickness > 8-11 mm; and c) 4.737 [p = 0.014, 95% CI = 1.712-12.840] times greater in patients with an endometrial thickness of > 11 mm (Table 5).

Discussion

The results of this study showed that with an increase in unit endometrium, the likelihood of malignancy increased by 1,178. Namely, an increase in the thickness of the endometrium by 1 mm relative to the control group significantly increases the likelihood of endometrial malignancy in patients with endometrial thickness from 5-8 mm in 1,012 times, in

those with an endometrium thickness of 8-11 mm in 1,769 times, while in patients with endometrial thickness > 11 mm, the likelihood of endometrial cancer is greatest and increases by 4,737 times in relation to control. The results of this study correlate with the relevant literature concerned.

Thus, in the study of Smith-Bindman et al. in which correlation between endometrial thickness and endometrial cancer risk was examined, a 6.7% risk of endometrial malignancy was found in patients with an endometrial thickness of over 11 mm and a 0.002% risk of endometrial thickness below 11 mm (10).

In conclusion, the thickness of the endometrium is a significant predictor of endometrial malignancy. With each millimetre, an increase in the endometrium significantly increases the likelihood of endometrial malignancy for:

-Enlargement for a unit of the endometrium, the risk of endometrial malignancy increases by 1.178 times.

-1.012 times [p = 0.002, 95% CI = 1.065-1.304] in the group with a thickness of the endometrium of 5-8 mm relative to the control.

-1.769 times [p = 0.041, 95% CI = 1.099-5.488] in the endometrial thickness group of 8-11 mm in terms of control.

-4.737 times [p = 0.014, 95% CI = 1.712-12.840] in the group with an endometrial thickness of over 11 mm relative to the control group.

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Role of Inflammation in the Pathogenesis of Diabetic Peripheral Neuropathy

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Abstract

BACKGROUND: Diabetic peripheral neuropathy (DPN) means the presence of symptoms and/or signs of peripheral nerve damage that occur to people with diabetes, excluding all other causes of neuropathy. Chronic hyperglycaemia leads to increased secretion of tumour necrotic factor-alpha (TNF-α), with the development of micro and macroangiopathy, damage to nerve fibres and local demyelination.

AIM: To determine the role of inflammation in the peripheral nerve damage process concerning people suffering from type II diabetes mellitus.

MATERIAL AND METHODS: The study included a total of 80 subjects, men and women, divided into two groups: an examined group (n = 50) consisting of subjects with DPN at the age from 30 to 80 years and a control group (n = 30) of healthy subjects aged from 18 to 45. In the investigated group, a neurological examination was performed using the Diabetic Neuropathy Symptoms (DNS) Score and Electroneurography. All the subjects had the blood plasma concentration of TNF-α by ELISA technique.

RESULTS: The average value of TNF-α in the test group was 8.24 ± 2.899 pg/ml, while the control group was 4.36 ± 2.622 pg/ml ($p < 0.0001$). The average value of TNF-α was correlated with the achieved DNS score in the investigated group ($p = 0.005$). Concerning the linear association of the concentration of TNF-α with the peripheral nerve velocity in the investigated group, no statistical significance was detected.

CONCLUSION: Inflammation can play a role in the pathogenesis of diabetic autonomic neuropathy and cranial neuritis.

Introduction

Diabetic peripheral neuropathy (DPN) is the most common microvascular complication in type I and type II diabetes mellitus, with an average in 30% of cases is with clinical manifestation as a painful neuropathy [1]. DPN means the presence of symptoms and/or signs of peripheral nerve damage that occur to people with diabetes, excluding all other causes of neuropathy. In the first years of diabetes, neuropathies develop about 5-10% of the patients, and after 20 years the duration of diabetes is thought to be about 60-70% of the patients develop some of the forms of diabetic peripheral neuropathy [2]. People who suffer from diabetic sensory neuropathy have a 25% greater risk of developing an ulcer on the feet and amputation of the limbs. The three-year survival of people with diabetic neuropathy is about 20% lower compared to people suffering from diabetes mellitus but have no peripheral neuropathy [3].

TNF-α (also known as cachectin) is a pro-inflammatory cytokine that plays a major role in the emergence of diabetic neuropathy; unlike other microvascular complications such as retinopathy or nephropathy (where the dominant role is played by IL-6 and CRP). In animal models, the role of TNF-α in the peripheral and central sensitisation mechanisms has been proven, and hence the occurrence of neuropathic pain [4]. Human TNF-α is synthesised as a 26 kDa type II transmembrane protein consisting of 35 amino acids in the cytoplasmic domain, 21 amino acids in the transmembrane segment and 177 amino

acids in the extracellular domain. In the extracellular domain, the human TNF-α possesses amino acid sequences that are 97% identical to those of rhesus monkeys; 71-92% identical to the amino acids detected in TNF-α in cows, dogs, horses, mice, rats. It creates various types of cells: immune, epithelial, endothelial, and tumor cells.

Chronic hyperglycaemia leads to the stimulation of macrophages, such as cells that dominantly secrete TNF-α and increased secretion of this cytokine, by developing micro and macroangiopathy. TNF-α increases the expression of endothelial cell adhesion molecules and thus accelerates the process of atherosclerosis [5]. The increased production of TNF-α secondary to hyperglycemia is a factor of exacerbation of insulin resistance in ill-controlled diabetes. The influence of TNF-α on Schwann cells should also be neglected, which explains local demyelination in the pathological process of peripheral neuropathy.

The aim of the study was to determine the role of inflammation in the process of peripheral nerve damage concerning people suffering from type II diabetes mellitus.

Material and Methods

A total of 80 examinees, men and women, were divided into two groups: a study group (n = 50) consisting of subjects with the symptomatology of DPN, aged 30 to 80 years, and a control group (n = 30) composed of healthy subjects, aged 18 to 45 years. Criteria for inclusion in the research were: respondents with diagnosed type II diabetes mellitus, lasting one to 40 years; on regular therapy with oral antidiabetics and / or insulin, with symptoms and signs of DPN. Criteria for exclusion from the study were: pre-diagnosed diabetic retinopathy and nephropathy; ischemic/haemorrhagic stroke or acute myocardial infarction over the past 12 months; acute and/or chronic skin infection, respiratory and gastrointestinal infection, known malignancy and autoimmune diseases, where higher plasma concentrations of TNF-α and diabetic foot or gangrene can be expected.

In the respondents from the investigated group (n = 50) clinical and neurological examination was first performed to determine the clinical type of DPN. To assess the severity of the clinical picture in our study, we used the Diabetic Neuropathy Symptom score-DNS, which assessed the following symptoms: pain, stiffness, tingling and the presence of ataxia. An objective electrophysiological assessment of the degree of damage to the peripheral nerves was made using electroneurography, which included the following peripheral nerves: n. medianus, n. ulnaris, n. peroneus profundus and n. suralis.

Subsequently, a sample of blood from the vein was taken for all subjects to determine the concentration of a TNF-α. Blood samples were first centrifuged to separate the blood plasma from the cellular elements, and then the blood plasma samples (about 1-2 ml) were frozen at -70°C. The concentration of the TNF-α was determined by the enzyme-linked immunosorbent assay (ELISA) technique. Anti-TNF-α antibodies that are absorbed in the wells bind to TNF-α which is present in the sample or standards. Subsequently, the addition of secondary biotinized anti-TNF-α antibodies that bind to TNF-α which is bound to the primary antibody. After incubation, unbound biotinised anti-TNF-α antibodies are removed by rinsing. The next step involves the addition of horseradish peroxidase (HRP) that is conjugated with streptavidin and which binds to biotin-conjugated anti-TNF-α antibodies.

After incubation, the unbound streptavidin HRP is removed by rinsing and a substrate solution for peroxidase, tetramethylbenzidine (TMB), which forms a colored product and whose coloring is proportional to the concentration of TNF-α in the sample or standard solution, is added. The reaction is stopped with 1 M phosphoric acid and the absorbance is measured at 450 nm. A standard curve of 7 standard solutions of TNF-α is formed.

For the analysis of the material were used the following statistical methods: frequency, percentage, contingency table, χ^2-Chi square test, Fisher Exact Test (FET), p index of statistical significance, mean value, standard deviation, Student t-test, Fisher F-test and Pearson correlation coefficient r.

Results

Based on the clinical presentation of DPN, the subjects (n = 50) were divided into 4 categories: 11 (22%) with a clinical picture of sensory neuropathy, 29 (58%) were with senso-motor neuropathy; 4 (8%) with cranial mononeuritis and 6 (12%) with dominant autonomic symptomatology (autonomic neuropathy). Regarding the achieved number of points on the DNS scale, the respondents were divided into four groups: with 1 point were 13 respondents, of which 6 (12%) were men, and 7 (14%) were women. With 2 points, there were 17 respondents, 7 (14%) men, 10 (20%) women. With 3 points on the DNS score were 10 respondents and 6 (12%) were men, and 4 (8%) were women. Ten respondents had 4 points on the DNS score with equal representation of men and women, respectively 5 (10%).

The average age of respondents in the study group was 65.5 years, while healthy subjects were 33.6 years old. The control group was composed of younger respondents because of the lower likelihood

of other chronic illnesses, which would give higher values of the investigated pro-inflammatory marker TNF-α. Based on the statistical analysis, it is noted that there is a statistically significant difference between the average age of subjects in the investigated and control group at the level $p < 0.0001$.

The average TNF-α blood plasma sample of the test group (n = 50) was 8.24 ± 2.899 pg/ml, while the control group (n = 30) was 4.36 ± 2.622 pg/ml ($p < 0.0001$). The average plasma TNF-α sample in the subjects from the test group (n = 50) was 8.24 pg/ml, 8.05 pg/ml for men and 8.40 pg/ml for women. The conducted statistical analyses showed that there was no statistically significant difference between the average values of TNF-α in men and women ($p = 0.671$). The average value of TNF-a does not depend on gender.

The average plasma concentration of TNF-α considering subjects with diabetic senso-motor peripheral neuropathy (n = 29) was 8.08 pg/ml, in subjects with diabetic sensory peripheral neuropathy (n = 11) was 6.81 pg/ml. The average TNF-α concentration in subjects with diabetic autonomic neuropathy (n = 6) was 11.2 pg/ml, while those with cranial mononeuritis (n = 4) was 8.85 pg/ml.

The average value of TNF-α was correlated with the achieved DNS score in the examined group ($p = 0.005$). The results are shown in Table 1.

Table 1: The average value of TNF-α dependence on DNS score in the examined group (n = 50) and statistical significance

DNS score	TNF-α (pg/ml) Average value ± S.D.	n	F (p)
1	6.138 ± 1.975	13	4.84
2	8.352 ± 3.058	17	
3	8.83 ± 3.27	10	P = 0.005
4	10.18 ± 1.503	10	

F-Fisher test; S.D. standard deviation; p-index of statistical significance.

Regarding the DNS score connection, the score with the DPN type was found a statistically significant difference at the level $p = 0.0244$. Respondents with autonomic neuropathy and cranial neuritis had higher scores on the DNS scale. This is shown in Table 2.

Table 2: Distribution by clinical type of diabetic peripheral neuropathy depending on the DNS score in the examined group (n = 50) and statistical significance

DPN type	DNS score				FET p
	1	2	3	4	
Sensomotor	6 (12 %)	11 (22 %)	8 (16 %)	4 (8 %)	P = 0.0244
Sensory	5 (10 %)	5 (10 %)	1 (2 %)	0 (0 %)	
Autonomous	0 (0 %)	1 (2 %)	1 (2%)	4 (8 %)	
Cranial mononeuritis	2 (4 %)	0 (0 %)	0 (0 %)	2 (4 %)	

FET-Fisher Exact Test; p-index of statistical significance.

Regarding the linear association of the concentration of TNF-α with the peripheral nerve velocity in the examined group, no statistical significance was found of the Pearson correlation coefficient r in any of the motor and sensory nerves conduction velocity.

Table 3: Correlation between TNF-α and the rates of nerve testing in the examined group (n = 50) and statistical significance

	Pearson correlation coefficient r	Relation	t (p)
n. medianus	-0.103	TNF-α = 9.699 – 0.030	-0.721 p = 0.474
n. ulnaris	-0.1	TNF-α = 10.125 – 3.841	-0.699 p = 0.488
n. peroneus	-0.178	TNF-α= 10.103 – 4.887	-1.257 p = 0.215
n. medianus	0.009	TNF-α = 8.182 + 1.419	6.559 p = 0.948
n. ulnaris	-0.093	TNF-α = 8.734 – 0.0129	0.653 p = 0.516
n. suralis	0.058	TNF-α = 8.044 + 9.932	0.405 p = 0.687

t-Student test; p-index of statistical significance.

Discussion

Diabetic peripheral neuropathy is the most common microvascular complication in diabetes mellitus. There are many different mechanisms involved in peripheral nerve damage in diabetic peripheral neuropathy, of which the key role in oxidative stress, inflammation and mitochondrial dysfunction [6]. Diabetes causes functional deficiency of nitric monoxide, activation of alternative metabolic pathways, accumulation of end-products of glycation, oxidative stress and inflammation, by activating inflammatory molecules. People with diabetes mellitus have an increased expression of pro-inflammatory cytokines such as C-reactive protein, TNF-α and IL-6. Chronic hyperglycemia leads to infiltration of cytokines into the vascular tissue and thus reduces the ability to repair.

Obesity increases the risk of developing neuropathy precisely because the fat tissue has increased expression of TNF-α, which in turn is involved in the mechanisms of insulin resistance. There is also a positive correlation between the increased plasma concentration of TNF-α and the number of macrophages with the progression of diabetic peripheral neuropathy [7].

In 2009, Herder et al. investigated the association of inflammation with diabetic peripheral neuropathy (by analysing 10 inflammatory markers of a total of 227 subjects who had type 2 diabetes mellitus). In this study, a high degree of association was found between CRP and IL-6 concentrations in people suffering from diabetic peripheral neuropathy [8].

In another study, an inverse association was found between the level of TNF-α and the nerves conduction velocities of n. suralis, n. medianus, and n. ulnaris, by analyzing the inflammatory marker in people who did not suffer from diabetic peripheral neuropathy and those who had undergone less than or more than 8 years of diagnosing diabetic neuropathy. Respondents who had diabetic neuropathy had higher serum TNF-α concentrations compared to others, with an upward trend in the duration of the disease [9].

In our study, subjects with diabetic peripheral neuropathy had higher concentrations of TNF-α in blood plasma related to healthy subjects, or individuals without diabetic neuropathy. This confirms the finding of numerous research studies where the role of TNF-α in the pathogenesis of DPN was examined [10], [11].

For a clinical assessment of the severity of DPN in our research, we used the DNS score as a simplified system for the diagnosis of distal diabetic neuropathy. Regarding the impact of the clinical type of diabetic peripheral neuropathy on the average value of TNF-α, in our study, higher TNF-α concentrations in the blood plasma sample had subjects in whom damage of the autonomic nervous system and cranial nerves were clinically dominant. The highest percentage of subjects diagnosed with diabetic autonomic neuropathy and cranial mononeuritis had a maximum number of points on the DNS score. The correlation between the levels of TNF-α with the severity of DPN has been proven in numerous research studies [7], [12], [13].

When it comes to the association of TNF-α concentration in blood plasma with the motor and sensory conduction velocities the examined nerves, no correlation was found in any of our motor and sensory implementation rates. That is, the increased plasma concentration of TNF-α as pro-inflammatory cytokine does not affect the neuropathic characteristics of the peripheral nerves. Regarding the link of the DNS score with the motor and sensory velocities of conducting the examined nerves, no positive correlation between these two parameters was found in our research study, that is, the severity of the clinical picture in subjects with diabetic peripheral neuropathy does not depend on the conduction velocities of the examined peripheral nerves.

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Evaluation of the Potential Association of Platelet Levels, Mean Platelet Volume and Platelet Distribution Width with Acute Appendicitis

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Abstract

BACKGROUND: The occurrence and early management of acute appendicitis among children are especially important due to the difficult diagnosis and nonspecific symptoms of the disease. Diagnosis of appendicitis in children is very difficult due to similarity of its symptoms to other diseases, and also its self-limiting nature Platelet indexes such as mean platelet volume (MPV) and platelet distribution width (PDW) have been suggested as a biomarker of inflammation.

AIM: Therefore, we examined the association of MPV and PDW with acute appendicitis in children.

METHODS: This cross-sectional study was conducted on 464 patients with suspected acute appendicitis under the age of 18 years referred to the specialised hospitals of the ten studied provinces between October 2014 and October 2015. All data obtained regarding patient's lab tests, i.e. platelet count, MPV and PDW and also radiological studies and surgical reports were gathered in datasheets and analysed to evaluate the potential association of platelet levels, mean platelet volume (MPV) and platelet distribution width (PDW) with acute appendicitis

RESULTS: Our results showed that the MPV was significantly higher in acute appendicitis in comparison to perforated appendicitis as well as acute gangrenous appendicitis. PDW was significantly higher in acute appendicitis in comparison to perforated appendicitis and acute gangrenous appendicitis. The current project indicated that PDW < 10.05 had a sensitivity of 35% and specificity of 75%, platelet count < 229500 had a sensitivity of 24% and specificity of 75% and MPV < 8.95 had a sensitivity of 70% and specificity of 71%.

CONCLUSION: Our study suggested that platelet indexes such as MPV and PDW could significantly correlate with acute appendicitis in pediatric patients. Hence, we believe that both MPV and PDW could use as a simple and low-cost lab test for diagnosing acute appendicitis. Also, this study revealed that the MPV lower than 8.95 could be a novel index for diagnosing acute appendicitis with sensitivity of 70% and specificity of 71%.

Introduction

Acute appendicitis is one of the most common reasons for emergent surgery in all age subgroups around the world, only in the United States; the annual incidence of pediatric cases suffering acute appendicitis is estimated to be around 70.000 with notably higher incidence in the developed countries [1], [2], [3]. The incidence of disease in children aged birth to 4 years is about 1 to 2 cases per 10,000 children per year, reaching 25 cases per 10,000 children per year in older children [4]. The prevalence of disease in boys is twice that of girls with a brief distinction in different countries [5], [6]. The prevalence of acute appendicitis in developed countries is higher probably due to low fibre diet, genetic susceptibilities, as well as different patterns in gastrointestinal flora [7]. From the perspective of pathophysiology, acute appendicitis has been identified as a complex disease with quite a bit of variability in clinical manifestation and pathophysiology. The dominant pathophysiology of disease includes obstruction of the blind-ending appendix frequently due to lymphoid hyperplasia of the submucosal follicles in children following viral infections, dehydration, or fecalith. Other rare causes for appendicitis are parasitic infections, foreign bodies, or inflammatory reactions [8], [9], [10].

Overall, the occurrence and early

management of acute appendicitis among children are especially important due to the difficult diagnosis and nonspecific symptoms of the disease. Diagnosis of appendicitis in children is very difficult due to the similarity of its symptoms to other diseases and also its self-limiting nature [11]. It may not have the classic features seen in adults, and therefore hence, doctors may be challenged in terms of timely diagnosis and treatment. Its late diagnosis is common in children that one-third of preoperative children may suffer from perforation with increased the likelihood of mortality and morbidity [12]. Moreover, about 10 to 30% of appendectomies seem not to be necessary for children that can increase morbidity and healthcare costs [13].

Typically, decisions about appendectomy in acute abdominal pain refer to the other causes expressed by the patient (anorexia, nausea, vomiting and pain migration), examinations and clinical symptoms (fever, tenderness, and rebound tenderness, guarding, psoas sign and etc.) and serum tests (white blood cell count and polymorphonuclear neutrophils) [14]. However, various studies have been reported negative appendectomy in 15-30% of cases, especially in children. Negative appendectomy is associated with severe complications such as wound infections, obstruction and infertility due to damage to the uterine tubes. Imposing an unnecessary surgery may also lead to complications such as intestinal adhesions and loss of efficacy [15], [16].

A recent study in Iran showed that the number of white blood cells, the percentage of neutrophils and CRP are some diagnostic factors of acute appendicitis [17]. Recently, it has been shown that platelet count and its morphological assay in the lab test can have valuable predictive values in different gastrointestinal disorders and their surgical outcomes [18]. Also, it has been shown that higher platelet counts are associated with negative outcomes in critically ill patients. Thus, considering the need for determining better diagnostic methods for appendicitis specifically in children, in this study we have evaluated the potential association of platelet levels, mean platelet volume (MPV) and platelet distribution width (PDW) with the incidence of acute appendicitis.

Material and Methods

This study was a descriptive cross-sectional conducted on 464 patients with suspected appendicitis under the age of 18 years referred to the specialised hospitals of the studied provinces between October 2014 and October 2015. Ten provinces (Tehran, Arak, Kashan, Tabriz, Bandar-e-Abbas, Karaj, Hamadan, Kermanshah, Zanjan, Mashhad and Shiraz) out of the 31 provinces were randomly selected from Iran. The criteria for suspected acute appendicitis were included fever (temperature > 38ºC), vomiting, history of anorexia and vague periumbilical pain following migration of pain to the right lower quadrant, tenderness, rebound tenderness in physical examination and increased white blood cell (elevation of the neutrophil or band count can be seen without elevation of the total WBC count). In abdominal radiography, the main criteria for acute appendicitis were convex lumbar scoliosis, obliteration of the right psoas margin, right lower quadrant air-fluid levels, air in the appendix, and localised ileus. In abdominal ultrasonography, main finding was a non-compressible tubular structure 6 mm or wider in the right lower quadrant with local tenderness. The diagnosis of appendicitis was finally approved according to the surgical findings and post-operative pathological assessments. The patient's data collected and entered into the checklist by reviewing the hospital records including gender, age, chief complaints and clinical manifestations, laboratory tests, histopathology reports, findings on physical examination and imaging assessments, preoperative prophylaxis, surgery report, in-hospital complications, and length of hospitalisation.

Descriptive analysis was used to describe the data, including mean ± standard deviation (SD) for quantitative variables and frequency (percentage) for categorical variables. Comparison of numerical data means was based on ANOVA, Tukey's post hoc test and student’s t-test. Also, Spearman correlation was used for indicated the correlation of study variables, i.e. platelet count, MPV and PDW with appendicitis incidence. Correlated variables were then analysed with the ROC curve to indicate their sensitivity and specificity for predicting appendicitis incidence and the decent cut-off values.

Results

In this study, 464 patients admitted to the university hospital with appendicitis symptoms were studied among whom 179 (38.6%) were female, and 285 (61.4%) were male. These patients had a mean age of 110 months which had a standard deviation of 40.8 months (range 1-216).

Through their admission, clinical and preclinical assessments confirmed the final diagnosis of appendicitis in 90.3% of these cases. Among these cases, 411 patients (88.6%) underwent open surgery while 52 were operated with laparoscopy (11.2%) and only one patient did not undergo surgical treatment (0.2%) due to denying consent for the operation.

Post-operative assessments on the obtained tissue were performed to determine the underlying pathology. As it is demonstrated in Table.1 acute appendicitis, perforated appendicitis, acute

gangrenous appendicitis and reactive follicular hyperplasia were the most frequent findings whereas 22 operated patients were revealed to have normal appendix tissue.

The obtained blood tests at the time of admission in these patients revealed a mean platelet count of 279271 ± 113806 platelets per microliter of blood, mean platelet volume (MPV) of 9.18 ± 4 fL and platelet distribution width (PDW) of 11.41 ± 2.2 Fl.

Table 1: Underlying pathologies in the study population

Pathology	Frequency	Per cent	Valid Percent
Normal	22	4.7	4.8
Acute appendicitis (Acute suppurative, early acute & acute appendicitis)	253	54.5	54.6
Eosinophilic appendicitis	2	0.4	0.4
Perforated appendicitis	39	8.4	8.4
Mucosal lymphoid follicular hyperplasia	1	0.2	0.2
Lymphoid follicular hyperplasia	12	2.6	2.6
Vermiform appendicitis	1	0.2	0.2
Obliteration appendix	1	0.2	0.2
Vermiform appendix	1	0.2	0.2
Acute gangrenous appendicitis	69	14.9	14.9
Serosal edema and congestion	1	0.2	0.2
Suppurative Appendicitis	7	1.5	1.5
Necrotic	1	0.2	0.2
Appendicitis	4	0.9	0.9
Congestion	6	1.3	1.3
Reactive follicular hyperplasia	41	8.8	8.9
Embedded	2	0.4	0.4
Total	463	99.8	100.0
Missing	1	0.2	
Total	464	100.0	

Comparison of platelet count, MPV and PDW between different acute appendicitis, perforated appendicitis and acute gangrenous appendicitis pathologies

Results of one-way ANOVA revealed a significant difference between the three types of acute appendicitis, perforated appendicitis and acute gangrenous appendicitis regarding PDW (F (2.355) = 27.57, p-value < 0.001) and MPV (F (2,354) = 26.22, p-value < 0.001). However, no significant difference was observed in platelet counts between these groups.

Furthermore, results of the Tukey's post-hoc test revealed that mean MPV was significantly higher in acute appendicitis compared to perforated appendicitis (p-value = 0.001) and acute gangrenous appendicitis (p-value < 0.001) while there was no significant difference between acute gangrenous appendicitis and perforated appendicitis in this regard (p-value = 0.248). Also, it was shown that mean PDW was significantly higher in acute appendicitis compared to perforated appendicitis (p-value < 0.001) and acute gangrenous appendicitis (p-value < 0.001) while there was no significant difference between acute gangrenous appendicitis and perforated appendicitis in this regard (p-value = 0.333). Table 2 represents the mean of platelet count, MPV and PDW in each group.

Table 2: Mean of platelet count, MPV and PDW in each appendicitis pathology group

Appendicitis pathology	Platelet		MPV		PDW	
	Mean	SD*	Mean	SD	Mean	SD
Acute appendicitis	271538	114966	9.1	1.0	10.9	1.5
Perforated appendicitis	311030	88228	8.1	1.1	12.3	3.5
Acute gangrenous appendicitis	289021	120619	8.4	1.0	13.0	3.0

*SD: Standard deviation.

Evaluation of correlation between platelet count, MPV and PDW and incidence of acute appendicitis, perforated appendicitis and acute gangrenous appendicitis pathologies

Results of spearman's correlation test for platelet count, MPV and PDW and incidence of acute appendicitis, perforated appendicitis and acute gangrenous appendicitis pathologies revealed a significant negative correlation between acute appendicitis and PDW (correlation coefficient -0.150, p-value = 0.001) and also the platelet count (correlation coefficient -0.1, p-value = 0.048). However, there was a significant positive correlation between acute appendicitis and MPV (correlation coefficient 0.188, p-value < 0.001). Moreover, it was shown that perforated appendicitis had a significant negative correlation with MPV (correlation coefficient -0.260, p-value < 0.001). Finally, analysis results revealed a significant positive correlation between acute gangrenous appendicitis and PDW (correlation coefficient 0.221, p-value < 0.001) and a significant negative correlation between this type of appendicitis and MPV (correlation coefficient -0.245, p-value < 0.001).

Predictive value of platelet count and PDW for appendicitis

To evaluate the Predictive value of platelet count, MPV and PDW for appendicitis ROC curve analysis was performed according to correlation analysis results. In this regard, as most of the correlations were negative, test was set as smaller test indicated more positive test, and for those with positive correlation, the 1/variable was considered for analysis.

Acute appendicitis

ROC curve analysis for variable correlated with acute appendicitis revealed that PDW, platelet count and 1 / MPV are sensitive and specific for determining the incidence of this type of appendicitis (Figure 1).

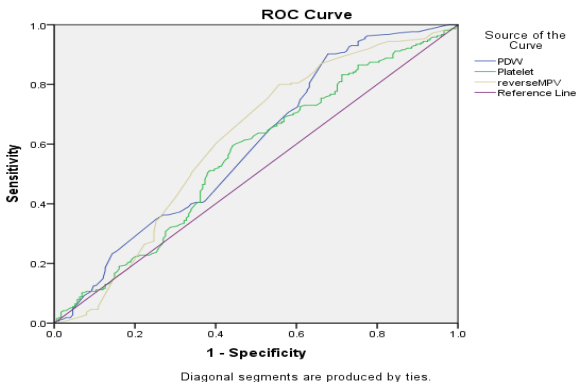

Figure 1: ROC curve analysis for variable correlated with acute appendicitis

In this regard the area under curve and p-value were as (AUC:0.594, p-value = 0.001) for PDW, (AUC:0.560, p-value = 0.041) for platelet count and (AUC:0.594, p-value < 0.001) for 1 / MPV.

Furthermore, the coordinate of the curves indicated that PDW < 10.050 had a sensitivity of 35% and specificity of 75%, platelet count < 229500 had a sensitivity of 24% and specificity of 75% and 1/MPV < 0.1058 had a sensitivity of 35% and specificity of 75%.

Perforated appendicitis

ROC curve analysis for variable correlated with acute appendicitis revealed that MPV is sensitive and specific for determining the incidence of this type of appendicitis (Figure 2)

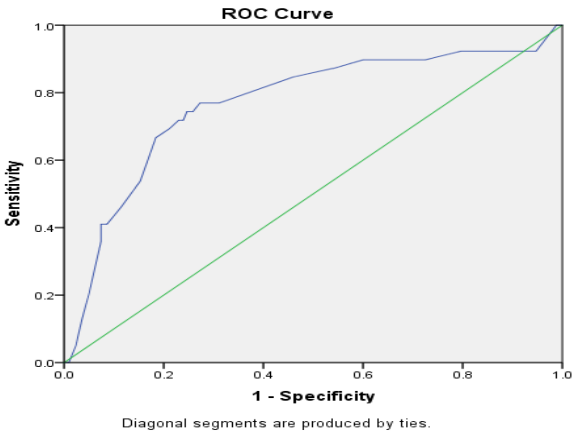

Figure 2: ROC curve analysis for variable correlated with perforated appendicitis

In this regard, the area under curve and p-value was as (AUC:0.768, p-value < 0.001) for MPV and coordinate of the curves indicated that MPV < 8.25 had a sensitivity of 70% and specificity of 80%.

Acute gangrenous appendicitis

ROC curve analysis for variable correlated with acute appendicitis revealed that MPV and 1/PDW are sensitive and specific for determining the incidence of this type of appendicitis (Figure 3).

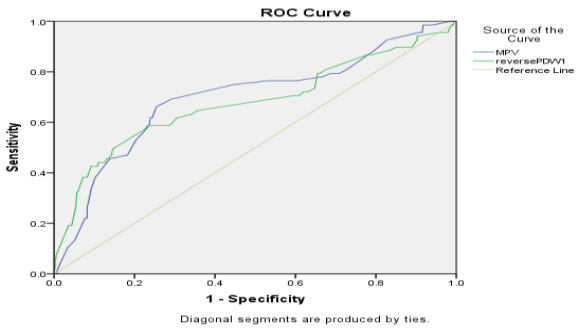

Figure 3: ROC curve analysis for variable correlated with acute gangrenous appendicitis

In this regard the area under curve and p-value were as (AUC:0.698, p-value = 0 < 001) for MPV and (AUC:0.679, p-value = 0 < 001) for 1 / PDW.

Furthermore, the coordinate of the curves indicated that MPV < 8.95 had a sensitivity of 70% and specificity of 71% and 1/PDW < 229500 had a sensitivity of 59% and specificity of 76%.

Discussion

According to our reports, on all children who were suspected of acute appendicitis according to initial manifestations, 10% suffered definitively from disease according to surgical findings as the gold diagnostic standard. Although clinical assessment along with different imaging modalities especially ultrasonography can be very helpful to discriminate abnormal from healthy conditions, but could not play a key role in this goal. The obtained prevalence of childhood appendicitis among suspected children is much closer to the rates previously reported. As shown by Wiersma et al. in 2009, the prevalence of appendicitis, proven by surgery and/or pathology, in this study was 34% [15].

The platelet factors such as MPV and PDW are markers that could be easily studied with complete blood count and which is an indicator of platelet function and activation. These markers volume were found to be associated with platelet function and activation [19], [20]. In general, platelet production increases as the platelet count decreases, and young platelets become larger and more reactive, and therefore, the MPV values are higher [21], [22]. In recent years, in some studies in which MPV was tested as a simple inflammatory marker, MPV was reported to have been affected by inflammation, and that it increases significantly in myocardial infarction, sepsis, cerebrovascular diseases, respiratory distress syndrome and chronic pulmonary diseases [23], [24].

In the literature, MPV has been reported to decrease in some inflammatory bowel diseases such as ulcerative colitis, especially in the active period, and that it could be used for determination of the disease activity [25], [26]. This condition is thought to have been related to the release of bioactive molecules of pro-inflammatory active platelets in the presence of inflammation. Also, numerous studies have been evaluated the role of platelet indexes such as MPV and PDW in acute appendicitis. In this regard, Boshnak et al., showed that Increased PDW combined with elevated white blood cells and neutrophil counts might be used as diagnostic tests in the cases of acute appendicitis, while MPV and RDW levels were not useful diagnostic markers, while studies are not in consist with each other [27].

Our results showed that the mean MPV was

significantly higher in acute appendicitis in comparison to perforated appendicitis as well as acute gangrenous appendicitis. Besides, current study demonstrated that the mean PDW was significantly higher in acute appendicitis in comparison to perforated appendicitis and acute gangrenous appendicitis. In contrast, Arian Nia et al. found no association between MPV and final diagnosis of acute appendicitis in children between the ages of 1 and 15 years; and also, they suggested that the MPV is not an effective index in the diagnosis of acute appendicitis and cannot be involved as a reliable index in making decision. However, Bilici et al. found that the mean MPV was found to be lower than normal in 48 cases in the acute appendicitis group (MPV = 7.55) [28]. However, the study by Uyanik et al. supports the findings which are in contrast with our study. They found that the mean MPV was 7.9 in the appendicitis group and 7.7 in the control group and that there was no statistically significant difference between the two groups [29]. Interestingly, MPV in Erdem et al., study was 7.4 in the appendicitis group and 9.1 in the control group [30]. Similarly, Fan et al. reported that the MPV value in gangrenous appendicitis group was significantly lower than in the controls group [31].

Although numerous studies have been reported the effectiveness of PDW and MPV in diagnosis of various types of appendicitis, few reports have been conducted to evaluate the sensitivity and specificity of platelet indexes for diagnosis of acute appendicitis. In this regard, MPV was evaluated by Bilici et al., [28]. In the recent mentioned study, the specificity was determined as 54%, and sensitivity was found as 87% for the decrease in MPV (< 7.4 fL). The current project indicated that PDW < 10.05 had a sensitivity of 35% and specificity of 75%, platelet count < 229500 had a sensitivity of 24% and specificity of 75% and 1/MPV < 0.1058 had a sensitivity of 35% and specificity of 75%. Furthermore, our results indicated that MPV < 8.95 had a sensitivity of 70% and specificity of 71% and 1 / PDW < 229500 had a sensitivity of 59% and specificity of 76%.

Finally, the results obtained from this study showed a significant negative correlation between acute appendicitis and PDW and also the platelet count. However, our results revealed that there was a significant positive correlation between acute appendicitis and MPV. Moreover, it was shown that perforated appendicitis had a significant negative correlation with MPV. Finally, analysis results revealed a significant positive correlation between acute gangrenous appendicitis and PDW and a significant negative correlation between this type of appendicitis and MPV.

In conclusion, our study suggested that platelet indexes such as MPV and PDW could significantly correlate with acute appendicitis in pediatric patients. Hence, we believe that both MPV and PDW could use as a simple and low-cost lab test for diagnosing acute appendicitis. Also, this study revealed that the MPV lower than 8.95 could be a novel index for diagnosing acute appendicitis with sensitivity of 70% and specificity of 71%.

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Clinical Science

The Difference of sVE-Cadherin Levels between Dengue Hemorrhagic Fever Patients with Shock and without Shock

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Keywords: sVE-Cadherin; Dengue Hemorrhagic Fever (DHF); Shock

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Abstract

BACKGROUND: Dengue virus infection is an infectious disease caused by the dengue virus and transmitted by the Aedes aegypti mosquito. Dengue virus (DEN-V) consists of 4 serotypes, namely DEN-1, DEN-2, DEN-3, and DEN-4. The most feared result of DHF is death. Death in children is caused by hypovolemic shock due to plasma leakage from intravascular to extravascular space due to endothelial dysfunction.

AIM: This study aims to analyse difference in sVE-Cadherin levels in Dengue Hemorrhagic Fever (DHF) with and without shock.

MATERIAL AND METHODS: The method of taking samples is consecutive sampling, namely the research subjects obtained based on the order of entry in the hospital with a comparative cross-sectional design. From the results of the calculation using the sample formula, the sample size for each group is set at 32 people. So that the total sample size used for both groups is 64 people. The serum sVE-Cadherin levels using the ELISA method. The statistical test used is the independent t-test. The value of $p < 0.05$ was said to be statistically significant.

RESULTS: The result showed that there was no difference in mean sVE-Cadherin levels between DHF patients with shock and without shock ($p > 0.05$).

CONCLUSION: This study concluded that there was no difference in mean of sVE-Cadherin level in DHF patients with shock and without shock.

Introduction

Dengue virus infection is an infectious disease caused by the dengue virus and is transmitted by the mosquito Aedes aegypti [1]. In dengue infection after the virus enters the body, the virus will infect Langerhans, dendrites, macrophages and B lymphocytes [2], [3], [4]. These infections produce various mediators that have an impact on endothelial cell function [5]. Langerhans, dendrites, macrophages and B lymphocytes that are infected will experience activation, securing mediators TNF-α, IL-8, IL-10, IL-15, IL-18, RANTES, MCP-Iα, MCP-Iβ, monokine, histamine and vascular endothelial growth factor (VEGF) [6], [7], [8].

Furthermore, MHC class II presents the dengue virus to T lymphocytes and T lymphocytes will stimulate macrophages to kill viruses that have been previously deposited. Infected B lymphocytes, after binding to T lymphocytes, will transform into plasma cells and then produce antibodies. Furthermore, antibodies will bind and neutralise circulating viruses, activate the complement system and cross-react with platelets, endothelial cells and hepatocytes (transient autoimmune) [9]. Antibodies that cannot neutralize the virus will bind the dengue virus and function as opsonin. The antibody-virus bond then binds to the Fc receptor on the surface of the macrophage to cause signals into the cell and activate macrophages [2].

Proinflammatory cytokines, VEGF, complement and antibodies released by the immune system including macrophages result in endothelial cells contracting actin filaments in the capillary endothelial cell cytoplasm. The contraction will pull in

the link protein between cells, JAMs and sVE-Cadherin that enter the cells resulting in widening of the gap between endothelial cells resulting in plasma leakage. Severe and prolonged plasma leakage can cause hypovolemic shock and even death of the patient [10].

Dengue research using endothelial tissue culture in patients with dengue infection showed endocytosis of sVE-Cadherin in endothelial cells that were activated. Endocytosis decreases levels of sVE-Cadherin, in endothelial cells which are directly proportional to the severity of plasma leakage. This shows that sVE-Cadherin plays an important role in maintaining the integrity of the link between endothelial cells and its level can be used as a parameter of plasma leakage [11].

This study aims to analyse difference in sVE-Cadherin levels in Dengue Hemorrhagic Fever (DHF) with and without shock.

Material and Methods

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

Study Population

The study population was patients with dengue virus infection (DHF and DSS) who were hospitalised at Dr M. Djamil Central General Hospital according to WHO 2011 criteria [12]. Subjects were part of the population that met the inclusion and exclusion criteria. The inclusion criteria were patients with dengue hemorrhagic fever who had received informed consent from parents to participate in the study with the age of 1-15 years. Exclusion criteria were patients suffering from other viral or bacterial infections based on clinical and laboratory examinations, receiving corticosteroid therapy, malnutrition and obesity.

Examination of sVE-Cadherin Levels

Blood samples ± 2-3 cc (which is checked in the critical phase) that were inserted into the serum tube were sent to the Biomedical Laboratory, Faculty of Medicine, Andalas University using media transport at 4°C. After that, prepare the microplate well as needed. Then, add 100 µL Diluent RD1-78 Assay into each well and add 50 µL of serum or standard or control into each well, cover with adhesive strip then incubate at room temperature and above the horizontal orbital microplate shaker set at 500 rpm + 50 rpm. The aspirations of each well and washing, do 3 times from a total of 4 washing times. Washing is done by entering 400 µL wash buffer. After that, add 200 µL conjugate sVE-Cadherin to each well. Then cover with a new adhesive strip and incubate for 2 hours. Perform the washing process again as in point 5. After that, add 200 µL Substrate Solution to each well and incubate for 30 minutes at room temperature and on benchtop avoid light and then add 50 µL Stop Solution to each well to stop the reaction. The colour inside the well must change from yellowish blue. Read using a microplate reader with a wavelength of 450 nm and a correction wavelength of 540 nm or 570 nm. Plot the standard curve and estimate the concentration of the sample against the curve.

Statistical analysis

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

Research Ethics

This study was already passed the ethics clearance and has been approved by the Ethics Committee of the Faculty of Medicine, Andalas University, Padang with registration number: 175 / KEP / FK / 2016.

Results

The difference in the results of sVE-Cadherin examination between dengue patients with shock compared to those without shock can be seen as follows.

Table 1: Difference in the results of the examination of sVE-Cadherin between DHF patients with shock and without shock

	DHF		
Variable	DSS (n = 62) mean ± SD	DHF (n = 48) mean ± SD	p-value
sVE-Cadherin (ng/ml)	5.93 ± 4.87	5.86 ± 4.811	0.956

Table 1 showed that the average sVE-Cadherin level in DHF patients with shock was 5.93 ± 4.87 ng/ml, while in DHF patients without shock 5.86 ± 4.811 ng/ml. From the results of statistical tests, there was no difference in mean sVE-Cadherin levels between DHF patients with shock and without shock ($p > 0.05$).

The cut-off point for sVE-Cadherin levels as a predictor of dengue patients with shock

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

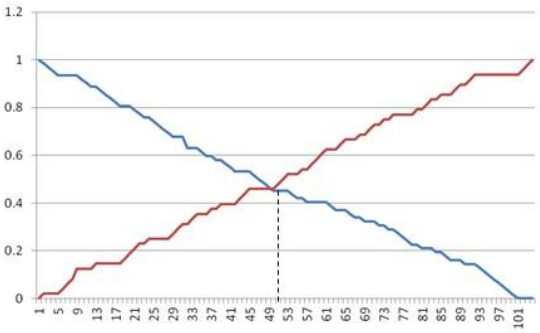

Figure 1: Cut-off of sVE-Cadherin levels as predictors of DHF patients with shock with A); Blue (sensitivity); B) Red (specificity)

Figure 1 shows that the optimal cut-off point on the intersection of sensitivity and specificity lines to determine the cut-off point of sVE-Cadherin levels as a predictor of DHF patients with shock is between point 50. Cut off points of sVE-Cadherin levels as predictors of DHF patients with shock can be explained as follows. Namely, subjects experiencing DSS, if the sVE-Cadherin level is ≥ 4.04 ng/ml and the subject has DHF if the sVE-Cadherin level is < 4.04 ng/ml

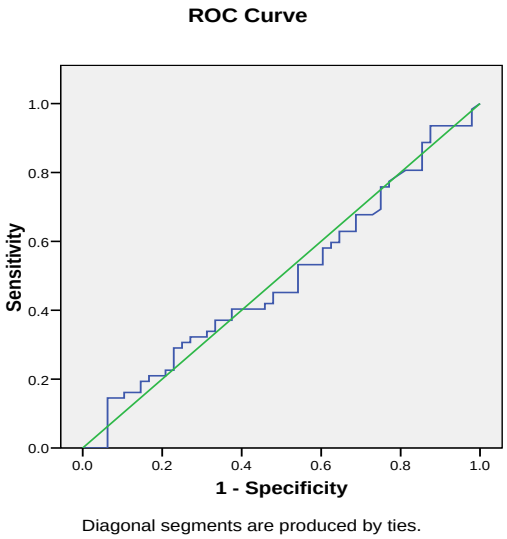

Figure 2: Accuracy of Cut-off point sVE-Cadherin levels as predictors of DHF patients with shock

The cut-off point of this sVE-Cadherin sensitivity was 45.1%, and specificity was 45.8%. The accuracy of the cut-off point of sVE-Cadherin levels as a predictor of DHF patients with shock is shown in Figure 2.

Figure 2 is known based on the receiver operating curve (ROC) analysis that the area under curve (AUC) value of 49.5% means that the cut-off point of sVE-Cadherin level of ≥ 4.04 ng/ml has poor accuracy in predicting DSS events.

Table 2: Selection of candidate variables in predicting payments in DHF patients

Variables	p-value
Long fever	0.274
Mucosal bleeding	0.001†
Abdominal pain	0.000†
Sedentary vomiting	0.000†
Hepatomegaly	0.000†
Hematocrit	0.005†
Platelets	0.000†
sVE-Cadherin	0.956

† qualify if $p < 0.25$.

Discussion

The difference in the results of sVE-Cadherin examination between DHF patients with shock compared to without shock

Inter-cell links that maintain the paracellular path are tight junction and adhering junction. From the two links the main one is the adhering junction. The large gap between endothelial cells is maintained constant by various proton adhesions in the gap between endothelial cells. Among these adhesion proteins, sVE-Cadherin is the main adhesion protein. sVE-Cadherin is embedded in the actin tissue of the cortex of the endothelial cell and forms a homophilic bond with neighbouring sVE-Cadherin cells. The movement of water and various molecules that dissolve in the blood, mainly through the paracellular pathway, the integrity of the protein sVE-Chaderin adhesion is very necessary [13], [14].

The Pober (2007) study found a statistically significant difference in the levels of sVE-Cadherin among DHF patients with and without shock ($p < 0.05$). Leukocyte interaction with the endothelium during inflammation can change the composition of endothelial permeability. The stimulation of proinflammatory cytokines will result in the emergence of adhesion molecules on the surface of the leukocytes and endothelium. Activated endothelial cells due to cytokine stimulation will express adhesion molecules such as FIK-1 (E-selectin), ICAM-1, VCAM-1, p-selectin and PECAM-1 on the endothelial surface [15], [16].

These adhesion molecules make leukocytes

stick to the endothelial surface and secrete free radicals, proteases and cause local inflammation and endothelial cell damage. Also, leukocytes that bind to ICAM-1, through SRC and Rho GTPase, interfere with sVE-cadherin adherens junction. PECAM-1 which is the most important molecule binds to leukocyte cells in the inter-endothelial gap, attracts and causes leukocyte migration. Endothelial damage that interferes with VE-cadherin adherent junction and migrated leukocytes widens the gap between the endothelium, causing and aggravating plasma leakage [17], [18].

The study of sVE-cadherin in dengue infection has so far only been in the in vitro research stage using endothelial tissue culture. This approach shows that the levels of sVE-cadherin decrease in leaky endothelial tissue (11). The release of proinflammatory cytokines, VEGF, antibodies and complement activation in the infection resulting in disruption of endothelial cell links, widening of the endothelial gap and leakage of plasma from the intravascular space to the extravascular space.

Cardozo *et al.,* (2017) investigating the effect of plasma leakage in patients with severe dengue infection getting vascular endothelial homeostasis plays an important role in plasma leakage, which is influenced by the immune response. Dengue virus affects endothelial cells to produce proinflammatory cytokines and chemokines such as IL-8, RANTES, MMP-2 and VEGF. Dengue infection also suppresses the production of TNF-α which mediates vascular hyperpermeability. PMBCs (peripheral mononuclear blood cells) also play a role in increasing endothelial cell permeability by decreasing the expression of sVe-cadherin. It can be concluded that the decrease in sVE-Cadherin values in individuals with dengue infection indicates an increased risk of becoming more severe infections [19], [11].

In vitro research by Yacoub *et al.,* (2016) and Kanlaya *et al.,* (2009) in the endothelial model found that the dengue virus can bind to EGL, reducing the expression of VE-cadherin and tight junction ZO-I proteins, causing an increase in plasma permeability [20], [21].

The difference of candidate variables in predicting payments in DHF patients

Fever, abdominal pain and vomiting are also symptoms that are often found in DHF and are a warning sign in dengue cases. Abdullah *et al.,* (2018) found that there were significant differences between persistent vomiting, fluid accumulation and mucosal bleeding with the severity of dengue infection and had high sensitivity and specificity in predicting the occurrence of severe dengue infection [22]. Nagaram (2017) found 73 cases with complaints of abdominal pain and 115 cases with vomiting. In DHF patients, 32.8% of cases of abdominal pain were obtained, and 60.4% of cases of vomiting in patients with DSS had 96% of cases reduced and 100% of cases of vomiting. Research conducted by researchers also found that there was a relationship between abdominal pain and vomiting with DHF in shock. Although dengue virus is a nonhepatotropic infection, liver injury often occurs, ranging from mild dysfunction to an increase in liver enzymes to those with severe yellow symptoms and even fulminant liver failure [23].

The Nagaram (2017) study obtained 100% hepatomegaly in the DSS case group and 77% in the DHF group [23]. Research by Zhang *et al.,* (2014) found hepatomegaly in children with dengue infection had a 5 times greater risk of death compared to children infected with dengue without the discovery of hepatomegaly. From the above review compared to this study, there was a relationship between mucosal bleeding, abdominal pain, persistent vomiting and hepatomegaly with DHF with shock ($p < 0.05$) [24].

This study concluded that there was no difference in mean levels of sVE-Cadherin in DHF patients with shock and without shock.

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Clinical Science

Chest Pain Characteristics in Cardiac Syndrome X Compared to Coronary Artery Disease

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Keywords: Chest pain; Cardiac syndrome X; Coronary artery disease

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Abstract

AIM: This study aimed to assess if clinical remarks gained by analysis of the present and past medical history of patients undergoing elective coronary angiography (ECA) due to typical chest pain can help to predict the outcome of ECA.

MATERIAL AND METHODS: One hundred and fifty-four ECA candidates with a history of typical chest were seen on the same day intended for ECA in the cardiac centre of AlShaab Teaching Hospital, Khartoum, Sudan. The details of the present complaints, characteristics of chest pain, past medical and socioeconomic history were recorded from each subject guided by a questionnaire. ECA confirmed CAD in 112 of the studied patients and were considered as the test group. The remaining patients (N = 42) were diagnosed as CSX after exclusion of significant narrowing of the coronary vessels and were considered as the control group.

RESULTS: Univariate analysis of pain characteristics among patients undergoing coronary angiography revealed that pain is less likely to radiate to the neck (OR = 0.44, 95% CI = 0.21 – 0.91, P = 0.027) and the back (OR = 0.48, 95% CI = 0.23 – 1.00, P = 0.049) in patients with CAD. Presence of shortness of breathing and/or dizziness significantly decrease the odds of having abnormal coronary angiography (OR = 0.30 and 0.48, 95% CI = 0.12 – 0.77 and 0.22 – 0.92, P = 0.013 and 0.030 respectively). Past history of diabetes mellitus significantly increases the odds of having abnormal coronary angiography (OR = 3.96, 95% CI = 1.68 – 9.30, P = 0.002). In contrast, past medical history of migraine decreases the odds of having positive finding in ECA (OR = 0.31, 95% CI = 0.13 – 0.72, P = 0.006).

CONCLUSION: Characteristics of chest pain are comparable in CAD and CSX. However, pain is less likely to radiate to the neck and/or the back in the first group. Presence of dyspnea and dizziness during angina attacks as well as the history of migraine significantly decreases the odds of having abnormal coronary angiography.

Introduction

Patients with typical chest pain and positive stress electrocardiography (ECG) or other cardiac tests are not certainly suffering from coronary artery disease (CAD) [1], [2]. Cardiac syndrome X (CSX) is frequently used to diagnose patients with typical chest pain, positive cardiac stress test(s) and normal coronary macrocirculation [3]. Dysfunction of coronary microcirculation [4] and abnormal perception of pain [5], [6] are the most acceptable explanations for CSX in the literature so far. In clinical practice, the relatively high percentage of patients with no significant angiographic findings following elective coronary angiography (ECA) raise a question whether ECA is overused in patients with suspected CAD [7]. Although there are a lot of studies investigating risk factors for CAD [8], [9], researches exploring the predictors of CSX are scarce [2], if any. This study aimed to assess if clinical remarks gained by analysis of the present and past medical history can help to predict outcome of ECA. Special care was given in comparing chest pain characteristics between patients with CSX and CAD.

Material and Methods

The present study was approved from the ethics review committee (ERC), Faculty of Medicine, University of Khartoum, Sudan. All candidates who agreed to join this study signed a written informed consent before being evaluated.

One hundred and fifty-four ECA candidates with a history of typical chest pain were seen on the same day intended for ECA in the cardiac centre of AlShaab Teaching Hospital, Khartoum, Sudan. The details of the present complaints, characteristics of chest pain, past medical and socioeconomic history were recorded from each subject guided by a questionnaire. The body mass index (BMI) and mean arterial blood pressure (MABP) were calculated for each subject by the formulae:

$$\text{BMI (kg/m^2)} = \text{weight (kg)/(height (m^2)}$$

and

$$\text{MABP} = \text{Diastolic blood pressure} + \frac{1}{3}(\text{Diastolic blood pressure} - \text{Systolic blood pressure})$$

respectively.

ECA confirmed CAD in 112 of the studied patients and were considered as the test group. The remaining patients (N = 42) were diagnosed as CSX after exclusion of significant narrowing of the coronary vessels and were considered as the control group.

Statistical analysis was performed using Statistical Package for the social sciences (SPSS) for Windows, version 16.0 (SPSS Inc., Chicago, IL, USA). Normal distribution of variables was examined using Shapiro-Wilk test. Unpaired T-test was used to assess statistical difference between mean (SD) of normally distributed scaled variables. Mann-Whitney U test was used to compare median (25^{th} – 75^{th} interquartile) of abnormally distributed scaled variables. Univariate analyses were carried out to evaluate characteristics of chest pain and past medical history as past predictors of CAD. Results of univariate analyses were expressed by odds ratios (OR) and their 95% CI. In all tables expressing results of univariate analysis, OR described the ratio of the odds of an event occurring in patients with CAD to the odds of the same event occurring in subject with CSX. P < 0.05 was considered significant.

Results

Coronary artery catheterization of the studied subjects (N = 154) revealed 112 patients with CAD (P (95% CI) = 72.7% (65.3 –79.3%), mean (SD) of age = 60.58 (10.26) years) and 42 subjects with normal coronary arteries (P (95% CI) = 27.3% (20.7–34.7%), mean (SD) of age = 50.95 (16.46) years).

Table 1 compares age, anthropometric measurements and blood pressures of subjects with CSX and CAD. Age was significantly higher in CAD patients compared to CSX (*P* < 0.001). In contrast, BMI was significantly higher in CSX compared to CAD patients (*P* = 0.004).

Table 1: Comparison of age, anthropometric measurements and blood pressures among patients undergoing ECA

	CSX N = 42 Mean (SD) Median (25 – 75 interquartile)	CAD N = 112 Mean (SD) Median (25 – 75 interquartile)	*P*
Age (years)	50.0 (42.5 – 53.3)	55.0 (60.0 – 68.8)	< 0.001*
Weight (Kg)	80.04 (13.77)	72.39 (13.67)	0.004*
Height (Cm)	164.65 (8.33)	165.38 (8.14)	0.640
BMI (kg/m^2)	29.61 (5.06)	26.32 (4.52)	0.001*
SBP (mmHg)	132.83 (20.16)	129.41 (22.86)	0.379
DBP (mmHg)	79.29 (11.99)	78.21 (11.94)	0.627
MABP (mmHg)	97.14 (12.82)	95.28 (13.92)	0.446

Univariate analysis of chest pain characteristics among patients undergoing coronary angiography revealed that chest pain is less likely to radiate to the neck (OR = 0.44, 95% CI = 0.21 – 0.91, *P* = 0.027) and the back (OR = 0.48, 95% CI = 0.23 – 1.00, *P* = 0.049) in patients with CAD (Table 2). In addition, presence of shortness of breathing and / or dizziness significantly decrease the odds of having abnormal coronary angiography (OR = 0.30 and 0.48, 95% CI = 0.12 – 0.77 and 0.22 – 0.92, *P* = 0.013 and 0.030 respectively).

Table 2: Univariate analyses of pain in patients undergoing ECA

	OR	95% CI	*P*
Duration since first chest pain attack > 2years	0.86	0.39 – 1.86	0.695
Duration of pain attack > 30 minutes	0.95	0.38 – 2.39	0.950
Severity of pain	0.92	0.56 – 1.52	0.750
Pain radiation			
Retrosternal	1.24	0.51 – 2.00	0.631
Left sided chest pain	0.70	0.32 – 1.55	0.378
Radiation of pain to the neck	0.44	0.21 – 0.91	0.027*
Radiation of pain to the right shoulder	0.74	0.35 – 1.53	0.410
Radiation of pain to the left shoulder	0.52	0.25 – 1.09	0.082
Radiation of pain to the right upper limb	1.19	0.52 – 2.70	0.686
Radiation of pain to the left upper limb	0.64	0.31 – 1.32	0.230
Radiation of pain to the Back	0.48	0.23 – 1.00	0.049*
Radiation of pain to other areas	1.16	0.55 – 2.42	0.702
Aggravating factors			
Exercise	1.34	0.57 – 3.15	0.503
Cold	0.56	0.26 – 1.23	0.148
Food intake	1.05	0.46 – 2.42	0.902
Others	0.75	0.13 – 2.24	0.743
Relieving factors			
Rest	1.07	0.35 – 3.25	0.905
Sublingual nitrates	1.05	0.52 – 2.12	0.883
Others	1.64	0.44 – 6.14	0.461
Associated factors			
Sweating	0.61	0.27 – 1.39	0.242
Nausea	0.80	0.38 – 1.67	0.552
Vomiting	0.92	0.42 – 2.02	0.841
Palpitation	0.58	0.28 – 1.20	0.143
Shortness of breathing	0.30	0.12 – 0.77	0.013*
Dizziness	0.45	0.22 – 0.92	0.030*
Loss of consciousness	0.84	0.34 – 2.11	0.714

Table 3 shows the results of univariate analyses of past medical and socioeconomic history in patients undergoing coronary angiography. Past history of diabetes mellitus significantly increases the odds of having abnormal coronary angiography (OR = 3.96, 95% CI = 1.68 – 9.30, P = 0.002). In contrast, past medical history of migraine decreases the odds

of having positive finding in ECA (OR = 0.31, 95% CI = 0.13 – 0.72, P = 0.006).

Table 3: Univariate analyses of past medical and socioeconomic history for patients undergoing ECA

	OR	95% CI	*P*
Past medical history			
Hypertension	1.29	0.63 – 2.63	0.490
Diabetes mellitus	3.96	1.68 – 9.30	0.002*
Peptic ulcer	0.45	0.11 – 1.76	0.249
Esophageal disease	0.50	0.23 – 1.07	0.072
Respiratory disease	0.87	0.21 – 3.52	0.841
Migraine	0.31	0.13 – 0.72	0.006*
Raynaud's phenomenon	0.37	0.02 – 6.01	0.482
Chronic inflammatory disease	0.58	0.18 – 1.89	0.366
Socioeconomic history			
Smoking			
- Active smoking	1.64	0.80 – 3.39	0.178
- Passive smoking	1.20	0.55 – 2.64	0.650
Socioeconomic status	0.97	0.54 – 1.76	0.923

Discussion

Univariate analysis of pain characteristics, namely onset and duration of pain attacks, severity, radiations, aggravating and relieving factors reveals only a few predictors for positive coronary angiography findings in patient undergoing ECA because of typical chest pain. According to the current results, distributions of chest pain are comparable in both studied groups. However, pain is less likely to radiate to the neck and/or the back in patients with CAD. Also, presence of shortness of breathing and/or dizziness significantly decreases the odds of having abnormal coronary angiography. Although previous reports on CSX patients suggest enhancement of their pain perception [10], the current study failed to demonstrate a significant difference in pain severity, duration or aggravating factors when CAD and CSX patients were compared. The special attention paid by cardiologists while evaluating the need of patients with chest pain for diagnostic coronary angiography may explain the limited difference in chest pain characteristics of studied groups. This is because typical features of angina are carefully evaluated by cardiologists while selecting patients who are in real need of diagnostic coronary angiography.

At least two previous studies explain the radiation of chest pain in patients with CSX [11], [12]. The first study was conducted by Lanza and his group in the late nineties of the last century [12]. Lanza *et al.* demonstrated cardiac adrenergic nerve dysfunction in 75% of patients with CSX patients suggesting cardiac origin of chest pain in these patients. Five years later, Rosen *et al.* used positron emission tomography (PET) and stress echocardiography studies to assess origin of pain in patients with CSX [11]. According to Rosen *et al.,* results, chest pain and ECG changes during attacks were not accompanied by demonstrable myocardial dysfunction. However, there was altered central neural handling of afferent signals suggesting that CSX might be a cortical pain syndrome. The hypotheses suggested by either Lanza *et al.,* or Rosen *et al.* can explain the great similarities in the areas of chest pain radiation in patients with CSX and CAD targeted by this study.

According to the results of the present study, the presence of dyspnea and dizziness in patients with typical chest pain significantly increases the probability of having normal rather than abnormal coronary angiography. This finding is not necessarily contradictory to what was reported before that dyspnea and dizziness are common during angina attacks of CAD patients [13], [14]. In contrast, it may indicate the higher frequency of these symptoms in patients with a separate pathology that causes typical angina in the presence of patent coronary vessels. Patients with CSX are at higher risk of neuroticism like anxiety and depression [15], [16]. Neuroticism could perpetuate to somatoform disorder with physical symptoms like shortness of breathing and dizziness; however, however, the present literature lacks scientific proof for this hypothesis and further researches are desirable to explore this area.

It is worth mentioning that previous studies suggested that patients with angina and normal coronary angiography may have a diffuse disorder of smooth muscle tone [17]. Cannon *et al.,* studied forced expiratory volume in the first second (FEV1) in the basal state and after methacholine inhalation to determine whether the bronchial smooth muscle is affected in CSX patients [18]. Fourteen per cent of patients with CSX had a basal FEV1 of less than 70% of that predicted and did not receive methacholine. Also, the product of the methacholine dose inhaled and the magnitude of decline in FEV1 from baseline was significantly lower in patients with CSX than in normal volunteers suggesting airway smooth muscle hyperresponsiveness in CSX patients. Cannon *et al.* findings may explain the shortness of breathing in patients with angina and normal coronary angiography during chest pain attacks. This is because the initiator of microvascular smooth muscle dysfunction, and hence angina, may at the same time trigger shortness of breathing by inducing airway hyperresponsiveness.

Previous researches that compare features of chest pain in patients with obstructive, non-obstructive and normal coronary vessels are scarce [19], [20]. The current study findings on the major differences of chest pain in patients classified according to ECA outcome are probably naïve and deserved to be explored further by additional researches.

Evaluation of past medical history in patients undergoing ECA shows that the odds of having CAD increases about four times in the presence of diabetes mellitus but decreases to about one-third if migraine exists. The results of the present study failed to link positive angiographic finding and common CAD risk factors like past medical history of hypertension [21], [22], Raynaud's phenomenon [23], chronic inflammatory diseases [24], smoking [25] and

socioeconomic status [26]. The current data also failed to demonstrate decreased odds of having positive angiographic finding in patients with past medical history of peptic ulcer, oesophagal or respiratory diseases, whose clinical presentations may mimic angina pain [27], [28].

The prevalence of migraine is significantly increased in either subject with spastic or normal coronary arteries compared with patients with CAD [29], [30]. Koh *et al.* conducted a prospective study on the prevalence of migraine in Korean patients with proven variant angina compared to two control groups: one group with CAD and another one with subjects without heart disease [30]. According to the results of Koh *et al.*, migraine was diagnosed in 40.0%, 20.0% and 38.7% of patients with variant angina, CAD and no heart diseases respectively. Five years later, a comparable study was conducted by Nakamura *et al.,* in Japanese [29]. The data of Nakamura *et al.,* showed that the prevalence of migraine in Japanese patients with vasospastic angina, angina with effort and subjects without known ischemic heart disease are 23%, 4% and 11% respectively. Although it is evident from the works of Koh *et* al., and Nakamura *et al.,* that there is possible link between migraine and normal coronary arteries, gender and age-specific prevalence of migraine mismatch with that of CAD, which could explain low risk of CAD in migraineurs [23]. This possible explanation for low prevalence of migraine among CAD patients warrants additional investigations and studies.

In conclusion, characteristics of chest pain are comparable in CAD and CSX, however, pain is less likely to radiate to the neck and / or the back in the first group. Presence of shortness of breathing and / or dizziness during angina attacks as well as history of migraine significantly decreases the odds of having abnormal coronary angiography.

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Clinical Science

Value of Combination of Standard Axial and Thin-Section Coronal Diffusion-weighted Imaging in Diagnosis of Acute Brainstem Infarction

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Abstract

AIM: To determine the value of the combination of thin-section 3 mm coronal and standard axial DWI and their impact in facilitating the diagnosis of acute brainstem infarction.

METHODS: A cross-sectional study conducted from the 1st of April 2017 to the end of February 2018 on 100 consecutive patients (66% were male, and 34% were female) with isolated acute ischemic infarction in the brainstem. The abnormal MRI findings concerning the ischemic lesions were interpreted on standard axial 5 mm and thin-section coronal 3mm DWI.

RESULTS: The mean age of the studied group was 69.2 ± 4.3 for male and 72.3 ± 2.5 years. The standard axial DWI can diagnose 20%, 6.7% and 6.7% of the infarctions in midbrain, pons and medulla oblongata respectively, while both axial and thin coronal sections together can diagnose 80% of midbrain infarctions, 93.3% of pons infarctions and 93.3% of medulla oblongata infarctions. Furthermore, the thin section coronal 3 mm section can diagnose very smaller ischemic lesion volume in comparison to the standard axial 5mm section (3.4 ± 0.45 / cm^3 versus 4.6 ± 0.23 / cm^3, P < 0.001)

CONCLUSION: The addition of thin-section coronal DWI can facilitate the detection of brainstem ischemic lesions. We suggest its inclusion in the stroke MRI protocol.

Introduction

The detection of acute brainstem ischemic infarction remains a challenge to both neurologist and radiologist in spite of the revolution of the imaging techniques. This type of infarction forms just about 10% of all acute ischemic strokes [1]. Pons is the most frequent site, followed by the medulla oblongata, and the midbrain [2]. Cranial nerve palsies, sensory loss, motor hemiparesis, vertigo, ataxia, and other specific brainstem syndromes represent the variable clinical presentation of this sort of infarction [1]. Vertebral arteries, basilar artery, anterior spinal artery, anterior inferior cerebellar arteries, posterior inferior cerebellar arteries, posterior cerebral arteries, and superior cerebellar arteries compromise the chief arterial supply of the brainstem [3]. Most frequent causes of brainstem infarction include large vessel disease of the vertebral arteries or basilar artery, small vessel disease of small perforating arteries, and cardiac thromboembolism [1]. For early diagnosis of brain small ischemic lesions, Diffusion-weighted imaging (DWI) regarded the highly sensitive method [4]. Accordingly, DWI becomes a good method to recognise and achieve the detection of acute brainstem infarction [5]. However, false-negative DWI is higher in the infratentorial due to the limited spatial resolution with standard axial 5 mm DWI [6], [7].

In this study, we attempted to assess the added benefit of combined standard axial and additional thin-section 3mm coronal DWI for the discovery of brainstem infarction.

Patients and Methods

Study design and setting

All patients with isolated acute ischemic infarction in the brainstem diagnosed by MRI in our hospital from the 1st of April 2016 to the end of February 2018 were included in this prospective cross-sectional study.

MRI protocols

Magnetic resonance imaging was performed on 3-Tesla MR system (Achieva, Phillips, Holland). Diffusion-weighted imaging using axial standard 5mm section and thin coronal section 3 mm, in addition to T1, T2, FLAIR and ADC sequences were performed on each patient. Table 1 exhibited the parameters of applied DWI sequences.

Table 1: Weighting imaging applied at the department of MR scanner

DWI sequence	Parameters	3-T Philips Achieva
Axial	FOV	230 x 230
	Matrix	152 x 121
	ST	5 mm
	Number of slices	24
	TR	3937
	TE	114
	b Value	0, 1,000
Coronal	FOV	230 x 230
	Matrix	152 x 152
	ST	3 mm
	Number of slices	26
	TR	4043
	TE	109
	b Value	0, 1,000

FOV: Field of view (mm x mm); ST: Slice thickness (mm); TR: repetition time (ms); TE: echo time(ms); b values (s/mm^2).

Images interpretation

The abnormal hyperintense signals in the brainstem were analysed on standard axial and thin-section coronal DWI. According to their site, the detected lesions were classified into (1) midbrain lesions; (2) pons lesions; and (3) medulla oblongata lesions. The discovery of ischemic lesions in standard axial and thin-section coronal DWI was independently assessed by two efficient specialized radiologists with more than 10 years' experience in brain imaging who were blinded to the clinical data and recognized as (1) better depiction on axial DWI; (2) better depiction on combined axial and thin section coronal DWI. Equivocal cases were reassessed by both readers and discussed until a unique decision was achieved. The size of the ischemic lesion was calculated on DWI by manually drawn ROI. The volume (cm^3) of each detected lesion was calculated by summation of the lesion areas (cm^2) on each section and multiplied by the slice thickness and inter-slice gap. This was achieved by the use of Radiant Dicom Viewer.

Ethical concern

Approval by the local institutional review board was obtained before the study commencement. The purpose and procedures explained to all participants and were given the right to participate or not; verbal consent was taken with the reassurance that interprets gained will be kept confidentially and not be used for another research object.

Statistical analysis

All patients' data entered using computerised statistical software; Statistical Package for Social Sciences (SPSS) version 21 was used. Descriptive statistics presented as (mean ± standard deviation) and frequencies as percentages. Kolmogorov Smirnov analysis verified the normality of the data set. Multiple contingency tables conducted and appropriate statistical tests performed, Chi-square test used for categorical variables. In all statistical analysis, level of significance (*P*-value) set at ≤ .05, and the result presented as tables.

Results

One hundred patients with isolated brainstem ischemia were enrolled in the current study. Males constitute 66% of the study sample, while females constitute 34% with a male to female ratio 1.9:1. The mean age of the studied group was 69.2 ± 4.3 for males and 72.3 ± 2.5 years for females. The main age group studied was 60-69 years (40%) then ≥ 70 years (30%), then 50-59 years (16%) and the last group was 40-49 years (14%).

Table 2: Distribution of brainstem infarction site according to the section of DWI sequence

	Midbrain	Pons	Medulla oblongata	
	No. (%)	No. (%)	No. (%)	*P*
Axial standard DWI section	2 (20.0)	4 (6.7)	2 (6.7)	
Both thin section coronal and standard axial section DWI	8 (80.0)	56 (93.3)	28 (93.3)	0.840
Total	10 (100)	60 (100)	30 (100)	

We identified 60 (60%) of the brainstem ischemia were located in the pons, 30 (30%) of the ischemia in the medulla oblongata and 10 (10%) in the midbrain.

Regarding the gender, it was found about 6 (60%) of the midbrain infarction were in male, and 4 (40%) were in female, same per cent were found for

pons 60% for male and 40% for female, while 80% of the infarctions located in medulla oblongata were in males and the rest 20% were in females.

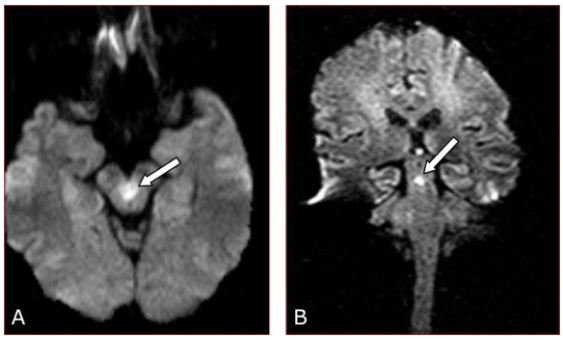

Figure 1: Acute midline midbrain infarction in a patient aged 60 years suffer from ataxia and vision problems; A) Standard axial-section 5 mm DWI; B) Thin-section coronal 3 mm DWI. The lesion is more delineated in the latter sequence image

Our results show that 92.0% of the lesions were easily seen by both axial section and thin coronal section sequence of DWI, and only 8% can be seen easily by axial section DWI.

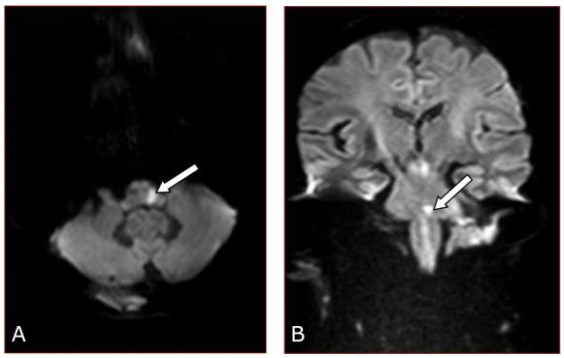

Figure 2: Acute left pons infarction in a patient aged 70 years suffered from dysphasia; A) Standard axial-section 5 mm DWI; B) Thin-section coronal 3 mm DWI. The lesion is more delineated in the latter sequence image

The standard axial DWI can diagnose 20%, 6.7% and 6.7% of the infarctions in midbrain, pons and medulla oblongata respectively, while both axial and thin coronal sections together can diagnose 80% of midbrain infarctions, 93.3% of pons infarctions and 93.3% of medulla oblongata infarctions (Table 2) (Figures 1, 2, and 3).

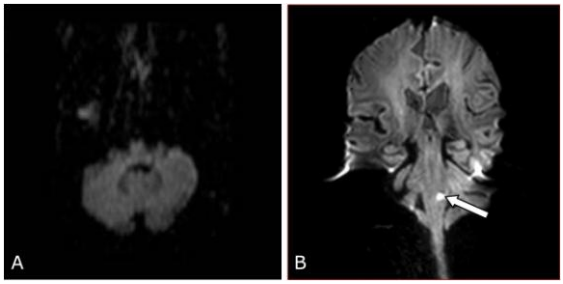

Figure 3: Acute ischemic infarction in the left medulla oblongata in a patient aged 62 years suffered from hemiplegia; A) On standard axial 5 mm DWI, the ischemic lesion is not visible; B) On thin-section coronal 3 mm DWI, the ischemic lesion is delineated

There was a non-significant association (P = 0.840) between the sequence of DWI and the site of the brainstem infarction where pons and medulla oblongata infarctions were seen better by combining of axial and thin coronal section of DWI sequence.

Highly significant association were found between the sequence of DWI and detection of the ischemic lesion were thin coronal section can diagnose very smaller ischemic lesion volume in comparison to the standard axial 5mm section (3.4 ± 0.45 / cm^3 versus 4.6 ± 0.23 / cm^3, P < 0.001) (Table 3).

Table 3: Correlation and evaluation of ischemic lesion volume to the section of the DWI sequence

	Mean ischemic lesion volume / cm^3	*P*
Standard axial section DWI	4.6 ± 0.23	
Thin section coronal DWI	3.4 ± 0.45	< 0.001

Discussion

Brainstem infarctions are usually small in size, round or oval, and demarcated from the adjacent tissue. Also, the tissue mainly consists of white matter and devoid of cerebrospinal fluid space. The heterogeneity of the tissue and the presence of CSF space in the tissue can affect the MRI indices [8]. Tiny ischemic lesion in the brainstem may cause important clinical symptoms due to its relatively small size and hardly arranged tissue composition. However, the diagnosis of brainstem infarction is incomplete if depend only on clinical bases [9]. Wardlaw et al., [4] mentioned that the gold standard for detection of acute ischemic stroke is the diffusion-weighted imaging. But DWI may fail to find the pathology of the very tiny brainstem ischemic lesion particularly located in the posterior fossa [6], [10], [11]. Besides, some studies establish high false-negative results particularly within the first 24 h [6], [12], [13]. To overcome these limitations, additional thin-section coronal DWI of the infratentorial has been proposed [14], [15].

The current study was showing regarding the gender that male was more than female represented about 2 / 3 of cases. Moreover, the mean age of the male was 69.2 ± 4.3 years, and the female were 72.3 ± 2.5. This is in agreement with Irimie CA et al., [15] which demonstrate the gender distribution of ischemic stroke risk factors in psychiatry and neurology hospital who mentioned similar findings.

In a study done by Felfeli P et al., [16] in 2017, only 2% of cases of acute brainstem infarction were detected by thin-section coronal DWI. Our study showed that (92.0%) of patients were easily seen by both sequence (standard axial and added thin coronal section) of DWI, and only (8%) can be easily seen by

axial DWI section. Although the detection rate still better by combining standard axial section and thin coronal section DWI sequences but from the volumetric statistical point of view lesions are seen more obvious by thin coronal section in which (mean ischemic lesion volume/cm^3) were 3.4 ± 0.45 compared to that measurement by standard axial section 4.6 ± 0.23 with *P* value of < 0.001 which is statistically significant.

Misdiagnosis of small brainstem ischemic lesion in an individual patient may have dangerous outcomes such as recurrent stroke and death [17]. Furthermore, very small ischemic lesions in the brainstem were much better identifiable on thin-section coronal DWI in comparison to standard axial section DWI from the volumetric statistical point. Therefore, for the discovery of very small brainstem infarctions, thin-section coronal DWI might be of help.

The current study results showed that combined coronal and axial DWI could better demonstrate the ischemic lesion of the midbrain in 8 (80%), of the pons in 56 (93.3%) and the medulla oblongata in 28 (93.3%). These values were more than that found in Felfeli P et al., [16] study where the thin-section coronal DWI was better identified the ischemic lesions of the midbrain in 8.6%, of the pons in 45.7%, and the medulla oblongata in 45.7%.

Highly significant association were found between the sequence of DWI in the study (thin coronal and standard axial sections) and ischemic size lesion (*P* < 0.001).

The results of this study would clarify the opportunity of early identification of brainstem ischemia if we added thin-section coronal DWI to the stroke MRI protocol. This point is of high clinical consequence for two reasons. First, as the diagnosis of brainstem infarction is imperfect if based exclusively on clinical grounds, this early and clear identification of ischemia allows easy differentiation of similar clinical syndromes. Second, quick detection of brainstem infarction could be a significant clue to a basilar thrombosis at an early stage that would manipulate additional therapeutic management.

Two main limitations associated with this study. First, this is a single institutional study. Second, a consensus reading instead of following up was the main determinant of the acute ischemic lesions.

In conclusion, the combination of both coronal and axial DWI section can be done from 2 points of views. First, the further information which can achieve by adding thin-section coronal DWI sequence is additive value especially when some of the lesions cannot be seen by standard axial section DWI sequence in the presence of strong clinical suspicion. Second, according to statistical analysis, the detection rate is superior in combined standard axial and thin section coronal DWI sequence, but using coronal thin-section DWI would be more useful from the volumetric point of analysis. Accordingly, we advise the inclusion of thin-section coronal DWI sequence in standard stroke MRI protocols for patients with suspected stroke in the posterior fossa, because this combination can probably facilitate the discovery of brainstem infarction.

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Differences in Total Score of Positive and Negative Syndrome Scale between Bataknese and Javanese Men with Schizophrenia Receiving Risperidone Treatment

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Abstract

BACKGROUND: Schizophrenia is a psychopathological syndrome clinic involving cognition, emotion, perception and other aspects from the individual which interferences. This interference is normally started before age 25, and it can affect all social classes.

AIM: To find out the difference in total positive and negative syndrome scale (PANSS) scores in schizophrenia between Bataknese and Javanese who have received treatment with risperidone.

METHODS: This study is a prospective study. This study used numeric comparative analytic two unpaired groups to observe the differences of PANSS score of the man with schizophrenia between Bataknese and Javanese who had received risperidone treatment.

RESULTS: Our study found that the average score of PANSS for Bataknese was 49.76 ± 12.65 and Javanese was 42.43 ± 9.05.

CONCLUSION: There was a difference score of PANSS for the man with schizophrenia between Bataknese and Javanese who had received risperidone treatment for 6 weeks (p = 0.037).

Introduction

Schizophrenia is a disturbing clinical syndrome of psychopathology that involves cognition, emotions, perceptions, and other aspects of behaviour. This disorder usually starts before the age of 25 and can attack people from various social groups [1]. According to Burroughs, race and ethnicity have different responses to treatment. The differences of response in race and ethnicity to the treatment are influenced by several factors including biological factors (age, gender, genetics, illness), cultural factors (attitudes, beliefs, family influences), and environmental factors (climate, parasites, pollutants, smoking, alcohol, medicines) [2].

Clinical studies and multicentre trials carried out by Buckley in Cleveland conclude that risperidone is an antipsychotic that effectively improves positive and negative symptoms in schizophrenia globally. The study compared risperidone and conventional antipsychotics (chlorpromazine) at the mean ± SD dose of risperidone at 6.8 ± 2.0 mg and equivalent dose of chlorpromazine at 1.295 ± 789 mg to improve positive and negative symptoms in schizophrenia [3]. Genetics has been known to affect the pharmacokinetics and pharmacodynamics of antipsychotic drugs. This is based on the time separation in the evolutionary trends in Africa. For example, genetics in Orientals and Caucasians are related to the cytochrome P450 enzymes (CYP 450), which have a relationship in the metabolism of psychotropic drugs in humans.

Recent studies on the pharmacokinetics and pharmacodynamics of antipsychotic drugs and sociocultural factors that influence the response of

antipsychotic drugs consistently provide racial differences in response to antipsychotic drugs [4]. Ethnicity also plays a role in responding to risperidone treatment among children and adolescents with the schizophrenic disorder [5]. In an experimental study by Sianturi [6] in Indonesia, there was a difference in the effectiveness of risperidone and haloperidol on the positive symptoms of schizophrenic patients in the Positive and Negative Syndrome Scale (PANSS) at the fourth week. The results showed that the mean difference of the PANSS positive scores for the risperidone therapy group was 13.1 (SD ± 3.4) whereas the mean difference of the PANSS positive scores for the haloperidol therapy group was 10.4 (SD ± 1.9), P = 0.005 (P < 0.05). There was also a difference in the PANSS positive delta scores based on the intervention groups [6]. Furthermore, a pilot study conducted by Frackiewicz *et al.,* [7] found that there was a significant difference in the PANSS scores between 10 Hispanic schizophrenic in-patients and 8 non-Hispanic schizophrenic in-patients who received risperidone (p < 0.02).

Men with schizophrenia usually experience a much earlier age onset. They tend to have primary negative symptoms associated with the occurrence of chronicity which is a large clinical case [8]. Schizophrenia is defined as a disorder that lasts for six months or more, in which the person has the notion, hallucinations, irregular speech, catatonic behaviour, or negative symptoms for at least one month [9]. Recent studies estimate that socioeconomic status might influence health risk factors of schizophrenic people to obesity and diabetes because lower middle class tend to consume excess calories [10]. The PANSS has become a standard tool for assessing clinical outcomes in the treatment studies of schizophrenia and other psychotic disorders and has been proven to be easily administered, reliable, and sensitive to changes with treatment. The PANSS scores of in-patients rarely exceed 80-150 [11].

Based on the information obtained from the first century AD, the Bataknese tribe originated from Greece and Chinese [12]. Indonesia has various ethnic groups, and the distribution of the ethnic groups is Sundanese with 36.7 million people (15.5%), Bataknese with 8.5 million people (3.6%), and other ethnic groups from Sulawesi with 7.6 million people (3.2%), and others [13]. The Bataknese people are known for their openness, spontaneity, and aggressiveness both physically and verbally. Previous research found that Bataknese people often choose to express their anger when they are angry while Javanese people tend to hide their anger [14]. Thus, Bataknese people mostly have low emotional regulation whereas Javanese people have polite behaviour in their speech and self-presentation by showing respect for others [15], [16]. Risperidone is effective for various psychopathology of schizophrenia and will be more effective than the First-Generation Antipsychotics (FGA).

Risperidone has been widely used and becomes the highest number of antipsychotics in the world. Risperidone has a high affinity for dopamine 2 (D2) and serotonin 2A (5-HT2A) receptors [17]. Differences between ethnic groups in drug metabolic enzymes and transporters have the potential to cause variability in the dose choice [18]. Allele gene polymorphisms that highly depend on ethnicity have a major role in the functions of 2D6, 2C19, 2C9, 2B6, 3A5, and 2A6 CYPs and lead to different pharmacogenetics phenotypes [19].

Bakare stated that genetics influence the pharmacokinetics and pharmacodynamics of antipsychotic drugs, but Patel reported that there were significant CBCL differences between African-American, Hispanic, but not Caucasian races. Also, Frackiewicz stated that racial factors affect the improvement of PANSS symptoms. However, there have been no previous studies in Indonesia that examined the relationship between ethnicity and risperidone effectiveness in men with schizophrenia. Therefore, the researchers were interested in conducting this research.

The present study was the first study conducted in Indonesia, especially in North Sumatra, which examined PANSS total score differences in men with schizophrenia between Bataknese and Javanese ethnic groups. To find out the sample size, therefore, a preliminary study was carried out from July to September 2017 by recruiting 20 subjects with 10 Bataknese and Javanese, respectively.

Methods

The present study was an observational study through a prospective cohort approach. This study was an unpaired numerical comparative analytical study of two groups to determine the difference in the PANSS total scores between Bataknese and Javanese men with schizophrenia who had received 6 mg of risperidone for 6 weeks. Subjects in this study were determined by consecutive sampling, which is a non-probability sampling type. The inclusion criteria in this study were male patients who had been diagnosed with schizophrenia at the Inpatient Installation in Prof. Moh. Ildrem Mental Hospital Medan North Sumatra. The patients with age 15-40 years received a fixed dose of 6 mg of risperidone per day and had the PANSS total score of 80-120. Also, the subjects had an inappropriate affect and dysphoric mood, cooperative (willing to be the research subject), normal body mass index, no history of liver disease and had a good liver function, had insight I or II when patients received 6 mg of risperidone per day. The exclusion criteria were having a history of general

medical illness and the use of prohibited substances except for nicotine and caffeine, having a family history of schizophrenia, and having a disease and history of kidney disorders.

The calculation of the sample size giving the highest number was 21 subjects for the Javanese ethnic group and 21 subjects for the Bataknese ethnic group. Data collection was preceded by finding patients from Bataknese and Javanese ethnicity who were diagnosed with schizophrenia in Prof. Ildrem Mental Hospital and received a fixed-dose at 6 mg of risperidone (in the first week of treatment). Structured interviews using MINI ICD-10 was performed to find people with schizophrenia determined by diagnostic guidelines based on PPDGJI-III. Screening using inclusion and exclusion criteria was done to determine the research subjects. In the interviews, several information was also collected from the patients including the age of the first attack, duration of attack, number of attacks, insight, education level, socioeconomic level, personality of the patients before diagnosed with schizophrenia, family history, and history of liver disease and liver function of patients from laboratory tests. Men with schizophrenia from Bataknese and Javanese ethnicity who fulfilled the inclusion criteria filled out written approval after getting detailed and clear explanations to participate in the study until the number of subjects in each Javanese and Bataknese group was 21 patients. Then, the PANSS total score of both Bataknese and Javanese patients who fulfilled the inclusion criteria and had received 6 mg of risperidone (first week of treatment) was measured. The patients were observed until the sixth week. After the sixth week of treatment, the PANSS total score was measured again. The PANSS total scores of Bataknese and Javanese male patients with schizophrenia in the sixth week were analysed to see if there were differences between the Bataknese and Javanese subjects. The research results were reported and presented in a table. Test analysis was performed on the PANSS total scores of the Bataknese and Javanese subjects. Data normality was tested using the Shapiro-Wilk test because the number of samples was 21 subjects for each Bataknese and Javanese group (less 50). The PANSS total score of the Bataknese and Javanese subjects after receiving 6 mg of risperidone for 6 weeks was normally distributed so that the analysis was continued with an unpaired t-test. The PANSS total scores of the Bataknese and Javanese subjects are shown in the mean and standard deviation.

Results

The difference in the PANSS total scores between the Bataknese and Javanese subjects is shown in Table 1.

Table 1: Difference in the Total PANSS Score between the Bataknese and Javanese subjects in the sixth week

PANSS total score of Javanese subjects	PANSS total score of Bataknese subjects
50	44
34	58
41	62
40	41
57	58
60	66
48	62
44	68
54	53
57	63
Mean ± Standard Deviation = 48.50 ± 8.618	Mean ± Standard Deviation = 57.50 ± 8.997

The characteristics of the research subjects can be seen in Table 2. Based on the age, the median (minimum-maximum) age of the Javanese subjects was 37.00 (26-40) years, whereas the median (minimum-maximum) age of the Bataknese subjects was 36.00 (24-40) years. Based on the education level, 7 Javanese subjects (33.3%) graduated middle school, 13 Javanese subjects (61.9%) graduated high school, and 1 Javanese subject (4.8%) graduated university. Similarly, 8 Bataknese subjects (38.1%) graduated middle school, 12 Bataknese subjects (57.1%) graduated high school, and 1 Bataknese subject (4.8%) graduated university. Regarding the employment status, 37 subjects (88.09%) were unemployed, and 5 subjects (11.91%) were employed from both groups. In the Javanese ethnic group, 1 subject (4.8%) was employed, and 20 subjects (95.2%) were unemployed. Similarly, in the Bataknese ethnic group, 4 subjects (19%) were employed, and 17 subjects (81%) were unemployed. The median (minimum-maximum) duration of illness of the Javanese subjects was 4.00 (1-5) years whereas the median duration of illness of the Bataknese subjects was 3.00 (1-5) years. Regarding the marital status, there were 9 married subjects (42.9%) and 12 unmarried subjects (57.1%) in the Bataknese ethnic group whereas there were 5 married subjects (23.8%) and 16 unmarried subjects (76.2%) in the Javanese ethnic group. The mean body mass index of the Javanese subjects was 22.50 ± 1.30 Kg/m^2 while the mean body mass index of the Bataknese subjects was 21.64 ± 1.57 Kg/m^2.

The median (minimum-maximum) onset of an attack of the Javanese and Bataknese subjects was 33.00 (24-35) years and 33.00 (23-35) years, respectively. Based on the frequency of attack, 21 Javanese subjects (100%) experienced attacks two times or less. Similarly, 18 Bataknese subjects (85.71%) experienced attacks two times or less, and 3 Bataknese subjects (14.29%) experienced attacks two to five times. In terms of insight, 2 Javanese subjects (9.52%) had insight I and 19 Javanese subjects (90.48%) had insight II whereas Bataknese subjects (52.38%) had insight I and 10 Bataknese subjects (47.62%) had insight II. Regarding the socioeconomic level, 11 subjects (80.95%) Javanese subjects were in the lower class, and 4 Javanese subjects (14.51%) were in the middle class while 16 Bataknese subjects (76.19%) were in the lower class, and 5 Bataknese subjects (23.81%) were in the middle class. The mean

PANSS total scores of the Javanese and Bataknese ethnic groups in the first week receiving a fixed dose of 6 mg of risperidone were 99.10 ± 9.88 and 104.19 ± 12.38, respectively.

Table 2: Distribution of Samples Based on Demographic Characteristics

	Javanese Men with Schizophrenia N (%)	Bataknese Men with Schizophrenia N (%)	p
Age {Median (Minimum-Maximum)}	37.00(26-40)	36.00(24-40)	0.559[b]
Education Level			
Middle school	7 (33.3%)	8 (38.1%)	0.747[c]
High school	13 (61.9%)	12 (57.1%)	
University	1 (4.8%)	1 (4.8%)	
Employment Status			
Employed	1 (4.8%)	4 (19.0%)	0.343[d]
Unemployed	20 (95.2%)	17 (81.0%)	
Marital Status			
Married	5 (23.8%)	9 (42.9%)	0.190[c]
Not married	16 (76.2%)	12 (57.1%)	
Duration of Illness {Median (Minimum-Maximum)}	4,00(1-5)	3,00(1-5)	0,078[b]
Body Mass Index in the first week receiving risperidone with a fixed dose of 6 mg (Mean ± SD)	22.50±1.30	21.64±1.57	0.061[a]
Onset of Illness {Median(Minimum-Maximum)}	33.00(24-35)	33.00(23-35)	0.759[b]
Frequency of Illness			
≤2 times of Illness	21 (100%)	18 (85,71%)	0,232[d]
3-5 times of Illness	-	3 (14,29%)	
>5 times of Illness	-	-	
Socioeconomic Level			
Lower class	17 (80.95%)	16 (76.19%)	1.000[d]
Middle class	4 (14.51%)	5 (23.81%)	
High class	-	-	
Insight			
Insight I	2 (9.5%)	11 (52.4%)	
Insight II	19 (90.5%)	10 (47.6%)	0.003[c]
PANSS Total Score in the first week receiving risperidone with a fixed dose of 6 mg	99.10 ± 9.88	104.19 ± 12.38	0.148[a]

[a]Independent T-Test; [b]Mann-Whitney U; [c]Chi Square Test with Yates correction; [d]Fisher Test.

As seen in Table 3, the mean PANSS total score in the Javanese subjects was 42.43 ± 9.05, whereas the mean PANSS total score in the Bataknese subjects was 49.76 ± 12.65. Based on the study results, there was a difference in the PANSS total scores of men with schizophrenia between the Bataknese and Javanese ethnic groups who had received 6 mg of risperidone treatment for 6 weeks (p = 0.037).

Table 3: The PANSS Total Score of Men with Schizophrenia from Bataknese and Javanese Ethnic Groups

	Javanese Men with Schizophrenia (n = 21)	Bataknese Men with Schizophrenia (n = 21)	P
PANSS total score in the sixth week after receiving 6 mg of risperidone (mean ± SD)	42.43 ± 9.05	49.76 ± 12.65	0.037*

*Independent T-Test.

Discussion

In this study, the median (minimum-maximum) onset of the attack of the Javanese and Bataknese subjects was 33.00 (24-35) and 33.00 (23-35) years, respectively. This is consistent with the statement of the American Psychiatric Association that the psychotic picture of schizophrenia usually appears between the late teens and middle thirties; the onset before adolescence is rare. The peak age during onset for the first psychotic episode was in the early to middle twenties for men and in the late twenties for women. The onset may be sudden or dangerous, but most individuals show a slow and gradual development of various clinically significant signs and symptoms. In a cohort study conducted in Melbourne by Gogtay in subjects at high risk for schizophrenia, the average age of onset was 19 ± 3.5 years and occurred in 58% of men. In the Buoli study, it was stated that the mean onset age of schizophrenia was 23.22 ± 5.97 years [20], [21].

Based on the education level of the Javanese subjects, 7 subjects (33.3%) graduated middle school, 13 subjects (61.9%) graduated high school, and 1 subject (4.8%) graduated university. Similarly, the education level in the Bataknese subjects showed that 8 subjects (38.1%) graduated middle school, 12 subjects (57.1%) graduated high school, and 1 subject (4.8%) graduated university. Most of the subjects for both ethnic groups in this study were at the high school level. This is consistent with the findings of the Arnold study. Similarly, Huang found that most of the schizophrenic patients were in the high school level with 39.5% (n = 43) while 34.7% (n = 38) was in the university level and 25.7% (n = 28) was in the middle school level [22], [23].

In this study, the median (minimum-maximum) duration of illness of the Javanese and Bataknese subjects was 4.00 (1-5) years and 3.00 (1-5) years, respectively. The mean body mass index of the Javanese subjects was 22.50 ± 1.30 Kg/$m^{2,}$ while the mean body mass index of the Bataknese subjects was 21.64 ± 1.57 Kg/m^2. Regarding the employment status, 1 Javanese subject (4.8%) was employed and 20 Javanese subjects (95.2%) were unemployed whereas 4 Bataknese subjects (19%) were employed and 17 Bataknese subjects (81%) were unemployed. This is by the literature [22]. Furthermore, a study by Huang and colleagues showed that there were 46 unemployed schizophrenic patients (42.2%) and 37 employed schizophrenic patients (33.9%) [22].

Based on the marital status, 9 subjects (42.9%) were married, and 12 subjects (57.1%) were not married in the Bataknese ethnic group whereas 5 subjects (23.8%) were married, and 16 subjects (76.2%) were not married in the Javanese ethnic group. This is also by the findings of Arnold and colleagues regarding patients who received antipsychotics [23]. Similarly, Huang and colleagues in China reported that 61 schizophrenic patients (56%) were not married, and 23 schizophrenic patients were married (21.1%) [22].

Regarding the socioeconomic level, 17 Javanese subjects (80.95%) were in the lower class,

and 4 Javanese subjects (14.51%) were in the middle class while 16 Bataknese subjects (76.19%) were in the lower class, and 5 Bataknese subjects (23.81%) were in the middle class. Huang and colleagues in China found that 29 subjects (26.6%) were in the lower class, 62 subjects (56.9%) were in the middle class, and 18 subjects (16.5%) were in the high class among schizophrenic patients [22]. The present study also found that the mean PANSS total score of Javanese subjects was 42.43 ± 9.05 while the mean PANSS total score of Bataknese subjects was 49.76 ± 12.65. Thus, there was a significant difference in the PANSS total score of men with schizophrenia receiving 6 mg of risperidone for 6 weeks between the Javanese and Bataknese ethnic groups ($p = 0.037$). This is consistent with a pilot study conducted by Frackiewicz and colleagues in the United States which found that there was a significant difference in the PANSS score between 10 Hispanic schizophrenic in-patients and 8 non-Hispanic schizophrenic in-patients who received risperidone ($p < 0.02$) [7].

One of the limitations in the current study was that this study did not examine the Bataknese ethnicity more specifically because this ethnicity consists of five sub-ethnicities. In addition, the study also did not explore the cause of the difference in the PANSS total scores between the Javanese and Bataknese subjects in the sixth week after receiving a fixed dose of 6 mg of risperidone, whether it was due to biological factors such as genetic influencing drug pharmacokinetics and pharmacodynamics, environmental factors, or cultural factors. The subjects in this study did not have the same belief because belief is a cultural factor that might influence the effectiveness or adherence to drug therapy [19].

In conclusion, the mean PANSS total score of the Javanese men with schizophrenia was 42.43 ± 9.05 while the mean PANSS total score of the Bataknese men with schizophrenia was 49.76 ± 12.65. Thus, there was a difference in the PANSS total score between Bataknese and Javanese men with schizophrenia in which Javanese patients were more responsive to the 6 mg of risperidone treatment for 6 weeks ($p = 0.037$), so the research hypothesis was accepted.

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Relationship between Vitamin D Level and Serum TNF-α Concentration on the Severity of Chronic Obstructive Pulmonary Disease

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Abstract

BACKGROUND: Chronic Obstructive Pulmonary Disease (COPD) is a chronic inflammatory disease and disturbed bacterial clearance. Vitamin D deficiency is sometimes observed in COPD patients and as significant roles in increasing inflammation of airway obstruction and systemic obstruction, increasing pro-inflammatory cytokine including TNF-α, reduction of bacterial clearance and increase exacerbation risk due to infection. Also, vitamin D plays significant roles in the metabolism of calcium and mineralisation of bones and regulation system of immune. TNF-α also has essential roles in pathogenesis and inflammation of COPD. Several studies that investigate the relationship between vitamin D level and serum TNF-α concentration in COPD patients are relatively uncommon, including in Indonesia.

AIM: This study aimed to assess the relationship between vitamin D level and TNF-α concentration in patients on the severity of the chronic obstructive pulmonary disease.

METHODS: This study was a hospital-based descriptive cross-sectional study. Total samples were 50 COPD patients with the average age of older than 60 years during their enrollments at the Department of Pulmonology and Respiratory Medicine of the Dr Wahidin Sudirohusodo General Hospital Makassar in September 2018-January 2019. All procedures of the present study were reviewed and approved by the Research Ethics Committee of Medicine Faculty of Hasanuddin University. The severity of COPD was assessed according to the combination of COPD assessment stages that referred to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) Guideline 2015 that consisted of the combination of scoring COPD Assessment Test (CAT), the modified Medical Research Council (mMRC) questionnaire and results of the spirometry measurement. Assessment of airway obstruction levels referred to the GOLD spirometry criteria. Determination of thoracic photographs was conducted to verify the COPD diagnosis of the severity of COPD. Determination of serum TNF-α concentration and vitamin D3 [1,25(OH)2D3] level used the ELISA method.

RESULTS: The majority of COPD patients were observed in the category of older than 60 years old accounted for 34 COPD patients (68%), and the majority of COPD patients were males accounted for 47 males with COPD (94%). The majority of COPD patients were observed in the group of D (38%). All the study subjects observed in this study were smokers, and 82% of them were in the category of heavy smokers. 21 study subjects had higher concentration of serum TNF-α (tertile 3 = 0.21-1.83 pg/dl), 20 study subjects and lower level of vitamin D (tertile 1 = 182.1-364.5 pg/dl). The majority of the study subjects (38%) were in the category of severe COPD (category D of the severity of COPD at the tertile 3) according to the GOLD Combine Assessment. Given the relationship between vitamin D level and serum TNF-α concentration on the airway obstruction, there were significant positive correlations between the increase of vitamin D levels and the increase of serum TNF-α concentrations on airway obstruction. Given the relationship between vitamin D level and serum TNF-α concentration on the severity of COPD, there were significant positive correlations between the increase of vitamin D levels (tertiles 1, 2 and 3) and the increase of serum TNF-α concentrations on the severity of COPD at p-value < 0.05. Overall, there were non-linear relationships between vitamin D level and serum TNF-α concentration on the severity of COPD.

CONCLUSIONS: Serum TNF-α concentration was positively associated with airway obstruction level and severity of COPD. Low level of vitamin D was negatively associated with airway obstruction level and severity of COPD. Vitamin D3 level (1,25(OH)2D) was negatively associated with serum TNF-α concentration and airway obstruction level and severity of COPD.

Introduction

Chronic Obstructive Pulmonary Disease (COPD) is a chronic inflammatory disease characterized by persistent airflow limitation of the lungs [1] that includes a number of combinations of asthma bronchitis (spasms or involuntary contraction in the bronchial passages), emphysema (decreased elasticity of the alveoli) and chronic bronchitis

(inflammation of the bronchi) [2], [3]. The mechanistic basis underlying COPD is complex and can involve recurrent inflammation, oxidative stress (i.e., oxidant / antioxidant imbalance), protease/antiprotease imbalance, environmental insult, and host genetics [4].

Cigarette smoking is the primary environmental risk factor for COPD. Also, other environmental (e.g., wood smoke) and occupational exposures, as well as genetics, contribute to COPD pathogenesis. Consequently, pathologic changes and clinical symptoms are linked to the interaction of host factors with the environment. These interactions generate the pathologic triad of COPD: persistent inflammation, protease-antiprotease imbalance, and oxidative stress. This triad results in mucous/goblet cell metaplasia and hyperplasia, hyper mucus secretion, fibrosis, smooth-muscle alterations, and lung-tissue destruction. Chronic smoking exposes the respiratory tree and lungs to reactive oxygen species (ROS), resulting in oxidative stress and injury. This triggers the production of other ROS and lipid peroxidation and subsequent pulmonary inflammation [5], mainly due to infections by bacteria and viruses [5], [6]. Based on a population-based survey in nine Asia-Pacific territories in 2012, the overall estimated prevalence of COPD was 6.2%, ranging from 4.5% in Indonesia to 9.5% in Taiwan [7]. COPD is a significant cause of morbidity and mortality worldwide, and it is the sixth leading cause of death world [8]. The determinants of COPD pathogenesis are not a separate entity by itself where smoking tobacco is the primary environmental risk factor for COPD and other determinants of COPD-associated inflammation related with smoking tobacco that includes reactive oxygen species (ROS), protease-antiprotease imbalance and genetic variations or polymorphisms [5], respiratory viruses and bacteria which infect the lower airway and increase airway inflammation [6].

Low level of vitamin D is sometimes found in patients with COPD. High prevalence vitamin D deficiency in COPD patients is caused by the reduction of synthesis of vitamin D in skin due to ageing, poor diet, low capacity to store at fat related to wasting [9]. Some publications show the inconsistency results about the role of vitamin D and COPD [10]; some studies reported a correlation between vitamin D deficiency and pulmonary function. In the placebo-controlled intervention study, Rafiq et al., [11] revealed that vitamin D had essential roles for the prevention of exacerbations (the increase of severity) in patients with COPD and vitamin D deficiency through vitamin D supplementation. Sank et al., [12] proved the positive relationship between vitamin D deficiency and the severity of Chronic Obstructive Pulmonary Disease.

Despite many mechanistic studies highlighting important anti-inflammatory and anti-infectious effects of vitamin D in laboratory experiments, the clinical evidence in cohorts of patients with COPD remains contradictory. Several studies have shown that vitamin D could regulate activities of immune cells [13], restore respiratory muscle strength and inflammatory responses [14]. In COPD, there is a down-regulation of local signalling of vitamin D, leading to insufficient control of pro-inflammatory processes in airways [15]. COPD is associated with chronic inflammation affecting predominantly lung parenchyma and peripheral airways and results in mostly irreversible and progressive airflow limitation. This inflammation is characterised by increased numbers of alveolar macrophages, neutrophils, and T lymphocytes, which are recruited from the circulation. Oxidative stress plays a crucial role in driving this inflammation. Pulmonary inflammation may enhance the development and growth of lung cancer. The peripheral inflammation extends into the circulation, resulting in systemic inflammation with the same inflammatory proteins. Systemic inflammation may worsen comorbidities. Treatment of pulmonary inflammation may, therefore, have beneficial effects [16].

Multiple cytokines play roles in inflammatory airway diseases, such as COPD, through the recruitment, activation, and survival of inflammatory cells [17]. This is proven from the increase of TNF-α concentration according to the examination of induced sputum and lung biopsy of COPD patients [18]. TNF-α is the most extensively studied cytokine in patients with COPD, and it is a potent activator of NF-κβ and amplifies the inflammatory response. TNF-α plays a significant role in many inflammatory diseases affecting the lung, such as chronic bronchitis (CB), COPD, asthma, acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) [19]. However, studies that investigate the relationship between vitamin D level and serum TNF-α concentration in COPD patients are relatively uncommon, including in Indonesia. For that reason, this study aimed to assess the relationship between vitamin D level and TNF-α concentration in patients on the severity of the chronic obstructive pulmonary disease.

Methods

This study was a hospital-based descriptive cross-sectional study. Total samples were 50 COPD patients with the average age of older than 60 years during their enrollments at the Department of Pulmonology and Respiratory Medicine of the Dr Wahidin Sudirohusodo General Hospital Makassar in September 2018-January 2019. All procedures of the present study were reviewed and approved by the Research Ethics Committee of Medicine Faculty of Hasanuddin University as stated in the Recommendation Letter of Research Ethics issued in the registration No.1052/H.4.8.4.5.31/PP36-KOMETIK/2018.

Inclusion criteria include COPD patients with dyspnea (difficulty in breathing) and chronic cough with sputum. There was a history of exposure of cigarette fog and other irritants, results of diagnosis using the spirometry test at $FEV_1/FVC < 70\%$ and post-bronchodilator (after the use of certain drugs to reduces the tone of smooth muscle in the lungs' bronchioles to increase their diameter) at $FEV_1 < 80\%$. The severity of COPD was assessed according to the combination of COPD assessment stages that referred to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) Guideline 2015 that consisted of the combination of scoring COPD Assessment Test (CAT), the modified Medical Research Council (mMRC) questionnaire and results of the spirometry measurement. Assessment of airway obstruction levels referred to the GOLD spirometry criteria. Determination of thoracic photographs was conducted to verify no other respiratory diseases except COPD. Determination of serum TNF-α concentration and vitamin D_3 [1,25(OH)2D_3] level used the ELISA method (Thermo Scientific Multiscan FC). Exclusion criteria include individuals with asthma, lung tuberculosis, bronchiectasis (dilatation of the bronchi) and patients with COPD in the category of acute exacerbation and the use of oral steroids.

Statistical analysis

The study data were processed and analysed using the Statistical Package for Social Science (SPSS) version 22. The analytical method used in this study was descriptive statistics using frequency distribution, whereas the statistical test used in this study was Kolmogorov-Smirnov's test used to determine the normality of data. Chi-square test was used to assess the strength of the correlation or relationship between two categorical variables. Kruskal-Wallis test was used to compare mean values of numerical data based on the categorical variables of more than two correlated variables. Results of statistical tests were significant when *p*-value < 0.05. Results of data analyses were presented in the form tables and graphs along with their descriptions.

Results

A total number of study subjects were 50 COPD patients who fulfilled the inclusion criteria. The majority of COPD patients were observed in the category of older than 60 years old accounted for 34 COPD patients (68%), and the majority of COPD patients were males considered for 47 males with COPD (94%). The study subjects were statistically distributed. The majority of COPD patients were observed in group D (38%). Vitamin D levels were classified into 3 tertiles, and the most significant frequency of COPD patients was seen at the tertile 1 of vitamin D level accounted for 20 COPD patients (40%). Serum TNF-α concentrations were also classified into three tertiles, and the most considerable frequency of COPD patients was noticed at the tertile 3 of serum TNF-α level accounted for 21 COPD patients (42%). Baseline characteristics of the study subjects are shown in Table 1.

Table 1: Baseline characteristics of the study subjects with COPD

Variable		n	%
Gender	Male	47	94.0
	Female	3	6.0
Age	40-59 years old	16	32.0
	≥ 60 years old	34	68.0
Smoking status	Mild	1	2.0
	Moderate	8	16.0
	Severe	41	82.0
Serum TNF-α concentration[a]	Tertile 1	12	24.0
	Tertile 2	17	34.0
	Tertile 3	21	42.0
Vitamin D Level[b]	Tertile 1	20	40.0
	Tertile 2	15	30.0
	Tertile 3	15	30.0
Severity of COPD	A	5	10.0
	B	18	36.0
	C	8	16.0
	D	19	38.0

[a] Tertile 1 (0. 00-0.06 pg/dl); Tertile 2 (0.07-0.20 pg/dl); Tertil 3 (0.21-1.83 pg/dl); [b] Tertile 1 (182. 1-364.5 pg/mL); Tertile 2 (364.7-630.7 pg/mL); Tertile 3 (632.6-3707.2 pg/mL).

TNF-α concentrations at the tertile 1 were dominant for COPD patients in the groups A and B (24%). TNF-α levels at the tertile 2 were prevailing for COPD patients in the groups B and C (34%) whereas TNF-α levels were dominant at the tertile 3 for COPD patients in the group D (42%).

Table 2: Frequency distribution of severity of COPD according to TNF-α level

Serum TNF-α Level			Severity of COPD				Total
			A	B	C	D	
	Tertile 1	n	3	9	0	0	12
		%	25.0%	75.0%	0.0%	0.0%	100.0%
	Tertile 2	n	2	9	6	0	17
		%	11.8%	52.9%	35.3%	0.0%	100.0%
	Tertile 3	n	0	0	2	19	21
		%	0.0%	0.0%	9.5%	90.5%	100.0%
Total		n	5	18	8	19	50
		%	10.0%	36.0%	16.0%	38.0%	100.0%

Relationship between vitamin D level and serum TNF-α concentration on the airway obstruction

Table 3 shows the statistical correlations of vitamin D level and serum TNF-α concentration on airway obstruction. There were significant positive correlations between the increase of vitamin D levels and the increase of serum TNF-α concentrations on airway obstruction according to the rise of GOLD values ($p < 0.05$) at the tertile 1 of vitamin D levels with the highest concentration of serum TNF-α at the tertile 1 was 0.24 pg/dl. There were also significant positive correlations between the increase of vitamin D levels and the increase of serum TNF-α concentrations on airway obstruction according to the rise in GOLD values ($p < 0.05$) at the tertile 2 of

vitamin D levels with the highest concentration of serum TNF-α at the tertile 2 was 0.93 pg/dl. In addition, there were positive significant correlations between the increase of vitamin D levels and the increase of serum TNF-α concentrations on airway obstruction according to the rise in GOLD values ($p < 0.05$) at the tertile 3 of vitamin D levels with the highest concentration of serum TNF-α at the tertile 3 was 0.48 pg/dl.

Table 3: Statistical correlations of vitamin D level and TNF-α concentration on airway obstruction

Vitamin D	Airway Obstruction	n	Mean TNF-α	SD	*p*
Tertile 1	Mild	3	0.04	0.02	0.024
	Moderate	11	0.06	0.05	
	Severe	5	0.24	0.19	
	Very severe	1	0.23	-	
Tertile 2	Mild	1	0.04	-	0.012
	Moderate	4	0.04	0.05	
	Severe	6	0.22	0.15	
	Very severe	4	0.93	0.63	
Tertile 3	Moderate	4	0.12	0.04	0.018
	Severe	4	0.31	0.11	
	Very severe	7	0.48	0.24	

Data in Table 3 also illustrate the relationship between serum TNF-α concentration and airway obstruction level. There was a positive significant linear correlation between vitamin D level and serum TNF-α concentration on the airway obstruction level with the correlation coefficient value = 0.502 at *p*-value = 0.000, and the most considerable airway obstruction was observed at the tertile 3.

Correlation pattern of vitamin D and TNF-α on the airway obstruction level airway was not linear, and the highest TNF-α concentration with vitamin D level was observed at the tertile 2 (Figure 1).

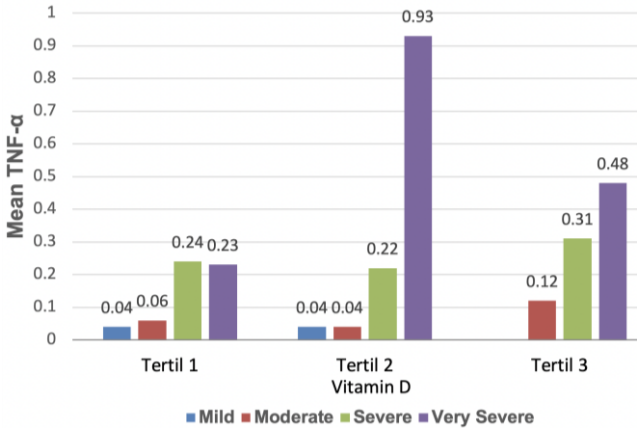

Figure 1: Linear relationship between vitamin D level and serum TNF-α concentration with the severity of airway obstruction

Relationship between vitamin D level and serum TNF-α concentration on the severity of COPD

On the whole, there were positive significant correlations between the increase of vitamin D levels (tertiles 1, 2 and 3) and the increase of serum TNF-α concentrations on the severity of COPD at *p*-value < 0.05 although the rise in serum TNF-α concentrations was not linear with the rise of vitamin D levels with the largest concentration of TNF-α was observed at the tertile 2 of vitamin D levels (Table 4).

Table 4: Statistical correlation between vitamin D level and serum TNF-α concentration with the severity of COPD

Vitamin D Level	Severity of COPD	n	Mean TNF-α	SD	*p*
Tertile 1	A	2	0.04	0.02	0.021
	B	12	0.06	0.05	
	C	3	0.15	0.12	
	D	3	0.33	0.17	
Tertile 2	A	2	0.06	0.03	0.008
	B	3	0.03	0.05	
	C	3	0.10	0.03	
	D	7	0.68	0.55	
Tertile 3	A	1	0.07	-	0.025
	B	3	0.14	0.03	
	C	2	0.24	0.14	
	D	9	0.46	0.22	

Overall, there were non-linear relationships between vitamin D level and serum TNF-α concentration on the severity of COPD (Figure 2).

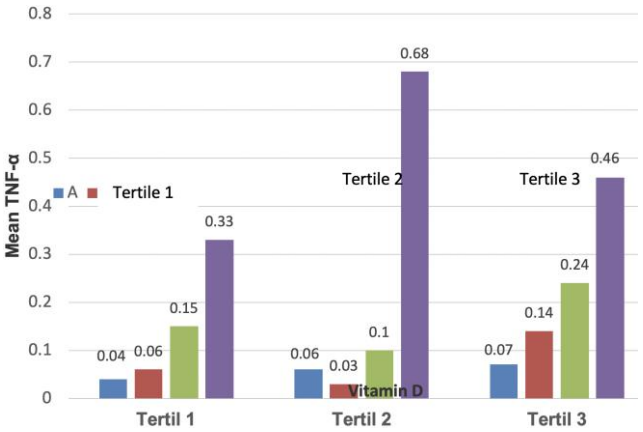

Figure 2: Non-linear relationships between vitamin D level and serum TNF-α concentration on the severity of COPD

Discussion

Characteristics of the study subjects

A total of the study subjects identified in this study were50 patients according to the history of the disease, clinical descriptions, and spirometry test. Of 50 study subjects, the majority of them were male accounted for 47 patients (94%) and 3 females (6%), and the majority of the study subjects are in the category of age older than 60 years old. Results of this study are consistent with those of the case-control study conducted by Sanket et al., for 81 COPD patients that consisted of 75 males (92.5%) [12]. The higher prevalence rate of COPD for the male group is associated with a higher prevalence rate of smokers for males compared to females [20]. All the study subjects observed in this study were smokers, and 82% of them were in the category of heavy smokers. Twenty-One study subjects had higher concentration of serum TNF-α (tertile 3 = 0.21-1.83 pg/dl), 20 study subjects and lower level of vitamin D (tertile 1 = 182.1-364.5 pg/dl). The majority of the study subjects (38%)

were in the category of severe COPD (category D of the severity of COPD at the tertile 3) according to the GOLD Combine Assessment.

Distribution of severity of COPD according to serum TNF-α concentration

As shown in Table 2, 12 study subjects were classified into two categories (A and B) according to the severity of COPD. The lowest TNF-α level was observed at the tertile 1 (= 0.00-0.06 ng/dl) accounted for, 17 study subjects are seen at the tertile 2 (0.07-0.20 pg/dl) and the tertile 3 with 21 study subjects. With refers to the analysis of above data distribution, mild COPD had the lowest serum TNF-α concentration whereas patients with high severe COPD and highest TNF-α level. These results coincide with those of the study of Maharaj et al., [21] that serum TNF-α concentration was positively associated with airway obstruction level and severity of COPD.

All the study subjects in this study are smokers, and 82% are heavy smokers. This coincides with the research of Watanabe et al., [22] for 142 non-COPD subjects that serum TNF-α concentrations of smokers increased compared to non-smokers and hypothesized that light smoking was associated with an increase in WBC counts, while heavy smoking was responsible for TNF-α activation in Japanese male subjects with standard glucose tolerance and the study of Tanni et al., [23] pertaining to the association between inflammation, smoking status, and disease and showed that serum TNF-α was higher in COPD current smokers [4.8 (4.2-5.8) pg/mL] and in current smoker controls [4.8 (4.2-6.1) pg/mL] when compared to COPD ex-smokers [4.3 (3.9-4.9) pg/mL; $p = 0.02$] and to never-smoker controls [3.7 (3.4-4.0) pg/mL; $p < 0.001$] and concluded smoking may influence TNF-α mediated systemic inflammation, which, in turn, may account for some of the benefits observed in patients with COPD who stop smoking. In view of the association between TNF-α concentration and severity COPD, results of this study are coherent with those of the research performed by Healing V et al., [24] that spontaneous TNF-α production was 5.0 times higher in patients with severe COPD compared to mild-to-moderate COPD ($p = 0.02$), and serum TNF-α was significantly elevated in patients versus controls (2.1 ±0.3 vs 1.1 ±0.1) at $p = 0.007$ and concluded that increasing airflow obstruction and hypercapnia was associated with an enhanced TNF-α response in COPD.A meta-analysis study conducted by Wei et al., [25] revealed that patients with stable COPD had higher serum IL-6 concentrations than healthy controls. No evidence showing a positive or negative association between IL-6 concentrations and the severity of pulmonary function impairment was found. The correlation between IL-6 levels and pulmonary function was weak in different severities of stable COPD patients.

Low level of vitamin D is sometimes found in patients with COPD. High prevalence of vitamin D deficiency in COPD patients is caused by the reduction of synthesis of vitamin D in skin due to ageing, poor diet, low capacity to store at fat related to wasting [9]. Active metabolism of vitamin D3 ($1,25(OH)_2D3$) increase the expression of an antimicrobial peptide, and reduce the expression of pro-inflammatory cytokines and can be used to explain the relationship between vitamin D and susceptibility of respiratory infection. Vitamin D deficiency also affects pulmonary function through various mechanisms, innate and adaptive immunity systems in the pathogenesis of COPD [27].

Overall, vitamin D level assessed in this study was not significantly associated with serum TNF-α concentration on airway obstruction level and severity of COPD. Various unobserved factors influencing vitamin D level and serum TNF-α concentration were not assessed in this study including physical activity, lifestyle, and nutritional status of the study subjects as investigated in the study of Bouillon et al., [28]. Moreover, levels of 1,25(OH)2D3 serum are also affected by serum Vitamin D Binding Protein (VDBP) level, phosphorus serum, parathyroid hormone [30]. In contrary, results of this study are consistent with those of the study Mekovet al., [31] (2015) and of the study Nishimura et al., [32] (2016) that there was not a significant positive correlation between vitamin D level and severity of COPD.

Results of this study were not consistent with the study of Sanket et al., [12] that COPD was associated with the risk increase of vitamin D deficiency, and there was a significant association between level vitamin D and severity of COPD. Zhu et al., (2015) [33] investigated the association between host serum 25-hydroxyvitamin D (25(OH)D) and the susceptibility and severity of COPD and found that low serum levels of 25(OH)D were not associated with COPD susceptibility, but the high deficiency rate of 25(OH)D was associated with COPD severity. Vitamin D supplementation may prevent COPD exacerbation. Vitamin D supplements could prevent COPD exacerbation as shown by Rafiq et al., (2015) [11] that a low level of vitamin D was positively associated with the severity of COPD.

This study has some limitations that did not evaluate other determinants influencing vitamin D3 status including lifestyles, eating behaviours, medications, serum vitamin D binding protein (VDBP), and serum vitamin D2 (25OHD). All information in this descriptive cross-sectional study was collected at the same time, and the study subjects were contacted only once. The temporal sequence of cause and effect could not be addressed in this study in assessing temporal relationships of vitamin D3 level, cytokine response, worsening of symptoms and severity of COPD, but it might be suggestive of an association that should be investigated more fully by further studies.

In conclusion, serum TNF-α concentration was positively associated with airway obstruction level and severity of COPD. Low level of vitamin D was negatively associated with airway obstruction level and severity of COPD. Vitamin D3 level {1,25(OH)2D} was negatively associated with serum TNF-α concentration and airway obstruction level and severity of COPD.

Further studies are necessary for determining more cytokines involved in pathogenesis and severity of COPD as well as search for determinants influencing vitamin D3 level to elucidate a more authentic relationship between the two pathological findings.

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Case Report

Neo Adjuvant Chemotherapy on Testicular Cancer after Scrotal Exploration: A Case Report

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Keywords: Neo Adjuvant Chemotherapy; Testicular Cancer; Scrotal Exploration

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Abstract

BACKGROUND: A case of testicular cancer treated with neo adjuvant therapy in a patient in male is rare. When testicular cancer presents, it is usually directly operated due to the size of cancer which presented within the order of operable size.

CASE PRESENTATION: Here, we report a rare case of a 5-year-old male patient presented to the emergency room (ER) due to mass on the scrotum without the difficulty of urination. From clinical findings, mass with a size of 15 x 10 cm was found with multiple abscesses on both sides of the scrotum. Pathological Anatomy Examination confirmed yolk sac tumour present within the scrotal mass. Initially, palliative chemotherapy took place to reduce the growth rate of the tumour. However, due to the responsiveness of testicular cancer to chemotherapy drugs, it reduced into operable size testicular cancer. Wide excision and Orchidectomy was then performed, followed by adjuvant chemotherapy.

CONCLUSION: This case report showed the possibility of using neo adjuvant chemotherapy as an alternative treatment when inoperable testicular cancer presented in hospital.

Introduction

In children, testicular tumours are uncommon, with an incidence of approximately 0.5 – 2.0 per 100,000 individuals and comprise about 1 – 2% of pediatric malignancies [1], [2], [3]. One study suggested that testicular tumours may be more common in Asian than in Caucasian children [4].

Testicular cancer represents the most common malignancy in males aged 15-34 years [5]. Histopathologically, testicular germ cell tumours are divided into two major groups: pure seminoma and nonseminoma. The pathogenesis of testicular germ cell tumours remains unknown; however, although recently questioned [6], cryptorchidism is the main risk factor, and molecular studies have shown strong evidence of an association between genetic alterations and testicular germ cell tumours [7]. Nearly 40% of the cases correspond to seminomas, and three-quarters of them are diagnosed with stage I of the disease [5]. Although testicular cancer has excellent cure rates, the choice of treatment centre is of utmost importance. Expert centres achieve better results for both the early-stage testicular cancer (lower relapse rates) and overall survival (higher stages within clinical trials) [8].

Seminomas are more sensitive to chemotherapy and radiation therapy; therefore, they are easier to cure than non-seminomas. The surgical treatment is either orchiectomy or orchidectomy plus lymph node dissection of the involved ganglia followed by adjuvant chemotherapy. Testicular cancer treated with neo adjuvant therapy was rare, due to the operable size of commonly found testicular cancer.

Case Report

A 5-year-old Indonesian male with a size of 15 x 10 cm soft tissue mass with multiple abbesses on

both side of the scrotum was presented to the emergency room. The patient stated that the lesion had been presented for 2 years. It appeared initially as an itchy mass appeared on the left side of the scrotum and grew bigger. The overlying skin surface was presented with erythema, multiple abscesses and sinus tracts. The lesion was indurated and tender (Figure 1). The bilateral testis cannot be identified even though penile and meatal can be identified. The patient had no difficulty of micturition.

Figure 1: Lesion of the Scrotum Pre

Secondary examinations such as blood test as well as Ultrasonography, Chest x-ray and Computed Tomography (CT) scan was performed to the scrotum. The blood test result showed increased zero marker value, which ensured the presence of testicular cancer. Ultrasonography of the scrotum showed enlargement of the left testis while right testis was found within normal limit. Abdominal CT-scan examination also showed enlargement on the left testis but limited only to the scrotum (Figure 5).

Figure 2: Lesion of the Scrotum Post Neo Adjuvant

Chest x-ray showed coin lesion as it was already metastasised to the lungs. Based on the examination results, we decided to do palliative chemotherapy due to the inoperable size of testicular cancer.

Figure 3: Lesion of the Scrotum Post

However, after four chemotherapy treatments, the size of testicular cancer was significantly reduced to operable size (Figure 2) as it was confirmed by the abdominal CT-scan (Figure 6).

Figure 4: Lesion of the Scrotum Post Adjuvant Chemotherapy

Therefore, we decided to do wide excision and orchidectomy on the left testis (Figure 3). Lymphadenectomy was also done in conjunction with previous surgery to remove an enlarged lymph node. It was then followed by four times adjuvant chemotherapy.

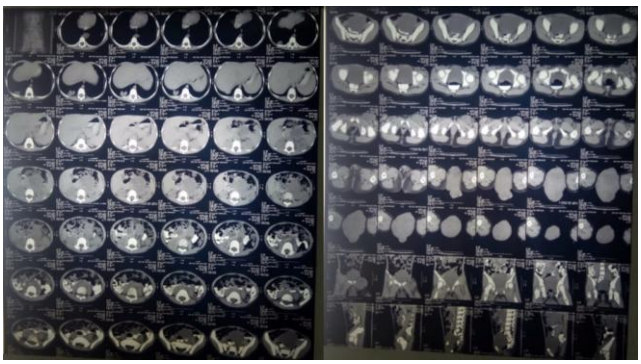

Figure 5: Computed Tomography of Lower Abdomen Pre

One month after the last chemotherapy, a patient came control to the outpatient clinic. The scrotum was appeared to be within normal limit. There was neither induration nor tenderness found in the scrotum.

Figure 6: Computed Tomography of Lower Abdomen Post Neo Adjuvant

The overlying skin surface was also presented within the normal limit (Figure 4). A chest x-ray also showed lungs within normal limit.

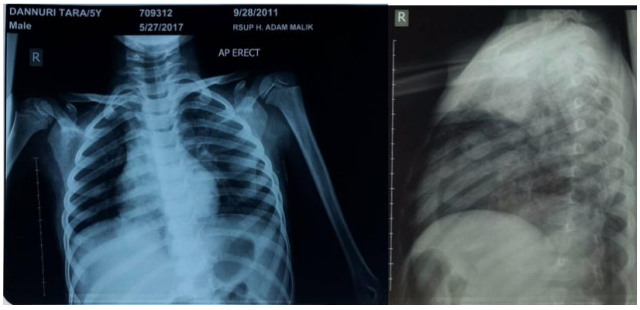

Figure 7: Chest X-Ray Pre

Discussion

This case suggested neo adjuvant chemotherapy usage in inoperable testicular cancer. The patient was delayed in presentation due to limited access to appropriate medical facilities as well as the patient's ignorance of the massive scrotal swelling that had been persisting for at least 12 months. In this case, the inoperable scrotal mass was then presented with multiple abscesses in the emergency room.

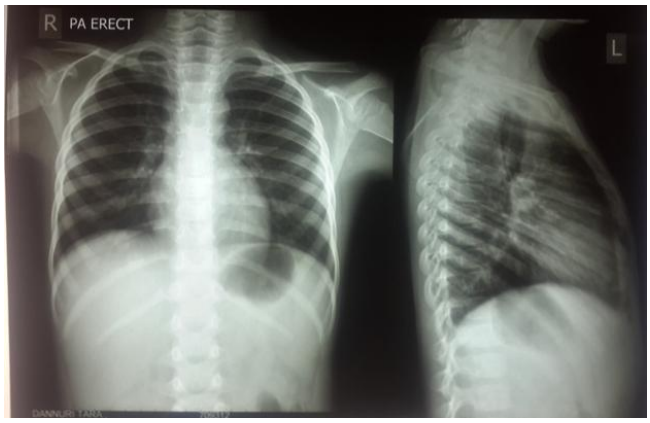

Figure 8: Chest X-Ray Post Operation

A review of the literature showed that this form of presentation is rare [9], [10], [11]. Although the recent diagnostic and therapeutic developments have altered the prognosis in this disease, the delay in diagnosis and occasional mismanagement of patients continue to inhibit further improvement in survival rate. A high index of suspicion and an aggressive approach to its management are advocated to improve long-term survival [12].

Testicular cancer has a higher responsiveness to chemotherapy compared to other cancer. The key to such success appears to lie in cancer's stem cells, which are more sensitive to chemotherapy than stem cells found in other types of cancer. Defining why testicular cancers are so susceptible to chemotherapy could eventually provide insights for treating other, more resistant cancers [13].

Most types of tumours contain distinct populations of cells. A small fraction of these is stem cells, which can grow new tumours from a single cell and are extremely resistant to therapy. Often, other types of tumour cells are killed off during treatment, but cancer stem cells survive, then drive relapse by re-growing new tumours. However, when testicular cancer stem cells are exposed to chemotherapy, those stem cells are more sensitive to it than other cells in the tumour [14].

The use of neo adjuvant chemotherapy has led to a dramatic improvement in the cure rate of patients with metastatic germ cell tumours (GCTs). The high responsiveness of testicular cancer was achieved even in inoperable size of testicular cancer. After that, the goals of the patient changed from palliative into curative by the introduction of neo adjuvant chemotherapy, followed by surgical treatment and adjuvant chemotherapy.

In conclusion, testicular cancer treated with neoadjuvant chemotherapy is unusual as the mainstay treatment for testicular cancer is directly surgical and followed by adjuvant chemotherapy. This case presented the inoperable size of testicular cancer in which palliative chemotherapy takes place to reduce the growth rate of testicular cancer. However, due to the responsiveness of testicular cancer to chemotherapy agent, it reduced the initial size into an operable size, which later on able to follow the treatment stated in guidelines. This case report showed the possibility of using neo adjuvant chemotherapy as an alternative treatment when inoperable testicular cancer presented in hospital.

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Case Report

Misidentification of *S. suis* as a Zoonotic Agent

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Abstract

BACKGROUND: *Streptococcus suis* is an emerging zoonotic pathogen. This bacterium commonly causes meningitis in human and is often associated with hearing and vestibular dysfunction. *S. suis* tends to be misidentified, leading to under-diagnosis.

CASE PRESENTATION: A previously healthy 50-year-old man was admitted to one of the district hospitals in Bali Province, Indonesia, due to meningitis. He had a history of consuming homemade raw pork product two days before the onset of illness. Streptococcus mitis was identified from the cerebrospinal fluid culture by using VITEX 2 COMPACT (Biomeriuex) with a 99% probability score. This patient had clinical symptoms and risk factor identical to *S. suis* infection. Therefore, we performed confirmation tests for the cerebrospinal fluid by PCR (using primer specific for *gdh* and *recN*) and sequencing of those PCR products. Both of the confirmation tests showed a positive result for *S. suis*.

CONCLUSION: There are few reports of *S. suis* infections in Indonesia, but we believe that the cases in Indonesia, especially Bali, are not uncommon. The under-reported cases are perhaps due to the difficulties in differentiating *S. suis* from other Streptococcus species by culture method, particularly *Streptococcus mitis*. Therefore, confirmation by PCR is necessary.

Introduction

Streptococcus suis (*S. suis*) is the main pathogen in pigs that can cause meningitis, endocarditis, and sepsis in humans [1], [2], [3]. *S. suis* infection is an emerging infectious disease whose incidence is reported to increase and causes public health problems [4], [5], [6]. There are two outbreaks of *S. suis* infection in China that affect more than 200 people with a mortality rate of almost 20%, which have since changed the views of experts on the threat of this pathogen to human health [1]. The first case of *S. suis* infection was reported in 1954 after an outbreak of meningitis, septicemia, and arthritis in piglets. The first case of *S. suis* meningitis in a human was reported in Denmark in 1968 [7], and it has occurred in Bali in 2014, which was followed by an increase in cases of infection in Bali [8].

S. suis is a gram-positive coccus, facultatively anaerobic, and based on Lancefield's classification it has a cell wall structure by group D *Streptococcus* [9]. These bacteria are often reported as *Streptococcus viridans* in their initial identification from CSF culture due to the similarity [10], [11].

Misidentification has an impact on the case finding and to control the transmission, which is highly important in the effort to comprehensively manage *S.*

suis as one of the zoonotic agents. The discussion of the following case is expected to enhance the understanding of the characteristics of *S. suis* and the possibility of identification errors that might occur.

Case Illustration

A 50-year-old male was brought to the emergency department in one of the regional hospitals in Bali with a decrease of consciousness accompanied by headache, fever, nausea, vomiting, and anorexia for 2 days before hospitalisation. He had a history of consuming homemade red *lawar* (a traditional Balinese food made by mixing chopped pork with fresh blood) three days before admission. He had no history of other acute infections. His past medical history was not significant.

On examination, his condition was confused with a Glasgow Coma Scale (GCS) E3V3M5, fever (38°C), and neck stiffness. Routine laboratory test showed leucocytosis (15.9 x 10^3/µL) with neutrophil predominant (93.6%) and thrombocytopenia (125 x 10^3/µL). A brain computed tomography scan (CT scan) demonstrated cerebral oedema. The Cerebrospinal fluid (CSF) had a turbid color, an increased leukocyte count (1080 cell/mm^3) predominating polymorphs (71%), an increased protein level (226 mg/dL) and a decreased glucose level (65 mg/dL) with a decreased CSF / serum glucose ratio (0.38). The CSF culture showed Gram-positive cocci, negative catalase, and colony on blood agar showed hemolytic alpha. Identification with VITEK 2 COMPACT (bioMériux) shows the results of *Streptococcus mitis (S. mitis)* with a probability of 99%.

The diagnosis of acute bacterial meningitis was established, and the patient was treated with 2 gram of ceftriaxone as empirical antibiotic at 12-hour intervals until the culture's result was done. After 3 days of treatment, the patient experienced hearing impairment. Based on those clinical features and history of consuming food containing raw pork products, *S. suis* meningitis was suspected. Furthermore, *S. mitis* as the culture result was known as a commensal bacteria and had never been reported as a cause of meningitis. We suspected there was a misidentification of the causative agent in this case. Hence, a confirmation examination was performed by PCR using the specific primers of the glutamate dehydrogenase (Gdh) and the recombination/repair protein (RecN) encoding gene in *S. suis*. The sequencing of those PCR products was also carried out to confirm whether the causative agent in these cases was *S. mitis* or *S. suis* [12], [13]. From the PCR results, it was found that the isolates showed the positive result in both amplified *S. suis* specific genes.

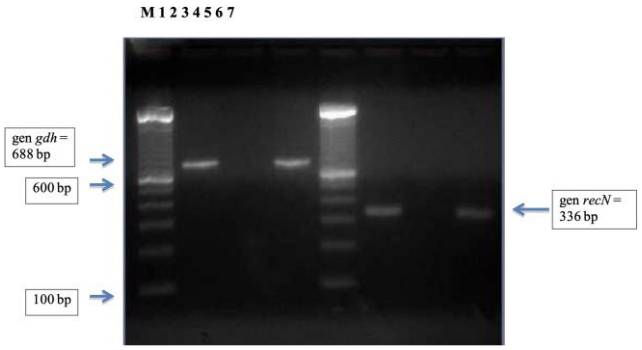

Figure 1: PCR gdh and recN gene detection. The amplicon was electrophoresed on 1 % agarose gel. The sample was positive for gdh gene (688bp) and recN gene (336bp). (M = marker 100 bp (Invitrogen); lane 1 = control positive for gdh gene; lane 2 = negative control; lane 3 = sample (gdh gene was positive); Lane 4 = Marker 100 bp (Invitrogen); Lane 5 = control positive for recN gene and lane 6 = sample (recN gene was positive)

The BLAST result of PCR product sequences showed 100% identity to *gdh* and 99% identity to *recN* of *S. suis* strains (CP020863.1). According to the performed confirmation tests, the patient was diagnosed with *S. suis* meningitis. After the patient had been hospitalized for 14 days, the patient was discharged from the hospital with the complications of hearing impairment.

Discussion

Regarding the case illustration above, our patient exhibits typical symptoms as acute bacterial meningitis including fever, headache, anorexia, hearing loss, and a history of consuming raw pork food products. *S. suis* can cause systemic infections in humans in the form of meningitis, sepsis, endocarditis, arthritis, endophthalmitis, uveitis, spondylodiscitis, ophthalmoplegia, and epidural abscess [7]. The most common manifestation of *S. suis* infection is meningitis that occurs in about two-thirds of patients [1], [2], [14]. One of the typical symptoms of *S. suis* meningitis is hearing impairment, or loss, which occurs in more than 50% of the cases [1], [2], [14]. Various risk factors associated with acquiring *S. suis* infection included the consumption of raw pork products, pig-related occupation, pigs, or pork exposure, alcohol drinking, skin injury especially due to exposure during pork processing, and underlying diseases contributing to immunocompromised conditions [2], [5].

Apparently, the incubation period of *S. suis* infection in humans varies depending on the route of transmission. A shorter incubation period occurs when infection happens through wounds on the skin which rapidly spread hematogenously, while the incubation period is longer if infected through oral consumption [7], [15], [16]. In the case of outbreaks in China, the

incubation period ranged from 3 hours to 14 days (median 2.2 days) [15]. *S. suis* infection often occurs in predominantly healthy adults, men with an average age of 51 years, but very rarely found in children [2], [16].

Microbiological examination plays an important role in establishing the diagnosis of meningitis, especially for identification of causative agents and antimicrobial sensitivity testing. The microbiological examinations of CSF fluid that were performed in this case — including the Gram staining, colony appearance on blood agar with alpha-hemolytic, and negative catalase test — matched the characteristics of *Streptococcus*. The result of bacterial identification using biochemical examination (VITEK 2 Compact) is *S. mitis*. However, the patient's history and clinical symptoms did not match with the bacterial identification result, *S. mitis*. This bacterium has never been reported as the causative agent of meningitis. Suspicion of other causative agents such as *S. suis* bacteria arose due to a history of contact with animals as hosts and hearing impairment that occur in the patient.

S. suis is a coccus Gram-positive bacterium, facultatively anaerobic, negative catalase, and forms alpha hemolysis in blood agar [17], [18]. If we review the results of the microbiological examination, it can be concluded that the bacteria have similarities with other Streptococcus bacteria such as *Streptococcus viridans*, *Streptococcus equid*, *Streptococcus mitis,* and other *Streptococcus* which often cause misidentification [4], [6], [11], [19]. In a study performed by Donsakul et al., from 1993 to 1999 (reported in 2003), 5 of the 8 cases were initially identified as *Streptococcus viridans* infections. The conventional biochemical examination has lower specificity than an examination of molecular biology [12].

In this case, PCR was performed using the specific primer to amplify two specific genes in *S. suis, gdh*, and *recN* [12], [13] and a positive result was shown, indicating that the bacteria isolated from the CSF is *S. suis*. The PCR method provides more specific result based on gene identification that encodes glutamate dehydrogenase (*gdh*) and the gene that encodes recombination, or repairs protein (*recN*) [11], [12], [20]. In 2015, Okwumabua et al. conducted a study that identified 306 *S. suis* isolates using PCR method through identification of the encoding gene *gdh*. The gene identification has been used in diagnosis of other bacterial infections and reported to have a very low mutation rate [12]. Identification of the *recN* gene is also an easy and accurate examination for *S. suis*. PCR based on the *recN* sequence is a better method for identification and detection of *S. suis* compared to *gdh* gene identification [11], because it has been shown to have a lower level of similarity at the species level and a higher divergence value at the subspecies level than other genes [21].

The diagnosis of *S. suis* meningitis is highly important, because it is one of the zoonotic diseases that can cause public health problems and potentially leads to an outbreak. It requires comprehensive prevention of the causative agents by breaking the chain of transmission through intermediary animals.

In conclusion, the low prevalence of *S. suis* meningitis cases reported in Indonesia, especially in Bali, does not indicate the actual condition of the cases. This could happen due to the misidentification of *S. suis* from other *Streptococcus sp.* from conventional cultural method. The choice of a more accurate type of examination such as PCR is highly important to consider as a confirmation test of CSF examination, especially in cases with clinical symptoms that were suspected as *S. suis* infection.

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Dental Science

Effect of a Self-Etch Adhesive Containing Nanobioglass on Postoperative Sensitivity of Posterior Composite Restorations - A Randomized Trial

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Keywords: Postoperative sensitivity; Bioglass; Composite restorations; Class II

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Abstract

BACKGROUND: Postoperative sensitivity is one of the major problems regarding posterior resin composite restorations that causes patient discomfort, maybe a reason for replacement of the restoration with an additional office time.

AIM: To evaluate the effect of the addition of a Nanobioglass to a self-etch adhesive on the reduction of post-operative sensitivity following composite restorations versus a self-etch adhesive that is free of Nanobioglass agent.

MATERIALS AND METHODS: Sixteen patients having class II carious lesions were included in the study. After cavity preparation, each tooth was randomised to one of the following restorative treatments. Teeth in the control group were restored using self-etch adhesive (OptiBond All-In-One, Kerr) that is free of nanobioglass agent, and nano-hybrid resin composite (Herculite Ultra, Kerr). Restoration of teeth in the experimental group was similar to the control except that a nanobioglass agent was added to the self-etch adhesive. Patients were contacted for evaluation of postoperative sensitivity at 1 day, 1 week, 1 month, and 3 months. Data were analyzed using friedmann test followed by fisher exact test.

RESULTS: The experimental group (self-etch adhesive containing nanobioglass) showed a significantly less postoperative sensitivity compared with the control group (Self-etch adhesive free of nanobioglass) at 1 day, and 1-week evaluation periods. While both groups did not possess any significant difference at 1 month, and 3 months periods.

CONCLUSION: The problem of postoperative sensitivity following resin composite restorations could be solved by the addition of bioglass nanoparticles into dental adhesives.

Introduction

Composite resins and adhesive technology have developed rapidly. Despite these developments, postoperative sensitivity following composite restorations is still a challenge for practitioners. Clinical studies revealed the presence of such complaints in 0-30% of the study populations [1]. It was reported that postoperative pain could be related to preparation trauma and microleakage of bacteria [2]. Other studies reported that polymerization shrinkage of composite leads to internal stresses, debonding and gap formation between the composite and tooth, leading to deformation of restorations under occlusal stresses which transmits hydraulic pressure to the odontoblastic processes causing pain [3], [4].

Several strategies have been presented in the literature tried to solve the problem of postoperative sensitivity, by using different light-curing modes [5], different adhesive strategies [6], applying cavity disinfectants and desensitizers before the bonding procedure [7], and implementing different techniques for placement of posterior composite restorations [8].

Now, post-operative sensitivity solution has been related to dentin adhesives' ability to seal up the gaps and open dentinal tubules that are present at the interface between the dentin adhesive and the dentin rather than the continuous trial to decrease polymerization shrinkage and its effects on cuspal deflections and marginal adaptation as was generally believed [9]. To address this solution, it was necessary to develop novel dental adhesives that contain nanobioglass having remineralising capabilities that could block theses gaps, thus decreasing postoperative sensitivity.

In the current literature, no randomised clinical trials have evaluated the effect of a self-etch adhesive containing nanobioglass on the incidence of postoperative sensitivity. In turn, this study would affect the feasibility of postoperative sensitivity reduction and consequently would benefit the patients.

Material and Methods

The materials used as well as their principal components, manufacturers and lot number are listed in Table 1.

Table 1: Materials' composition, manufacturers, and Lot number

Material	Specifications	Composition	Manufacturer	Lot Number
OptiBond All-In-One	One-Step, Self-Etch Adhesive system	Acetone, ethyl alcohol, uncured methacrylate ester monomers, inert mineral fillers, ytterbium fluoride, photoinitiators, accelerators, stabilisers, water		5811789
Herculite Ultra	Visible light cured Nano-Hybrid resin composite	Organic part: Bis-GMA[1], TEGDMA[2], Bis-EMA[3] Inorganic part: Barium glass (0.4 lm; silica, 20-50 nm); pre-polymerized filler (barium glass and silica) Filler load 78% wt (57% vol)	Kerr, Italia S.r.l	2391712

Abbreviations: Bis-GMA, Bisphenol A diglycidylmethacrylate; TEGDMA, Triethyleneglycoldimethacrylate; BIS-EMA: Bisphenol A polyethylene glycol diether dimethacrylate.

Preparation and Characterization of Nanobioglass Powder Particles

The bioglass nanoparticles used in this study were prepared using the sol-gel technique [10]. Nitric acid in water (1 Mole solution) was added to tetraethyl orthosilicate (TEOS) for a final H2O: TEOS molar ratio of 18. The solution was subjected to hydrolysis for 60 minutes while stirring at room temperature. The following reagents were added and allowed to react for 45 minutes in the following sequence: triethyl phosphate, calcium nitrate tetrahydrate, and sodium hydroxide. The solution was stored in a sealed container for 3 days at room temperature to allow gel formation. The gel was aged for 3 days at 70°C and then dried in an oven at 150°C for 3 days. Finally, the dried gel was calcined in a high alumina crucible (furnace) at 700°C for 3 hours resulting in white bioglass nanoparticles.

The principal components and manufacturer of the nanobioglass are listed in Table 2. The resultant particles were characterised using Transmission electron microscope (TEM) (JEOL JEM-2100, Tokyo, Japan) to assess its size and shape. Scanning Electron Microscope (SEM) &Energy Dispersive Analytical X-ray (EDAX) (Fei Company, model: Quanta 250 FEG, Germany) was also used to examine its surface topography, and ensuring the purity of the resultant powder.

Table 2: Nanobioglass composition, and manufacturer

Preparation	Composition	Manufacturer
Nanobioglass powder	45% SiO_2, 25% CaO, 25% Na_2O and 5% P_2O_5	Nanostreams Company, 6th of October City, Egypt

Incorporation of Nanobioglass Into The Self-etch Adhesive

The optimum amount of nanobioglass that could be added to the self-etch adhesive without affecting its viscosity was determined by measuring the viscosity of the self-etch adhesive before and after nanobioglass incorporation according to previous studies [11], [12]. The whole self-etch adhesive bottle containing the nanobioglass was then sonicated in the ultrasonic mixer to produce a homogenous mixture.

Study Design

This was a double-blinded (operator and patient), randomised clinical trial.

Patients Recruitment

This study was revised and approved by the research ethics committee, Faculty of Oral and Dental medicine, Cairo University. Patients were recruited from the outpatient clinic of the Operative dentistry department, Faculty of Dentistry, Cairo University. They were then assessed for eligibility according to the inclusion and exclusion criteria listed below. Patients were informed of the nature of the study, consented to participate and signed a consent form.

Inclusion and Exclusion Criteria

Selected patients had to have a moderate to the deep proximal primary carious lesion in posterior teeth as diagnosed by clinical examination and a periapical radiograph. Selected teeth should have an occlusal contact with natural or a crowned antagonist tooth so that it could be tested for post-operative pain during food mastication. Patients had to have healthy gingival tissues, without gingival recession or alveolar

bone loss. Patients were excluded if they had any signs or symptoms of pulpal and periapical disease, spontaneous dental or orofacial pain, defective restorations that need replacement. Patients are taking medications and analgesics that could alter their normal pain perception level, and patients having medical, psychiatric histories, including the use of anti-inflammatory, psychotropic drugs were also excluded.

Randomisation

Patients were randomly allocated into two groups according to the investigated restorative treatment provided: 1. Experimental group: single-step self-etch adhesive containing nanobioglass and 2. Control group: a single-step self-etch adhesive that is free of nanobioglass. Randomisation depended on two interrelated aspects: Sequence generation, and allocation concealment. Sequence generation involved the allocation of each patient to one of the restorative treatment options according to a randomisation list generated using "random.org". Thus, the randomisation list defined the type of the used self-etch adhesive (either containing or free of nanobioglass). Allocation concealment involved unrevealing and concealing the randomisation list by placing them in an opaque and sealed envelope. At the day of restoration, each patient was allowed to pick up an envelope randomly. Each envelope contains a numbered paper that corresponds to one of the restorative treatment options, according to the previously created randomisation list.

Blinding

The current study was double-blinded. The patient did not know what treatment she/he was taking. The operator was blinded to the treatment option given; self-etch adhesive (containing or free of nanobioglass), as they were masked in two identical bottles and was given codes (A & B) (Figure 1). A researcher not involved in any of the experimental phases performed the procedures of Sequence generation, allocation concealment, and blinding.

Figure 1: Restorative treatment options masked in identical bottles

Interventions

Patients' general information, including name, gender, occupation, age, and phone number, were recorded. Also, medical and dental histories were taken. The diagnosis of caries was depending based on clinical examination that was done tentatively using a diagnostic mirror and an explorer aided by the light from the dental unit. Pre-operative radiographic examination (Figure 2) was routinely taken to evaluate cavity proximity to the pulp and any sign of periapical radiolucency that could preclude the patient inclusion into the study

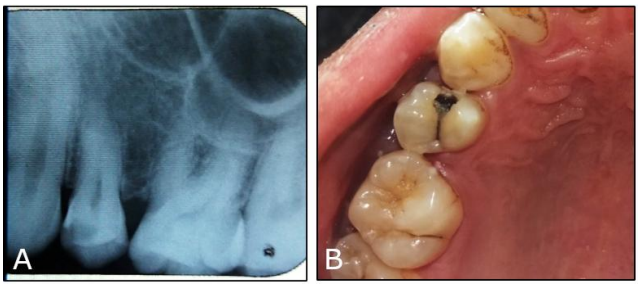

Figure 2: A): Periapical radiograph showing proximal caries; B): clinical picture

The patient was anaesthetised using a local anaesthetic, Mepecaine-L local anaesthesia, using a dose of 20mg Mepivacaine hydrochloride U.S.P., 0.06 mg Levonordefrin hydrochloride (Alexandria Co. for Pharmaceuticals, Alexandria, Egypt) to control tooth pain during caries removal. The field of operation was isolated with the application of a rubber dam. Entrance to the lesion and lateral extension through the cavity was then done using an inverted cone bur # 35 (MIDWEST, DENTSPLY) under air-water coolant. In case of deep cavities with a large amount of carious dentin, the highly softened dentin was removed using an excavator. A caries detector dye (Kuraray America) was then used to disclose the residual infected dentin at the pulpal floor and surrounding walls, by applying the dye using a disposable applicator brush for 10 seconds, rinsed off, then stainable (red) tissue was removed using a spoon excavator (Becht, Germany).

Figure 3: Finished cavity preparation

The procedure was repeated two times until the dentin surface was stained pale pink and was relatively hard. Teeth were excluded from the study in case of a pulp exposure, where calcium hydroxide agent was placed. The cavo-surface angle of the prepared cavity was entirely in enamel without beveling (Figure 3). The cavity walls were then smoothed with a straight fissure bur # 57 (MIDWEST, DENTSPLY).

Sectional metal matrices with rings (TOR VM LTD, Moscow, Russia) and wooden wedges were placed before the bonding and restorative procedures (Figure 4). OptiBond was applied according to the manufacturer's instructions as follows: a generous amount of the bonding agent was applied to the enamel and dentin surfaces using a disposable applicator brush. Scrubbing of the surfaces was then done with a brushing motion for 20 seconds. A second application of the bonding agent was then performed with a brushing motion for 20 seconds. The adhesive was then dried for 5 seconds with oil-free air, and light-cured for 10 seconds using Elipar LED curing light (3M ESPE) at a light intensity of 1200 mw/cm^2.

Figure 4: Sectional metal matrix

Cavity preparations were restored using Herculite Ultra nano-hybrid resin composite (Kerr, Italia). Proximal boxes were initially restored using oblique incremental packing technique. The increments were light-cured from both the occlusal surface and indirectly through the cusps using Elipar LED curing light for 10 seconds. After the final build-up, the restoration was further polymerised for 10 seconds in three directions: occlusal, buccal and lingual. After final polymerisation of the restoration, the rubber dam was removed. Premature contacts were detected with an articulating paper and removed with a flame shape finishing carbide bur # 7106 (MIDWEST, DENTSPLY) in a high-speed handpiece under air-water coolant. Restorations were then finished using a flame shape finishing carbide bur # 7106 (MIDWEST, DENTSPLY) and polished using rubber cup and flame polishers (HiLuster polishers, KerrHawe). Figure 5 illustrates the final restoration after finishing and polishing.

Figure 5: *Final restoration after finishing and polishing*

Postoperative Sensitivity Evaluation and Testing

Post-operative sensitivity was evaluated using a Visual Analog Scale (VAS). It is 100 mm horizontal line with a descriptor at its far-left end indicating no pain, and at its far-right end indicating the worst possible pain. Illustration of facial expressions with colour codes was added below the 10-centimetre line Visual Analog Scale (Figure 6).

Figure 6: Visual Analog Scale

Participants were instructed to rate the pain level using VAS scale as follows: If the pain were the worst possible, the participant would mark at the far-right end of the line, and in the absence of pain he would mark at the far-left end. For pain levels between the two extremes, participants made a mark at a point along the line that best represented their pain. The distance in millimetres from the far-left end of the line to the marked point of intersection was measured and recorded.

POS was tested against normal daily life stimuli, patients were requested to report their pain levels against cold, hot, sweet stimuli during drinking or eating, and pressure stimuli during their masticatory routine. The participants were instructed to avoid taking any analgesic or anti-inflammatory drugs during the whole study period.

Follow-up evaluation periods were scheduled at 1 day, 1 week, 1 month and 3 months intervals. At each evaluation period, patients were contacted via telephone calls to remind them to rate their sensitivity

levels on the VAS scale. They were also verbally questioned regarding the presence of spontaneous pain, and whether it is prolonged or not. Pre-operative sensitivity levels were assumed as zero as asymptomatic teeth were selected [13].

Statistical Analysis

Data were presented as mean and standard deviation (SD) values. Data were explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests. Friedmann test was used to compare between different follow-up periods for different tested groups, followed by Fisher exact test for Pairwise comparison. Mann Whitney test was used to compare between different tested groups. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® (SPSS Inc., IBM Corporation, NY, USA) Statistics Version 24 for Windows.

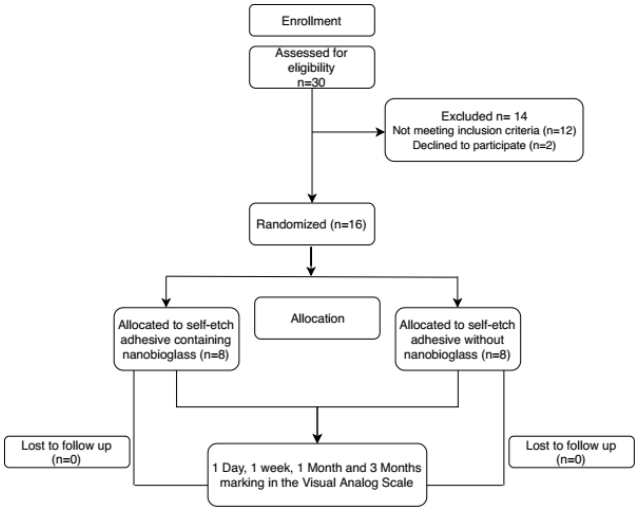

Figure 7: Participant flow diagram in the different phases of the study design

Results

The experimental protocols were implemented exactly as planned, and no modifications were performed. Figure 7 shows the participant flow diagram at the different phases of the study design. A total of 30 patients were recruited and assessed for eligibility. 14 patients were excluded (2 patients declined to participate, and 12 patients did not meet the inclusion criteria). 16 patients were then subjected to the randomisation procedure and allocated to one of the treatment options to be 8 patients in every group. All patients returned to a 3 months recall.

None of the subjects needed an analgesic drug to reduce postoperative sensitivity (POS). Regardless of the group, most of the postoperative sensitivity complaints occurred within the 1 day and 1-week evaluation periods. Mean and standard deviation (SD) of Visual Analog Scale (VAS) scores for different groups at all follow-up periods are shown in Table 3 and Figure 8.

Table 3: Mean and SD values of VAS scores for tested materials at different evaluation periods

	Group A (Nanobioglass)		Group B (Control)		p-value
	Mean	SD	Mean	SD	
Pre-operatively	0.00^{a}	0.00	0.00^{a}	0.00	1.00 NS
1 Day	2.06^{b}	2.24	4.75^{b}	1.75	0.038*
1 Week	1.38^{b}	1.69	3.69^{b}	1.51	0.015*
1 Month	0.44^{c}	0.62	0.63^{c}	0.74	0.721 NS
3 Months	0.00^{a}	0.00	0.00^{a}	0.00	1.00 NS
p-value	≤ 0.001*		≤ 0.001*		

Means with different letter within each column indicates significant difference; * = Significant, NS = Non-significant.

The mean VAS scores for the nanobioglass and control groups at 1 day and 1-week evaluation periods was higher than the 1 month, and 3 months periods with a significant difference ($p \leq 0.001$; Fisher exact test). This dictates that the peak of POS was in the first week after treatment. The mean VAS scores for both groups declined at the 1 month, and 3 months period. All patients reported no sensitivity after 3 months.

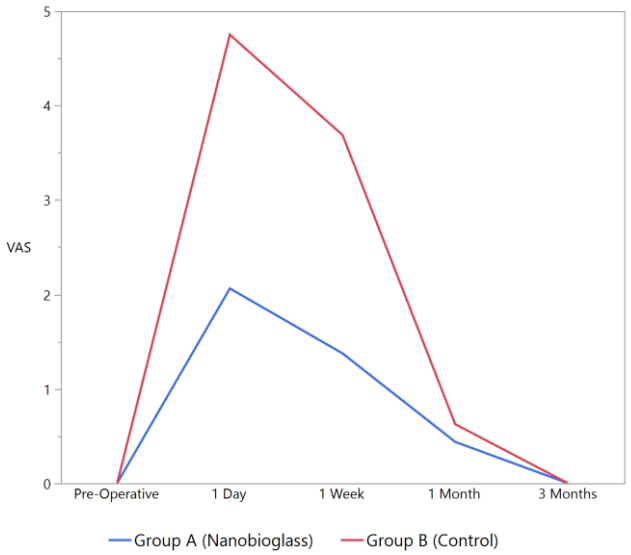

Figure 8: A-Line Chart showing the mean VAS Scores for tested materials at different evaluation periods

As for the comparison between the two groups, the mean VAS scores for the nanobioglass group was lower than the control group at the 1-day (2.06 and 4.75 respectively), and 1-week (1.38 and 3.69 respectively) evaluation periods with a statistically significant difference. While at the 1 month, and 3 months, the difference between the mean VAS scores for both groups was statistically insignificant. This indicates that nanobioglass was effective in decreasing POS.

Discussion

Placing successful posterior composite restorations is challenging and technique sensitive, so any mistake during placement of such restorations will lead to postoperative problems. It is well reported that POS is one of the main problems regarding this type of restoration. The postulated theory for POS following composite restorations include gap formation that predisposes to microleakage. This, in turn, causes compressibility of the restoration during loading, causing fluid to be forced in and out from underneath the restoration causing pain. The current theory of pulpal tooth pain dictates that any change in the hydraulic pressure within the dentinal tubules stimulates the pain receptors within the pulp, causing pain. Consequently, how efficient the dentin adhesive seals the cut dentinal tubules seem to be the winning factor in decreasing POS.

In this regard, a nanobioglass was incorporated into the dental adhesive in the current study. Concerning its nano-sized bioactive components, those gaps in the dentinal tubules could be sealed. Besides, its high surface area allows the release of more calcium and phosphate ions at low concentrations. This is important because a low filler concentration could be used for adhesives so that the adhesive could maintain its viscosity and ability to flow into the dentinal tubules.

Another important reason for using nanobioglass particles is that its average size of about 20 nm that could infiltrate into the dentinal tubules more easily than the traditional particles of several microns to tens of microns in size [11]. In addition, nanobioglass is a precursor that can convert to an appatite, similar to the minerals in tooth enamel and dentin. Bioglass, when exposed to a physiological fluid, cation exchange of Na+ and Ca2+ by protons (H+ or H3O+) occurs on the bioglass surface to form microporous silica (SiO2- rich layer) upon which Hydroxy Carbonate Appatite (HCA) layer forms. Changes in pH and weight loss happen with the change in the ionic dissolution and consecutive precipitation of hydroxy carbonate appatite [11].

A single step self-etch adhesive strategy was selected for the current trial. Early clinical studies [14], [15] attributed the cause of POS to the use of etch and rinse adhesive systems. This was not the case for the more recent studies [16], [17] who believed that self-etch adhesive systems lowers the risk of POS as they do not remove, but incorporate the smear layer in the hybridised area. Furthermore, because dentin conditioning and resin infiltration occur simultaneously, dentin tubules are more likely to remain sealed. Nevertheless, a recent systematic review [18] has helped to support or refute these findings; they stated that the type of adhesive strategy, either etch and rinse or self-etch for posterior resin composite restoration does not influence the risk and intensity of POS.

class II cavities were chosen for this study because of the incidence of post-operative sensitivity in class II cavities is higher than that of other cavity preparations [19], as the increased amount of destruction of dental structure that is found in class II cavities seems to be the determinant factor in the occurrence of POS, this was explained by a series of cuspal contraction and expansion that occurs during the bonding procedure [13].

Only moderate to deep cavities were included in the current study. As the dentin is prepared closer to the pulp, the tubule density and diameter increase, thus increasing both the volume and flow of pulpal fluid (hydrodynamic effects) when teeth are subjected to stimuli [20] which is perceived by patients as pain. In other words, it would be expected that restorations placed in deep cavities are associated with more postoperative sensitivity [21]. Therefore, it was logical to determine the ability of the tested materials to occlude those tubules under the circumstances of such hydrodynamic effects. Shallow cavities were not included in the current study, since post-operative sensitivity is low or infrequently detected in shallow cavities, as reported by other clinical studies [22], [23].

Resin composite restorations in the current study were placed directly in posterior teeth without the use of liners and bases in accordance with a systematic review [24] that linked the use of liners to the reduction in postoperative sensitivity, they revealed that there is an inconsistent evidence regarding the difference in POS between resin composite restorations placed with or without liners. Resin composite was placed using an incremental filling technique and indirect curing through the cusps, to minimize the deleterious effects of polymerization shrinkage stresses on the marginal integrity of the composite restorations as well as on the microscopic integrity of the adhesive bond to dentin [22].

The Visual Analog Scale (VAS) method that was used to evaluate POS in the current study offers participants a broader range of responses and more uniform instructions by avoiding descriptors such as mild, moderate and severe, which can be interpreted quite differently from one participant to another [25]. Furthermore, it provides a more accurate and effective statistical test than tests based on fixed categories [26]. Besides, its ability to detect minor changes in pain intensities over time or due to treatment [27].

Illustration of facial expressions with colour codes was added below the 10- centimetre line Visual Analog Scale, in an attempt to make it better understood by patients. Furthermore, more cooperation from the patients was experienced when facial expressions were added rather than a plain 10-centimetre line was used alone [28]. POS testing in the current study, has typically been based on the patient's day-to-day experiences (real life) to various stimuli like pressure, cold and sweet stimuli during

drinking, eating and chewing. This has provided a more realistic scenario for POS testing rather than a standardised, controlled stimulus that the patient may not encounter throughout his life [16].

The 3 months evaluation period that was assigned for the current study might have provided a more reasonable scenario for testing the effectiveness of the investigated materials, giving them more time to block the incompletely sealed dentinal tubules present in the hybridised layer, thus decreasing POS on longer periods. This was by a previous study [13] who evaluated postoperative sensitivity after 33 months despite the study design was to evaluate it at 48 hours and 1 week only, as they were contacted by patients complaining of post-operative sensitivity after 33 months of their study. All clinical work in this study was carried out by one clinician to reduce the variability among clinicians in handling and manipulating materials.

Post-operative sensitivity (POS) results revealed that the nanobioglass group has lower VAS scores than the nanobioglass free one with a statistically significant difference. This might be due to the presence of nanoparticles of calcium and phosphate in the composition of the nanobioglass. Furthermore, the presence of an acidic self-etching primer in the composition of the self-etch adhesive has demineralised the peritubular dentin. The dissolved ions from the peritubular dentin, in addition to the calcium and phosphate ions from the bioglass, all together have formed a precipitate that might have occluded the cut dentinal tubules, thus reducing POS.

These results were similar to the findings of a previous study [11] who incorporated nanoparticles of amorphous calcium phosphate (NACP) into dental adhesives. They found numerous NACP nanoparticles in the adhesive layer, in the hybrid zone, and inside the dentinal tubules. NACP was not only able to infiltrate with the adhesive into straight tubules but also into bent and irregularly shaped tubules without impairing the adhesive bond strength to dentin.

In conclusion, within the limitations of this clinical trial, we have concluded that the problem of postoperative sensitivity following posterior resin composite restorations could be solved by the addition of bioglass nanoparticles into dental adhesives.

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Dental Science

Comparison of Salivary Cortisol Level in Type 2 Diabetic Patients and Pre-Diabetics with Healthy People

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Abstract

BACKGROUND: Cortisol is one of the important enzymes of saliva. Control of this hormone is an effective way to adjust the glucose level in diabetic patients.

AIM: The aim of this research is to compare the salivary cortisol level in type 2 diabetic patients and pre-diabetics with healthy people.

METHODS: In this case-control study (2018), the unstimulated salivary samples were collected from 44 patients with type 2 diabetes, 44 pre-diabetic people (case group), and 44 healthy subjects (control group), matched for age and gender. The samples were transferred to the laboratory, and salivary cortisol level was measured using ELISA. Data were analysed using SPSS 22 and Chi 2 tests.

RESULTS: The mean salivary cortisol level in type 2 diabetic patients was 3.14 ± 1.17, in pre-diabetic cases was 1.83 ± 0.68, and in healthy controls was 0.86 ± 0.43 ($P < 0.001$). The mean DMFT in type 2 diabetic patients was 19.6 ± 6.5, in the pre-diabetic group was 13.43 ± 4.5, and in healthy controls was 9.38 ± 3.72 ($P < 0.001$).

CONCLUSION: With regards to the results, salivary cortisol level in type 2 diabetic patients is more than pre-diabetic people, and in pre-diabetic people is more than healthy people. Also, there was a significant relation between salivary cortisol level and DMFT index.

Introduction

Diabetes mellitus is a multi-agent metabolic disease characterised by increased blood glucose and metabolic disorders of carbohydrates, fat and protein [1]. Increased blood glucose results from impaired secretion of insulin and liver gluconeogenesis [2], [3]. Pre-diabetes is a condition in which blood glucose is somewhat high, but the patient does not have all the criteria for diabetes. Fasting blood glucose in pre-diabetes is 100 to 125 mg/dL, while in diabetes, fasting blood glucose is 126 or above 126 mg/dL [4]. The two main types of diabetes include type 1 diabetes (insulin-dependent) and type 2 diabetes (non-insulin dependent). The global prevalence of diabetes is increasing, and it's predicted to reach from 180 million in 2000 to 320 million in 2025 [5], [6]. The prevalence of diabetes in Iran is close to its global value as about 5.5% of the population [7].

Diabetes Mellitus has a very complex clinical presentation and is associated with complications such as nerve, kidney and retina damage and cardiovascular disease [8], [9]. The oral manifestations of diabetes are variable and are commonly manifested in patients with poorly controlled blood glucose. Some oral complications of diabetes include dry mouth, gingivitis, periodontitis, dental abscesses and soft tissue lesions of the tongue and oral mucosa [10], [11].

Diabetic patients need to control blood

glucose levels to diagnose, treat and track their illness. Typically, a blood sample for analysis is obtained through the vein or by finger stick or other aggressive methods which often result in physical and mental stress and pain in the patient. Because of this, the use of other biological fluids, such as saliva, is desirable to determine the level of glucose in the blood and avoid these invasive methods [12]. Salivary glands dysfunction is one of the problems that is commonly referred to by diabetic patients. This can be a direct result of the patient's medical condition and inadequate control of their blood glucose [13]. Therefore, many salivary compounds, such as enzymes, can be reliably considered for diagnosis and prognosis because saliva is readily available [14], [15], [16], [17].

Studies have shown that the determination of salivary combinations in diabetic patients can be useful in the detection and control of oral complications of diabetes [18], [19], [20]. One of the compounds in the saliva is cortisol. Cortisol is a glucocorticoid hormone secreted from the adrenal cortex and plays a role in regulating mineralocorticoids, immune system function, blood pressure and metabolism. Conditions such as hypertension, hypercholesterolemia, central obesity and glucose intolerance are associated with increased levels of cortisol. Following the changes in blood cortisol levels, the amount of this hormone also changes in the saliva [21], [22]. Salivary cortisol level has recently been suggested as a valuable recommendation for blood cortisol analyses. Given that this method is non-invasive and requires not much laboratory procedures, it can be investigated in unlimited cases [23]. Shirzaii et al., a study in 2016 showed that salivary cortisol level is higher in people with type 2 diabetes compared to healthy subjects [24].

Dental caries is the most common disease which itself is the most common dental disease. In many cases, dental caries leads to tooth pulp infection, along with pain in the patient [25]. In 1938, a person by the name of Palmpour presented the DMFT index which was suggested by World Health Organization (WHO) and the International Dental Federation (FDI) to evaluate the variability of teeth health [26].

There are controversial reports about the role of cortisol in the aetiology of diabetes, and few studies have been conducted on the evaluation of salivary cortisol levels, and also there have been no studies on the evaluation of salivary cortisol levels in pre-diabetic patients and the relationship between salivary cortisol level and DMFT index. Therefore, the present study aimed to compare salivary cortisol level in diabetic and pre-diabetic individuals with healthy subjects and its relationship with DMFT index.

Material and Methods

This is a case-control study which was conducted on patients referring to the health centre of Sari city in 2018. The sample size was estimated as 31 subjects for each group based on Shirzaii et al., study while considering the results of the study (mean and standard deviation of salivary cortisol levels in diabetic patients were 1.73 and 1.017 and 1.08 and 0.643 in the control group respectively), confidence level of 95% and power of 90% were estimated using the formula for comparing the two meanings in the G-power software. Regarding the fact that the number of studied groups was 3, the final sample size was adjusted according to the following formula, and it was determined as 132 subjects (44 in each group) [24].

$$n' = n * \sqrt{g-1}$$

The ethics code to this research was considered as IR. MAZUMS. REC. 1397.1188 after being approved by the Medical Ethics Committee. Samples were selected by simple sampling method, and written consent was obtained from patients for participation in the study. In this study, subjects were divided into three groups with 44 subjects in each. The first group consisted of all people with type 2 diabetes whose fasting blood glucose was 126 or greater than 126 mg/dL and had complete medical file and records and at least 5 years history of diabetes, had no other systemic diseases and weren't taking any other medications other than glucose controlling ones. The second group consisted of pre-diabetic subjects whose fasting blood glucose was 100 to 125 mg/dL and the third group consisted of healthy subjects without symptoms of diabetes (obesity and familial history of diabetes) or history of taking medications in the last three months that were matched with case group in terms of age and gender.

Exclusion criteria included: Body Mass Index more than 30, pregnancy, tobacco use, drugs and alcohol, Addison's systemic disease, Cushing's syndrome and thyroid disorders, history of injury and surgery in the past four weeks, history of malignancy, use of corticosteroid or hormone therapy and mental and sleep disorders.

All the participants had a blood test in the recent last month. All data, including gender, age, FBS, HbA1C and DMFT, were recorded. To evaluate the DMFT index, dental surfaces were dried using air syringe and carefully examined for the presence of tooth-coloured and non-tooth-coloured restorations. In this study, M represents the number of teeth lost due to decay. Whenever there is a lesion that appears on the smooth dental surfaces or inside pits and grooves that are embedded in the enamel or is softened on the floor and around it, the tooth is considered decayed (D). In this study, teeth that have cavities and are

dressed with temporary filling materials, as well as teeth with restorations that still contain caries are classified as Decayed teeth. Damaged teeth or treated decayed teeth whose appearance, function and size are restored to normal, are considered as Filled (F) which is referred to as the number of teeth that have undergone root canal therapies or are filled with restorative materials. After recording the mentioned items, the obtained numbers are summed, and the result determines the DMFT in each individual. The examination of teeth was conducted under the light on a dental chair [27].

To determine salivary cortisol level, subjects were asked not to eat, drink and brush 90 minutes before sampling. Subjects' total non-stimulatory salivary samples (by spitting) were obtained while sitting on a chair upright with their head slightly tilted forwards in a calm environment between 10 to 12 A. M. (near the peak of serum cortisol level). The collected samples were immediately put into the numbered test tubes and transferred to the immunology laboratory of the Baghban clinic of Sari. At the laboratory, saliva samples were centrifuged at centrifugal speeds of 2000 rpm for 10 minutes, and then the supernatant was stored at -20°C until the test. The level of salivary cortisol was measured by ELISA method using the Diameter kit (made in Italy).

Results

In this study, 44 diabetic patients, 44 pre-diabetics and 44 healthy people participated. There were 34 females (77.3%) and 10 males (22.7%) in all groups. The mean age in the diabetic group was 54 years and 48/07 in the pre-diabetic group, and the healthy group it was 42.86 years. The mean FBS in the diabetic group was 188.91 mg/dL, in the pre-diabetic group, it was 108.75 mg/dL, and in the healthy group it was 88.59 mg/dL. The mean salivary cortisol level in people with diabetes was reported as 3.13 mmol/dL, in pre-diabetics as 1.83 mmol/dL and healthy subjects as 0.85 mmol/dL. The mean DMFT index in people with diabetes was 19.56, in pre-diabetic individuals was 13.43, and in healthy subjects it was 9/38 (Table 1).

Table 1: Specifications of participants in the study

Title	Range	Mean ± Standard deviation	Groups	p-value
Age (year)	40-60	54 ± 5.70	Diabetics group	< 0/100
	31-60	48.07 ± 8.68	Pre-diabetics group	
	30-60	42.86 ± 11.90	Healthy group	
FBS (mg/Dl)	126-342	188.91 ± 48.91	Diabetics group	< 0/100
	100-124	108.75 ± 7.78	Pre-diabetics group	
	70-99	88.59 ± 7.49	Healthy group	
Cortisol (mmol/Dl)	1.4-6.8	3.14 ± 1.17	Diabetics group	< 0/100
	0.2-3.1	1.83 ± 0.68	Pre-diabetics group	
	0.3-1.8	0.86 ± 0.43	Healthy group	
DMFT	10-28	19.6 ± 6.5	Diabetics group	< 0/100
	6-28	13.43 ± 4.5	Pre-diabetics group	
	4-18	9.38 ± 3.72	Healthy group	

In a comparison between the two genders, the mean cortisol level in males was 2.34 mmol/dL, and in women it was 1.83 mmol/dL. According to the results, there is a significant difference in the cortisol levels between male and female diabetics (P = 0.005), but there are no significant differences in the cortisol levels between males and females in pre-diabetic and healthy groups (P = 0.051 and P = 0.624). In a comparison of cortisol levels between the three groups, the differences in salivary cortisol levels were significant among males (P < 0.001) and females (P < 0.001) (Table 2).

Table 2: Comparison of cortisol levels in both genders

Gender / Group	Female	Male	p-value
Diabetes	2.88 ± 0.97	4.02 ± 1.42	*0.005
Pre-diabetes	1.73 ± 0.69	2.2 ± 0.46	0/051
Healthy	0.88 ± 0.44	0.8 ± 0.39	0/624
Total	1.83 ± 1.09	2.34 ± 1.59	0/046*
p-value	* < 0.001	* < 0.001	------

*p < 0.05.

In a comparison of DMFT index between males and females, the mean DMFT index is 15.3 in males and 13.79 in females. According to the table below, there are no significant differences in the DMFT index between males and females in any of the participating groups. With the DMFT compared in the three groups, the DMFT index was significantly different among males (P < 0.001) and among females (P < 0.001) (Table 3).

Table 3: Comparison of DMFT index in both genders

Gender / Group	Female	Male	p-value
Diabetes	18.5 ± 6.7	22 ± 5.39	0/183
Pre-diabetes	13.1 ± 4.8	14.5 ± 2.72	0/396
healthy	9.41 ± 3.7	9.3 ± 3.97	0/935
total	13.79 ± 6.49	15.6 ± 6.65	0/280
p-value	* < 0.001	* < 0.001	---

*p < 0.05.

Regarding HbA1C, diabetic subjects were divided into two subgroups of controlled diabetes (7 > HbA1C) and uncontrolled diabetes (7 < HbA1C) and cortisol levels and DMFT index were evaluated. In the comparison between the two subgroups, 12 subjects were in the controlled group and 32 in the uncontrolled group. The mean cortisol level in the controlled group was 3.03, and in the uncontrolled group it was 3.49, and its P-value was reported as 0.94 by the Mann-Whitney test, which is statistically not significant. In a comparison of DMFT between the two groups, the mean DMFT was 16.83 in the controlled diabetes group and 20.6 in the uncontrolled diabetes group. Using the Mann-Whitney test, the P-value was reported to be 0.86, which is statistically not significant (Table 4).

Table 4: Comparison of cortisol levels and DMFT in diabetics group based on HbA1c levels in patients

	Number	DMFT	Cortisol level
Controlled diabetes	12	16.83 ± 6.10	3.03 ± 0.74
Uncontrolled diabetes	32	20.59 ± 6.47	3.49 ± 1.29
p-value	---	0.89	0.124

*p < 0.05.

Comparing the studied groups for age, FBS, salivary cortisol level and DMFT index, the results of the study showed a significant difference between the studied groups and the results are presented in Table 5.

Table 5: Significance level of inter-group relations

Treatment groups P-value	Age	FBS	Cortisol	DMFT
Healthy subjects & pre-diabetic patients	*0.022	*< 0.001	*< 0.001	*< 0.001
Healthy subjects & diabetic patients	*< 0.001	*< 0.001	*< 0.001	*< 0.001
Diabetic & pre-diabetic patients	*< 0.001	*< 0.001	*< 0.001	*< 0.001

*p < 0.05.

According to the results presented in Table 6, cortisol level was 0.97 units (P < 0.001) in pre-diabetic subjects and 2.28 (P < 0.001) in diabetic subjects higher than healthy subjects which are statistically significant. By adjusting the effects of variables such as age, gender and FBS, cortisol level in pre-diabetic subjects is 0.33 and in diabetic subjects is 0.62 units higher than healthy subjects, which is statistically significant. Based on the results of Kruskal-Wallis test, cortisol, as a dependent variable, as compared with independent variables in this study including gender, age, and FBS and significant results were reported. Based on the results of single-variable linear regression, for each unit of increase in age, cortisol level increases by 0.04 units, which was statistically significant (P < 0.001), but the effect of age on the cortisol level after adjusting other variables in this study was not statistically significant and considerable, and for each unit of increase in age, cortisol level decreases by 0.2 units (P = 0.713). In the assessment of the relationship between gender and salivary cortisol level, cortisol level in females was 51.1 mmol/dl less than males, which is statistically significant (P = 0.046). After adjusting other variables in the study, cortisol level in females was 0.15 units lower than males, which is still statistically significant (P = 0.01). In the assessment of the relationship between FBS and salivary cortisol level, for each unit of increase in FBS, the cortisol level increases by 0.02 units, which is reported as a significant relationship (P < 0.001). By adjusting the effects of other variables, the cortisol level increases by 0.28 units for each unit of increase in FBS, which is statistically significant (P = 0.006).

Ultimately, in the assessment of the relationship between cortisol level and DMFT, the correlation coefficient between cortisol level and DMFT was 0.5, and this correlation is statistically significant (P < 0.001).

Table 6: Investigation of factors related to cortisol level

Title	Multi-variable analysis		Single-variable analysis	
Variables	P-value	Beta factor	P-value	Beta factor
Pre-diabetics group (reference=healthy people)	*<0.001	0.33	*<0.001	0.97
Diabetics group (reference=healthy people)	*<0.001	0.62	*<0.001	2.28
Age	0.713	-0.02	*<0.001	0.04
Gender	*<0.010	-0.15	*0.046	-0.51
FBS	*0.006	0.28	*<0.001	0.02

*p < 0.05.

Discussion

This study was conducted to compare the salivary cortisol levels between the diabetic, pre-diabetic and healthy groups and also to investigate its relationship with DMFT. According to the results, the mean cortisol level in diabetic patients was higher than the pre-diabetic group and also in pre-diabetic group it was higher than in healthy subjects. These differences were statistically significant. Also, cortisol levels in patients with uncontrolled diabetes were more than those with diabetes, but this difference was not statistically significant. In a comparison between the two genders, the cortisol level in diabetic males was higher than diabetic females, but there were no significant differences in cortisol level between the two genders in the pre-diabetic and healthy group.

Investigating the DMFT index, the mean DMFT in diabetic patients was higher than pre-diabetic patients, and in pre-diabetic patients, it was higher than healthy subjects making these differences significant. In a comparison between the two genders, there were no significant differences in the DMFT index between males and females. There is a significant relationship between the mean DMFT and salivary cortisol levels in the studied subjects.

There have been some studies about the relationship between the blood glucose and salivary cortisol level increase, which are reviewed:

According to a study by Shirzaii et al., in Zahedan in 2016 with the aim of comparing salivary cortisol levels in type 2 controlled diabetic patients with healthy subjects, the mean salivary cortisol level in type 2 diabetic patients was 1.73 and in healthy subjects it was reported as 1/08 and it has been stated that there is a significant relationship between them and the mean cortisol level in diabetic patients was higher than healthy subjects. This result is similar to the present study. There have been no significant differences in the cortisol level in Shirzaii study between the two genders. Also, in this study cortisol levels in diabetic patients have been reported higher than Shirzaii study which is due to differences in sampling conditions, geographical location, and living conditions of the studied subjects [24].

In a study by Chiodini et al., In Italy in 2007, with the aim of evaluating salivary cortisol secretion in type 2 diabetic patients, the hypothalamus-pituitary-adrenal axis activity and cortisol secretion in diabetic patients was higher than healthy subjects (0001 > P) and it's been stated that it depends on the complications of diabetes. In this study, factors such as gender, duration of diabetes and HbA1c levels are deemed affect cortisol levels. These results are similar to the results of the current study with the difference that in Chiodini study no tests were conducted about pre-diabetic subjects [28].

In a study by Roy et al., 1990 regarding

hypothalamic-pituitary-adrenal axis disorder in diabetic patients, glucocorticoid and cortisol secretion were higher in patients with type 2 diabetes mellitus and insulin resistance. Regarding this study, an increase in cortisol secretion leads to diabetes and makes metabolic control difficult. In the current study, similar to Roy study, cortisol secretion has been observed to be higher in people with diabetes than healthy subjects, but no results on the effects of cortisol secretion on diabetes occurrence have been achieved [29].

In a study by Liu et al., in the United States in 2005 regarding the level of salivary cortisol in soldiers with diabetes, similar to the current study, there is an increase in cortisol levels in diabetic patients [30].

In 1998, Roy et al., in a study on cortisol levels in diabetic subjects, found that diabetic patients with retinal damage and cardiovascular complications had higher levels of cortisol. They have pointed to rising cortisol levels in diabetic patients. However, the current study suggests that cortisol levels may still increase even if no diabetes complications occur, and blood glucose control is adequate [31].

In another study by Hackett et al., In 2014 about the association between the daily pattern of cortisol and type 2 diabetes, it was concluded that salivary cortisol levels in patients with type 2 diabetes increase only during sleep and is not much changed during the day, while the current study showed that salivary cortisol levels increase significantly in the morning compared to non-diabetic subjects. Measuring salivary cortisol levels frequently in a day can justify these differences [32].

Radahmadi et al., in a similar study in 2004 on the effects of psychical stress on exacerbation of diabetes mellitus, serum glucose, cortisol levels and body mass in rats found that cortisol secretion levels in people with diabetes mellitus increased significantly and the effects of diabetes on cortisol secretion is more than mental stress. Diabetes mellitus is a strong stimulant for the physiological system of the body [33].

All of the studies mentioned above have been based on the correlation between cortisol and blood glucose in diabetic patients and no studies have been conducted on pre-diabetic patients. According to our study, an increase in blood glucose levels in pre-diabetes may also increase salivary cortisol levels.

One of the oral complications of diabetes is dry mouth and increased glucose level of gingival crevicular fluid, which can increase dental caries occurrence. Another result of our study is the increase in DMFT in diabetic patients, which is reviewed in the following in some similar studies.

According to a study by Behbahani and Yasin in Ahvaz in 2017 with the aim of determining the relationship between DMFT index, FBS and HbA1C in type 2 diabetic patients, it is stated that there is a significant relationship between DMFT and blood glucose which is similar to the results of the current study. The prevalence of DMFT in the Behbahani study was 51.51, which is slightly lower than the current study. This difference can be due to differences in culture and the degree of oral hygiene in that area [34].

In 2010 in a study on the impact of diabetes on the prevalence of dental problems, Miko et al. stated that poor blood glucose control and preterm diabetes could increase the risk of dental caries. This finding is consistent with the results of the current study [35].

In a study by Miralles et al., in 2006 on the effects of systemic factors of diabetes on the development of dental caries, diabetes mellitus has been shown to increase dental caries, and dental caries has been more common in the diabetic population. These results are similar to the results of the current study [36].

All of the above studies have been conducted to investigate the relationship between diabetes and DMFT, but no studies have been done about the relationship between diabetes and DMFT on pre-diabetic subjects. According to the present study, it seems that increased blood sugar even in pre-diabetics may increase DMFT and pre-diabetes is a risk factor for dental caries.

Another finding from the present study is the relationship between salivary cortisol levels and DMFT. In a study by Golestannezhad et al., in Isfahan in 2014 on dental caries rampancy in patients with migraine, it has been stated that during migraine attacks, due to increased activity of the hypothalamus-pituitary-adrenal axis, salivary cortisol levels increase. It has also been stated that there is a significant relationship between migraine and increased dental caries [37].

The mentioned study above indirectly points out the relationship between cortisol and DMFT, but no studies have been done to assess the relationship between salivary cortisol levels and DMFT index so far. According to the findings of the current study, there is a significant relationship between salivary cortisol levels and DMFT index, and it seems that increased salivary cortisol levels increase the risk of dental caries. The exact mechanism of this association is unclear, but increased cortisol levels may increase the risk of dental caries by increasing the amount of glucose in the gingival crevicular fluid in diabetic and pre-diabetic subjects.

In a study by Farahat et al., in Yazd in 2013, on the status of DMFT index in patients with type 2 diabetes and its relationship with HbA1C, it has been observed that DMFT index in patients with uncontrolled diabetes is significantly higher than patients with controlled diabetes. In the present study, DMFT is higher in patients with uncontrolled diabetes than in patients with controlled diabetes, but this

difference was not statistically significant [38]. Also, no significant differences were found between salivary cortisol levels in these two groups, and no studies have been done on this issue so far.

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Dental Science

Influence of Medicinal Plant Extracts on the Growth of Oral Pathogens *Streptococcus Mutans* and *Lactobacillus Acidophilus*: An *In-Vitro* Study

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Abstract

AIM: This study investigated the antibacterial efficacy of five plant extracts, as well as the combinations of the two most effective plant, extracts either with or without commercial varnish (MI varnish) on the in vitro growth of *Streptococcus mutans* and *Lactobacillus acidophilus* in comparison to MI varnish using agar disk diffusion and broth dilution methods.

METHODS: Methanolic extractions of five plants (Cinnamon, Turmeric, Ginger, Clove and Black seed,) were tested against the growth of the two oral pathogens. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined for the two most effective extracts, and their combinations with different ratios were evaluated against the growth of the two oral pathogens, followed by incorporating the two effective plants or each into commercial MI varnish to be assessed against the oral pathogens in comparison to MI varnish.

RESULTS: Only Cinnamon and Clove produced inhibition zones against *Streptococcus mutans* and *Lactobacillus acidophilus* growth. MIC for the two plants showed equal antimicrobial activity against *Streptococcus mutans*, while Cinnamon had a higher sensitivity to *Lactobacillus acidophilus* than Clove. A mixture of Cinnamon and Clove in a ratio 1:2 exhibited the highest antibacterial activity. Integration the mixture of both plants into MI varnish in a ratio of 1:1:1 presented the highest antibacterial activity. Meanwhile, the lowest one was recorded for the MI varnish alone.

CONCLUSION: Methanolic extract of Cinnamon and Clove has considerable antimicrobial activity against *Streptococcus mutans* and *Lactobacillus acidophilus* and a new tool for minimally invasive and adhesive dentistry avenues.

Introduction

Bacteria Dental caries is one of multifactorial infectious disease caused by acids from bacterial metabolic activity diffusing into enamel and dentine. Even as caries is a profoundly preventable disease that has seen a decline in most developed international locations these days, it stays a noteworthy public fitness problem [1], [2]. The principal etiologic factor included in the presence of specific bacteria particularly Streptococcus mutans and other non-streptococcus species like Lactobacillus acidophilus which produce acid and bring the plaque to the critical pH [3]. Numerous preventive strategies have been attempted and tested yet none is ended up being 100% powerful for the cessation of dental caries and the journey still goes on. Right now, Chlorhexidine is thought to be the gold standard level among chemotherapeutic agents

against the most cariogenic pathogen *Streptococcus mutans*, however, the occurrence of oral side effects, for example, teeth staining, bad taste, dryness, and burning sensation debilitate patients to utilise it [4]. More critically, most antibacterial agents can also promote the development of resistant bacterial strains [5]. Therefore, it becomes a necessity for the current therapeutic research to investigate naturally available products which are safe for humans and specific for dental caries, as their side effects are insignificant, and the patient is dealt with comprehensively. Several investigations have been done to decide the utilisation of normal regular household natural basic oils including Cloves and Cinnamon as solutions for managing dental diseases like toothache and gum swelling [6], [7]. More recently, scientific research demonstrated the potential antibacterial properties of concentrates from these herbs [6], [8], [9]. Use of the improvement of such bioassays in clinical science may offer valuable learning and a way to manage oral disease. One therapeutic plant in selected group is Cinnamon (Cinnamomum zeylanicum) which is thought to have medical advantages [10] and has been utilised as a part of the conventional prescription for colds, flatulence, nausea and diarrhoea, also improves vitality, circulation and energy [11].

Additionally, studies have discovered that Cinnamon and Ginger (Zingiber officinale) may have antibacterial and antifungal properties [12], [13]. Turmeric (Curcuma longa) belongs to the Ginger (Zingiberaceae) circle of relatives. Since the time of Ayurveda (1900 BC) many therapeutic sports had been assigned to Turmeric for more than one illness and situations, like the ones of the pores and skin, pulmonary, and gastrointestinal structures, aches, pains, and liver problems [14]. Although several plants have demonstrated antibacterial activity however, to date, their antibacterial activity against cariogenic bacteria is still under research. Hence in search for novel anti-cariogenic agents, five plants which are known for their medical applications have been chosen in this study; Cinnamon, Ginger, Turmeric, Cloves and Black seed [15], [16]. Therefore, in the present study, we found it interesting to investigate the antibacterial effect of the chosen five plants in terms of bacterial growth inhibition of *Streptococcus mutans* and *Lactobacillus acidophilus*. In addition, the current study aimed to investigate the antibacterial activity of the two most effective plants in combination with commercial varnish [5% sodium fluoride varnish with Recaldent (CPP–ACP), GC America, USA] where various reports have been published on the anti-cariogenic action of casein phosphopeptide amorphous calcium phosphate (CPP-ACP) paste/solution [17], [18], [19] and the synergistic impact of CPP-ACP and fluoride [17], [18], [19], [20], meanwhile no trials have been made on medicinal plants antibacterial effect in combination with CPP-ACP.

Material and Methods

Different plants preparation

Five plants; (Cinnamon; bark) Cinnamomum zeylanicum, (Turmeric; rhizome) Curcuma longa, (Ginger; rhizomes) Zingiber officinale, (Clove; fruits) Syzygium aromaticum, and (black seed; seeds) Nigella sativa were tested.

Tested plants were purchased from local stores in Ismailia, Egypt. The collected plants were re-identified, and the nomenclature was rechecked and confirmed by the help of plant taxonomist. All raw substances had been washed with clean tap water, then through sterilised distilled water and air-dried. An exception for Ginger rhizomes which had been reduced wiped clean then dried in a vacuum oven at 80°C for two days. All dried plant life had been powdered the usage of the sterilised grinding system. The obtained powder was immediately subjected to the extraction procedure [21], [22].

Extracts preparation

Fifty grams of each plant powder were packed in the Soxhlet thimble then extracted successively with an organic solvent 90% methanol. Extraction carried out for each plant separately for forty-eight hours using Soxhlet extractor [23]. Each extract was filtered through filter paper (Whatman No.1) then concentrated by complete evaporation with a rotary evaporator under reduced pressure. The resulting dry extracts were re-weighed, and the percentage of the resultants were calculated from the quantity of the initial plant material (50 g). Crude extracts stock solutions were prepared by mixing dried extracts with an appropriate amount of DMSO (100%) and stored at 4°C in an airtight sterilised bottle till use [24].

Tested bacterial strains

Two bacterial strains were tested; *Streptococcus mutans* Serotype c. Carious dentin ATCC 25175 type strain which was purchase from Microbiological Resources Centre (MIRCEN), Cairo, Egypt and *Lactobacillus acidophilus* CH-2 from Chr. Hansen's Lab, Denmark. *Streptococcus mutans* was streaked on tryptic soy agar (TSA) while *Lactobacillus acidophilus* MRS agar. Both cultures were incubated for twenty-four hours at 37°C.

Antibacterial assessment

Antibacterial activities of methanolic extracts of different plants were carried out by disc diffusion assay according to the standard method [25], [26].

Disc diffusion method

The inoculum was prepared as recommended by the Clinical and Laboratory Standards Institute by direct colony suspension method [27]. Colonies of an overnight culture of both *Streptococcus mutans* and *lactobacillus acidophilus* were suspended in Mueller-Hinton broth (Oxoid) and adjusted to 0.5 McFarland standards to reach a final inoculum corresponding to approximately 1 x 10^8 CFU/ml.

Total of sixty sterilised (6 mm) filter paper discs were divided into six groups which had been loaded with the different plant extracts (G1; Cinnamon, G2; Turmeric, G3; Ginger, G4; Clove, G5; Black seed, G6; positive control) (n = 10). Each group was further subdivided according to the type of bacterial strain that was tested on into two main subgroups (n = 5). The discs were saturated with 10 µl of different plant extracts separately under aseptic conditions and 0.12% Chlorohexidine Digluconate liquid (CHX) (Sigma Aldrich, Steinheim, Germany) as a positive control. Loaded discs were dried in laminar flow for 15 minutes at room temperature and were used for the disc diffusion assay. 100 µl from each bacterial strain was streaked separately using a sterile swab to Muller Hinton agar plates. Plates were let to dry for 15 minutes then used for the sensitivity test. All loaded discs were placed on the surface of the inoculated Mueller-Hinton agar (Figure 1). Plates were incubated for overnight at 37°C, and the antibacterial activity of each extract was expressed by measuring the diameter of the inhibition zone (mm). The experiment was done in triplicate to ensure consistency.

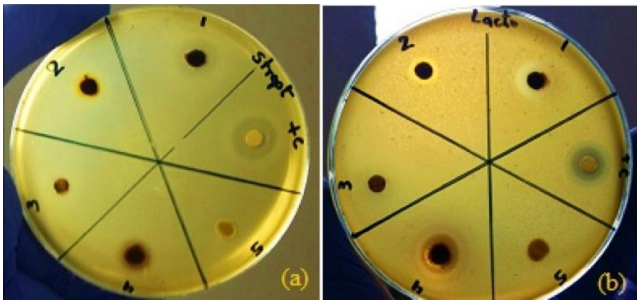

Figure 1: Antimicrobial activity of the five plant extracts against A) Streptococcus mutans estimated by the agar-Disc diffusion method and B) Lactobacillus acidophilus estimated by the agar-Disc diffusion method

Broth dilution method

The two plant extracts; Cinnamon and Clove that showed effective antimicrobial activity, were selected to demonstrate their minimum inhibitory concentrations (MICs) and Minimum Bactericidal Concentrations (MBC) according to the Clinical and Laboratory Standard Institution strategies by serial two-fold micro broth dilution technique [27]. Mueller Hinton broth (MHB) was prepared and poured into sterile test tubes. One colony of each tested bacterial strains (*Streptococcus mutans* and *Lactobacillus acidophilus*) were inoculated separately in 2.5 ml MHB and incubated overnight at 37°C on a shaker (250 rpm). 0.5 ml of each overnight culture were inoculated into 5 ml pre-warmed MHB then incubated at 37°C on a shaking incubator for about 18 h to a final optical density (OD600) of 1. A serial dilution was done to different plant extracts in MHB medium to reach concentrations ranging from 100 to 1.563 mg/ml. 100 µl of each prepared bacterial strain was inoculated to the tubes with different concentrations of plant extracts. A tube of MHB supplemented with different plant extracts was left uninoculated and used as a negative control for each dilution. For positive control, 100 µl of both bacterial strains were inoculated to MHB tubes without plant extract. Tubes were incubated at 37°C for overnight and the lowest concentration that inhibits the bacterial growth was considered as the MIC value for each of the tested bacteria strain. To determine MBC value, sterilised Muller–Hinton agar (MHA) was poured into a Petri dish and was let to solidify. Samples that showed no obvious bacterial growth were streaked on the surface of the agar separately then incubated for twenty-four hours at 37°C. The lowest concentration which displayed no growth on the MHA plates was recorded as the MBC.

Combination test

The two selected plant extracts Cinnamon and Clove were combined in three different concentrations by weight: Cinnamon: Clove; C1: 1:1, C2: 1:2, and C3: 2:1 respectively. Three discs loaded with 10 µl of each combination were tested against the growth of each tested bacterial strains (*Streptococcus mutants* and *Lactobacillus acidophilus*) using disc diffusion method as previously mentioned and utilise CHX and DMSO as a positive and negative control.

Another three different combinations and commercial MI Varnish' [5% sodium fluoride varnish with Recaldent (CPP–ACP), GC America, USA] were assessed against the growth of each tested bacterial strains using disc diffusion method. Each plant extract was incorporating into commercial MI Varnish separately by weight (V1; 1:1 = Cinnamon: MI, V2; 1:1 = Clove: MI) and a third combination was prepared by mixing two plant extracts with MI Varnish (V3; 1:1:1 = Cinnamon: Clove: MI). Ten µl of each mixed combination was added to a sterile disc, and their antibacterial activity was tested in comparison to MI varnish (V4).

Statistics have been explored for normality the usage of Kolmogorov-Smirnov and Shapiro-Wilk assessments; statistics confirmed parametric (ordinary) distribution.

Repeated measure ANOVA changed into used to examine among extra than groups in related samples. Paired wise sample t-test turned into used to

compare among two groups in related samples. One-way ANOVA accompanied by way of Tukey post hoc test was used to compare among greater than two groups in non-related samples. The importance level became set at $P \leq 0.05$. Statistical evaluation becomes carried out with IBM® SPSS® statistics model 20 for windows.

Results

Inhibition zones results

Regarding the antibacterial activity of the tested five plant extracts in the disc-well diffusion method; the methanolic extracts of Cinnamon (G1) and Cloves (G4) showed antibacterial activity with inhibition zones diameters of 14.00 mm and 12.67 mm against *Streptococcus mutans* and 16.67 mm and 18.67 mm against *Lactobacillus acidophilus*, respectively, while the other plant extracts did not demonstrate any antibacterial activity against the two strains except methanolic extract of Turmeric showed antibacterial activity against *Lactobacillus acidophilus* with inhibition zones diameters of 9.33 mm. For the antibacterial activity against *Streptococcus mutans*, the highest inhibition mean value was found in the positive control group (G6) followed by (G1) and (G4) respectively. Meanwhile, the lowest mean value was recorded for (G2, G3 and G5) where ($P < 0.001$). While for the antibacterial activity against *Lactobacillus acidophilus*, the highest mean value was found in (G4) followed by (G6) followed by (G1) followed by (G2) respectively, meanwhile the lowest mean value was found in (G3), (G5) where ($P < 0.001$) (Figure 2).

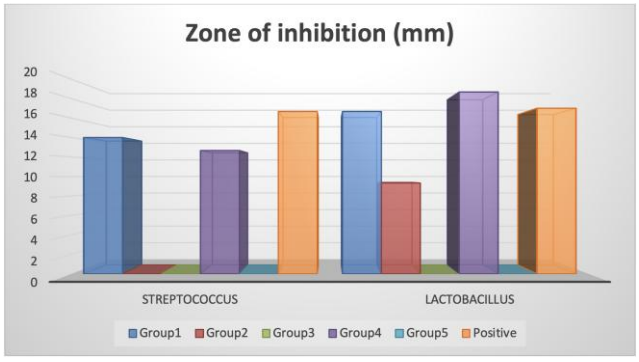

Figure 2: Bar chart representing Comparison in the mean value of inhibition zones with the different plant extracts against Streptococcus mutans, and Lactobacillus acidophilus

MIC and MBC results

The MIC and MBC were carried out for the Cinnamon (G1), and Clove (G4) extracts which were the most effective extracts according to disk diffusion test results. No statistically significant difference was found between (G1) and (G4) in MIC with *Streptococcus mutans* where ($P = 0.364$). While the MIC for *Lactobacillus acidophilus* was exhibited a statistically significant difference between (G1) and (G4) where ($P < 0.001$). A statistically significant difference ($P < 0.001$) was found in the MBC value for both strains between (G1) and (G4). Where the highest mean value of MIC and MBC for positive inhibitory effects with *Streptococcus mutans* and *Lactobacillus acidophilus* was found in (G4) while (G1) showed the lowest mean value (Table 1).

Table 1: MIC and MBC results of Cinnamon and Clove extracts (mg/mL)

Variables	MIC				MBC			
	S. mutans		*L. acidophilus*		*S. mutans*		*L. acidophilus*	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Group 1	13.44	4.09	5.18	0.77	23.60	1.52	16.40	2.43
Group 4	15.56	2.73	13.92	1.34	48.25	1.71	51.20	3.11
P-value	0.364ns		< 0.001*		< 0.001*		< 0.001*	

*; significant ($P \leq 0.05$) ns; nonsignificant ($P > 0.05$).

Antibacterial activity of the three different combinations between Cinnamon and Clove extracts

A statistically significant difference was recorded between the cinnamon extract mixed with Clove extract in the combination ratio of 1:1 (C1) and 2:1 (C3) where the mean inhibition zone diameter was (18.33 ± 0.58) and (19.00 ± 1.00) for C1 and (14.00 ± 1.00) and (16.33 ± 0.58) for C3 against *Streptococcus mutans* and *Lactobacillus acidophilus* respectively. Also, a statistically significant difference was found between C2 (1:2) and C3 where the mean inhibition zone diameter was (18.67 ± 0.58, 19.67 ± 0.58) respectively. While there was no statistically significant difference between the combinations (C1) and (C2) against the two oral pathogens where ($P < 0.001$). The highest mean value was found in (C2) followed by (C1), (positive) and (C3) while the lowest mean value was found in the negative control group (Figure 3).

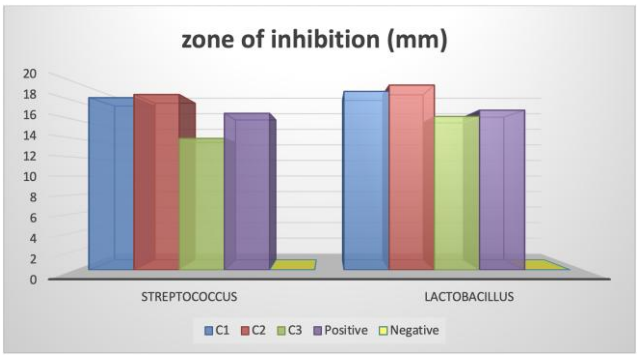

Figure 3: Bar chart representing Comparison in the mean value of inhibition zones with the three different combinations between Cinnamon and Clove extracts against Streptococcus mutans and Lactobacillus acidophilus

Antibacterial activity of the two-plant extract incorporating each/ both into MI varnish

From Figure 4 regarding the inhibition zones against *Streptococcus mutans* growth showed a statistically significant difference between the mixture of Cinnamon and Clove extract with MI Varnish in a ratio 1:1:1 (V3) and each of V1 (Cinnamon: MI = 1:1) and V2 (Clove: MI = 1:1) where ($P = 0.003$), and ($P < 0.001$). Also, a statistically significant difference was found between V3 and V4 (MI varnish) where ($P < 0.001$). While there was no statistically significant difference was found between (V1) and (V2) ($P < 0.001$). The highest mean inhibition zones value was found in (V3) followed by positive, (V1), V2 and (V4) groups while the lowest mean value was noted in the negative group. For the inhibition zones against *Lactobacillus acidophilus* growth, A statistically significant difference was found between group (V1) and each of (V2), (V3), and (V4) groups where ($P = 0.042$), ($P = 0.002$), and ($P < 0.001$). While there was no statistical significance difference between (V2) and (V3) where ($P < 0.001$).

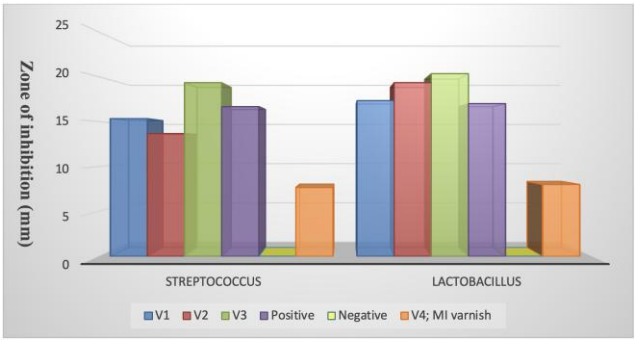

Figure 4: Bar chart representing Comparison of the zone of inhibition with one of two plant extract (Cinnamon, Clove) and their mixture each incorporating the commercial varnish separately

Discussion

Dental caries is one of the major causes of the destruction of mineralised tissue of the teeth. *Streptococcus mutans* (*S. mutans*) and *Lactobacillus acidophilus* (*L. acidophilus*) species are dominant microorganisms in the lesion of advanced caries, and these two are considered as a principle microorganism in the pathology of dental caries. *S. mutans* is the organism causing initiation of caries, whereas *L. acidophilus* causes progression of dental caries [28], [29], [30]. Thus, the existence of *S. mutans* and *L. acidophilus* in dental structure is a signal of a cariogenic biofilm and any chemical substance which can use to decrease these bacteria levels can offer additional means of stopping dental caries [31]. In recent years, researchers gave attention to the use of plant extracts against cariogenic bacteria regarding their effect on growth. For this reason, the present study selected five plants (Cinnamon, Ginger, Turmeric, Cloves and Black seed) [15], [16] which are known for their medical applications to evaluate their effect on *S. mutans* and *L. acidophilus* bacteria. Sensitivities of these two cariogenic pathogens to the five different methanolic plant extracts in comparison to chlorhexidine gluconate (0.12%), were tested using Agar disc technique.

The results obtained from our study shows a very good antibacterial activity of two extracts; Cinnamon and Cloves extract against *S. mutans* and *L. acidophilus*. While no antibacterial activity was exhibited by Ginger and Black seeds extracts, while Turmeric has only antibacterial potential against *L. acidophilus*. According to a literature data on the effectiveness of plant extracts the results are inconsistent probably because of differences in extract preparation methods [32], therefore we assumed that the solvent used for the experiment could influence the result. In this study, we used methanol extract for each tested plant as it could allow releasing of active ingredients from Cinnamon and Cloves extract causing their antibacterial efficiency against *S. mutans* and *L. acidophilus*. The previous investigation could be supported by Cowan [33] who stated that approximately all the identified components from plants showed activity against microorganisms are saturated organic compounds or aromatic, and they are most frequently acquired through initial methanol or ethanol extraction. On the other side, using methanol solvent in our study might corrupt the effective ingredients of the Ginger and Black seed extract which masking their antibacterial potential against the two oral tested pathogens or due to the high resistance of tested strains [34]. While the methanolic extraction of Turmeric had high potential to inhibit only *L. acidophilus* growth as it might inhibit the growth in a dose-dependent manner [35] and so *L. acidophilus* could be suppressed at lower concentrations of Turmeric extraction than for *S. mutans*.

According, to our study results, *L. acidophilus* was found to be most sensitive pathogen to the methanolic extract of Cinnamon, with MIC of 5.18 mg/ml followed by the methanolic extract of Clove (13.92 mg/ml). While methanolic extract for both plants showed almost equal antibacterial activity against *Streptococcus mutans*. The high antibacterial activity of the Cinnamon extract is mainly attributed to its secondary metabolites. It has been shown that Cinnamon antimicrobial properties are mainly related to its cinnamaldehyde which is highly electronegative which interferes in biological processes including electron transfer and react with nitrogen-containing components, such as nucleic acids as well as proteins, therefore inhibits the microorganism's growth [36]. Concerning the antibacterial of Clove, Shoji *et al.,* [37] presented that the methanol and aqueous extracts of this plant contain flavonoids and saponins. Though further studies are needed to identify the

active agents responsible for other biological and pharmacological activities of these plants is requisite.

In our experiment the combination, which consisted of Cinnamon to Clove at ratio 1:2 in the group (C2) was found the most active ratio compared to the other ratios in the group (C1) (1:1), and group (C3) (2:1). This latter result seemed to reflect the amount of Clove extract as the activity of combination increases by increasing Clove amount, and this could be due to the presence of active components in inadequate quantities in the Cinnamon extract to show the activity with the used dose levels [38]. Moreover, the acetonic extraction for Cinnamon has greater antimicrobial activity than water and alcohol extraction [36].

Although the high antibacterial activity of CHX (positive control) which was reported by previous studies [39], [40], the result of the present study demonstrated that incorporating Cinnamon (V1), Clove (V2) extracts separately or both (V3) into commercial varnish (5% sodium fluoride varnish with Recaldent (CPP–ACP), GC America, USA) was able to achieve a higher antibacterial activity. Also, they showed a more significant effect on the suppression of *S. mutans* and *L. acidophilus* compared with commercial varnish only (V4). Anywise, the synergistic effect between Cinnamon and Clove extract in the component of commercial varnish (V3) did not suppress the antimicrobial activity of each extract, and this could be explained the maximum inhibition zone for group V3. Thus, from the overall result, it is evident that the methanolic extract has been found to have good antimicrobial activity for Cinnamon and Clove extract against *S. mutans* and *L. acidophilus*. Also, Cinnamon and Clove extracts containing varnish can be beneficial clinically against the dental caries pathogens.

In conclusion, within the restrictions of this study, the accompanying conclusions were proposed that Cinnamon and Clove methanolic extracts would be useful compounds for the development of antibacterial agents against *S. mutans* and *L. acidophilus*, though, the latter needs higher concentration of the Clove extract to reach MIC. Although their effectiveness was less than Chlorhexidine, they may have a potential role in dental varnish for dental caries prevention.

Therefore, the present results could display; a scientific basis for the traditional use of Cinnamon and Clove on oral pathogens, contribute to the enhancement of oral health and lessen the side effects and cost of the treatment with allopathic medicine. However, additional clinical trials seem necessary to assess their safety and efficacy. Also, further studies should be carried out on the effect of both on the remineralising ability of MI varnish and enamel colour.

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Evaluation of Post-Operative Sensitivity of Bulk Fill Resin Composite versus Nano Resin Composite: A Randomized Controlled Clinical Study

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Keywords: Postoperative sensitivity; Class II posterior restorations; Bulk fill resin composite; Incremental Nano resin composite; Adhesive system

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Abstract

BACKGROUND: Despite recent advances in restorative dentistry adhesive restorations may cause postoperative sensitivity which leads to restoration failure.

AIM: This study aimed to compare and evaluate the incremental and bulk fill resin Composite postoperative sensitivity in class II posterior restorations bonded with two adhesive systems (self-etch and etch-and-rinse).

METHODS: Sixty patients were randomly selected, their age range from twenty-five to forty years old, divided into two groups according to the packing technique of resin composite material; incremental Tetric Evoceram and Tetric Evoceram bulk-fill resin composite. Thirty patients (n = 30) for incremental Tetric Evoceram resin composite restorations and according to the adhesive systems used they were equally divided (n = 15 teeth).Thirty patients (n = 30) for Tetric Evoceram bulk-fill resin composite restorations and according to the adhesive systems used (etch and rinse or self-etch), they were equally divided (n = 15 teeth). Post-operative pain assessed at 24 hours, 1 week and 1 month using the Visual Analog Scale Score (VAS). Each patient was instructed to put a mark on the VAS line at home to point out the intensity of pain at each assessment period. The problem of measuring the pain that pain tolerance of individuals may be different from the others. This may be due to different reasons, and it is not always because of a problem in the restoration

RESULTS: After 1 day, 1 week as well as 1 month, no statistically significant disagreement between the two resin composite types using self-etch adhesive strategy and total-etch adhesive strategy. Also, when the two adhesive systems were compared using Bulk Fill resin composite and incremental Nano resin composite no statistically significant disagreement between the two adhesive systems after 1 day, 1 week as well as 1 month.

CONCLUSION: The post-operative hypersensitivity is related to many factors as the procedure of cavity preparation, adhesive approach, and type of resin composite used and placement technique of the resin composite.

Introduction

New technologic development is continuing to minimise the shrinkage and polymerisation stress of resin composites. The recent group of bulk-fill resin composites provide the effectual use of 4 or 5 mm thick increment as the first increment of the restoration claiming full conversion of this increment with minimal polymerization stresses providing leak-proof margin to minimize microleakage and a remove it an easy quick application of the composite in difficult compound and complex restorations [1]. The lack of marginal integrity of resin composite restorations results in dentinal sensitivity and secondary caries formation. Dentinal sensitivity present as a sharp, well-defined pain. [2]

Studies have shown that bulk-fill resin composite has some degree of conversion, high depth of cure resins, and low volumetric shrinkage [3]. Furthermore, clinical researches have reported that the postoperative sensitivity increases lead to cuspal deflection and increase stresses at the interface when placing 4or 5 mm-thick increments of resin composite [4].

Self-etch (single step) and etch-and-rinse adhesives using phosphoric acid were used with resin

composite; after rinsing phosphoric acid, postoperative sensitivity may increase due to resin monomers can't be infiltrated into the dematerialised dentin and also can't seal dentin tubules so hydration of dentin should be managed. Self-etch adhesives don't demand multiple steps for bonding which may lower postoperative sensitivity when compared to etch and rinse technique [5]. However, the result of different adhesive techniques on postoperative sensitivity in posterior teeth is still debatable [6].

The aim of this double-blind, randomised clinical trial was to assess and compare the clinical results of postoperative sensitivity after incremental nano resin composite and bulk-fill resin composite placement techniques in posterior restorations bonded with two different adhesive systems.

Material and Methods

Ethical considerations and approval

This study was approved by the Ethics Committee of Scientific Research-Faculty of Dentistry-Cairo University, Approval no: 150405.

Registration

This study was registered at the ClinicalTrials.gov; registration number is NCT03792178.

Study design

Trial Design: Randomized clinical trial.

Participants

All patients were enrolled from the Operative dentistry clinic, Faculty of Dentistry Cairo University. The selection was completed according to the patients need for class II cavity preparations followed by final resin composite restorations. A total of patients was enrolled for this study from April 2016 till April 2017. Medical and dental histories were taken from all patients (Table 1).

Table 1: Inclusion and Exclusion criteria

Inclusion criteria	Exclusion criteria
Patients must not show any signs of voluntary dental pain.	Increasing pre-operative sensitivity of the selected teeth.
The existence of molar and premolar class II teeth requiring resin composite restorations for the treatment of primary carious lesions	Teeth with very deep caries.
The selected teeth should have an occlusal contact with natural or crowned antagonist teeth	Patients have old restorations.
The selected teeth should have a proximal contact with the adjacent teeth.	Teeth with spontaneous pain.
Shallow and mid-sized cavity depths will be included in the study.	Patients with temporomandibular joint problems involving pain.
	Patients are taking analgesics.

Sample size calculation

The total sample size of 42 will be effective to observe this effect size of 0.2, a power of 80%, and a significance level of 5%. This number has been raised to a total sample size of 48, to modify for using a nonparametric test. The number is raised again to sample size of 60 (30 for each group) to permit for losing around 25%. The sample size was calculated using G * Power program (University of Düsseldorf, Düsseldorf, Germany) [7].

Randomisation

Randomisation was accomplished using (https://www.randomizer.org/) in the Center of Evidence-Based Dentistry, Cairo University. Each patient will choose a number from sequentially numbered opaque sealed envelopes after cavity preparation. They will be then allocated into one of the set-ups using a randomisation table (Random.org). All patients who give consent for participation and who fulfil the inclusion criteria will be randomised.

Allocation concealments

Concealed allocation carried out using a set of random numbers placed in sealed opaque envelopes. The operator unlocked the envelope containing the procedure to be carried out on each patient. Sealed envelopes waiting for new subjects remained in a secured place and stated to the operator as the sessions are scheduled.

Implementation

The allocation sequence produced by the statistician who forwarded the sealed opaque envelops to the operator the day before the intervention and the envelope was opened containing the procedure that accomplished on each patient immediately before intervention.

Blinding

Double-blinded study since the participant and the investigator be unaware of the type of resin composite, self-etch adhesive and total-etch that is used. The 2 types of resin composite had the same shade guide. The investigator is blinded since each type of resin composite, the self-etch adhesive and total-etch was given a code that is known only by the data manager, and is placed in very similar bottles.

Intervention

Wholly clinical steps were achieved by only one operator.

Procedure methodology

After consent was acquired, collect the data of the patients retrospectively by using a well-designed questionnaire. The questionnaire involved Medical history, dental history, age, gender, nationality, social status, Occupation [8].

Cavity preparations were done, Participants were stated a short explanation about the examinations and all consent to participate and sign a consent form, Sensitivity tests were performed with hot gutta-percha stick and cold (ice stick) stimuli in order to initiate pulp condition and determine whether there was be any abnormal pulpal responses which could jeopardize the final sensitivity results [9].

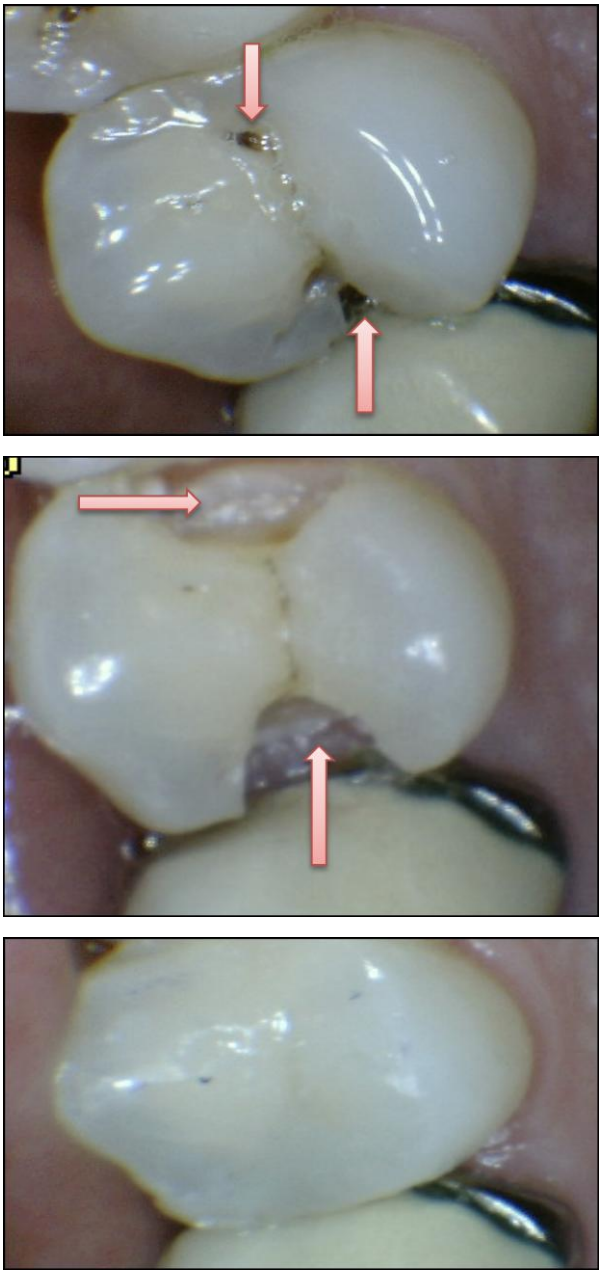

Figure 1: Clinical Case 1. Arrows showing Cl. II (Mesial and distal surface) in upper 4 (top); Arrow showing prepared cavity after caries removal and excavation (middle); Tooth after Tetric Evoceram Bulk Fill resin composite placement and finishing (bottom)

Periapical radiographs were taken for each selected tooth to evaluate cavity proximity to the pulp and any sign of periapical radiolucency. A Local anaesthetic was used for the operative procedures; the working field throughout the whole procedure was be insulated with cotton rolls and saliva aspirator [10].

Figure 2: Clinical case 2. Arrows showing Cl.II (Mesial surface) in left upper 4 (top); Arrow showing prepared cavity after caries removal and excavation (middle); Tooth after Tetric Evoceram universal nano-hybrid resin composite incremental (bottom)

The cavo surface angle of the prepared cavity was entirely in enamel without any beveling. The depth of each cavity preparation was estimated against the mesial and distal marginal ridges, using a periodontal probe to be 3 mm and 5 mm but not greater than 5 mm in occlusal and proximal parts respectively with no lining material under resin composite restorations. The cavity was cleaned with a

water spray from the dental unit. Tooth surfaces were treated with the total self-etch and self-etch adhesives according to the directions provided by the manufacturer.

Sectional matrices (Palodent plus, Dentsply) were placed before the restorative procedure.

The treated cases Separated into 2 equal groups according to the packing technique of resin composite material; incremental Tetric Evoceram and Tetric Evoceram bulk-fill resin composite were as follows:

Group A: Incremental Tetric Evoceram resin composite restorations and they equitably divided according to the adhesive Strategies used (Total etch or single-step self-etch adhesive strategy).

Group B: Tetric Evoceram bulk-fill resin composite restorations and they equitably divided according to the adhesive strategies used (Total etch or single-step self-etch adhesive strategy).

Post-operative pain assessed at 24 hours, 1 week and 1 month using the Visual Analog Scale Score (VAS). The VAS is a measurement instrument for subjective characteristics or reaction that cannot be directly measured (Figure 3). A 10 cm line with the anchor words “no sensitivity” at one end and “intolerable sensitivity” at the other end. Each patient was instructed to mark a vertical mark on the VAS rule at home to point out the intensity of pain at each assessment period [11].

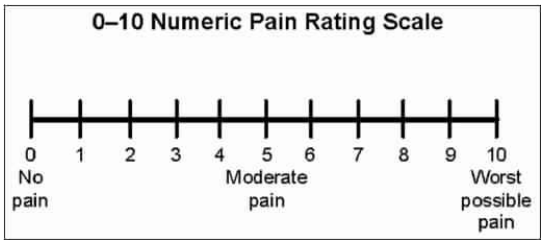

Figure 3: Visual Analog Scale

Statistical analysis

Data were analysed using IBM SPSS advanced statistics (Statistical Package for Social Sciences), version 21 (SPSS Inc., Chicago, IL). Numerical data were described as median and range, while qualitative data were described as number and percentage. To evaluate and compare the post-operative sensitivity of patients with class II cavities using bulk-fill resin composite containing self-etch adhesive and free self-adhesive versus those with Nano resin composite containing self-etch adhesive and free self-etch adhesive, Mann-Whitney nonparametric test was performed. To compare the different measurements within each group, Friedman repeated-measures analysis of variance on ranks was done. To adjust for inflation of the type I error, these tests were followed by the Bonferonni corrections. A p-value less than or equal to 0.05 was considered statistically significant. All tests will be two-tailed.

Results

After 1 month of follow up was found that when the two resin composite types using single-step self-etch adhesive and total-etch adhesive strategies were evaluated; there was no statistically significant difference between the two resin composite types after 1 day, 1 week as well as 1 month. When the two adhesive systems were compared using Bulk Fill composite & incremental Nano resin composite there was no statistically notable difference between the two adhesive systems after 1 day, 1 week as well as 1 month.

Using Bulk Fill composite with self-etch adhesive; there was a statistically notable reduce in the prevalence of hypersensitivity after 1 week. There were no cases with hypersensitivity after 1 week as well as 1 month. Using Bulk Fill composite with Total etch adhesive; there was a statistically notable reduce in prevalence of hypersensitivity after 1 week as well as from 1 week to 1 month.

Using Incremental Nano resin composite with self-etch adhesive; there was a statistically notable reduce in the prevalence of hypersensitivity after 1 week as well as from 1 week to 1 month. Using Incremental Nano resin composite with total-etch adhesive; there was a statistically notable reduce in prevalence of hypersensitivity after 1 week as well as from 1 week to 1 month.

Table 2 shows the frequencies, percentages and results of Chi-square and Fisher's exact tests for comparison between the prevalence of hypersensitivity after using the two resin composite types. Results showed postoperative sensitivity recorded in three patients at one day using bulk fill packing technique with self-etch adhesive system, at one week and one month all cases had no sensitivity.

Table 2: Show the frequencies, percentages and results of Chi-square and Fisher's exact tests for comparison between the prevalence of hypersensitivity after using the two composite types

Adhesive system	Time	Bulk Fill (n = 15)		Incremental Nano Resin (n = 15)		P-value
		N	%	N	%	
Self-etch	1 day	3	20.0	6	40.0	0.427
	1 week	0	0.0	3	20.0	0.224
	1 month	0	0.0	0	0.0	Not computed
Total etch	1 day	4	26.7	7	46.7	0.256
	1 week	2	13.3	4	26.7	0.651
	1 month	0	0.0	2	13.3	0.483

*: Significant at P ≤ 0.05.

For bulk fill packing technique with the total-etch adhesive system; at one day four patients had sensitivity; at one week two patients only had

sensitivity, at one month all cases had no sensitivity. However post-operative sensitivity recorded in four patients at one day using Incremental Nano resin composite packing technique with self-etch adhesive system, at one week two patients had sensitivity and at one month all cases had no sensitivity.

For Incremental Nano resin composite packing technique with a total-etch adhesive system, seven patients recorded postoperative sensitivity at one day, four patients at one week and two patients at one month. Results showed that bulk-fill composite had less post-operative sensitivity when compared to incremental Nano resin composite.

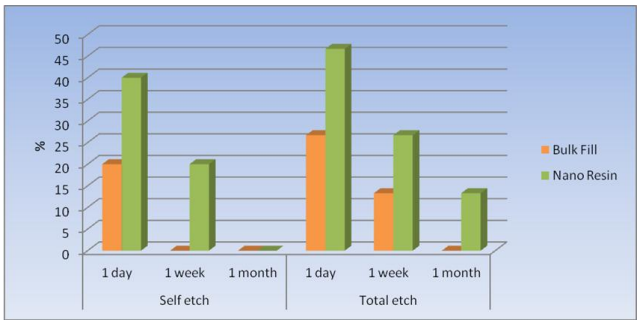

Figure 4: Bar chart representing the prevalence of hypersensitivity after using the two composite types

Table 3 reveals the frequencies, percentages and results of Chi-square and Fisher's exact tests for comparison between prevalence of hypersensitivity after using the two adhesive systems.

Table 3: Shows the frequencies, percentages and results of Chi-square and Fisher's exact tests for comparison between the prevalence of hypersensitivity after using the two adhesive systems

Composite type	Time	Self-etch (n = 15)		Total etch (n = 15)		P-value
		n	%	n	%	
Bulk Fill	1 day	3	20.0	4	26.7	0.666
	1 week	0	0.0	2	13.3	0.483
	1 month	0	0.0	0	0.0	Not computed
Incremental	1 day	6	40.0	7	46.7	0.713
Nano Resin	1 week	3	20.0	4	26.7	1.000
	1 month	0	0.0	2	13.3	0.483

*: Significant at P ≤ 0.05.

Results revealed that when Bulk Fill composite was used; there was no statistically notable difference between the two adhesive systems after 1 day as well as 1 week. After 1 month, all cases had no sensitivity when incremental Nano resin composite was used; there was no statistically significant difference between the two adhesive systems after 1 day, 1 week as well as 1 month.

Results showed that postoperative sensitivity was reduced using a one-step self-etch adhesive strategy compared to total etch adhesive strategy.

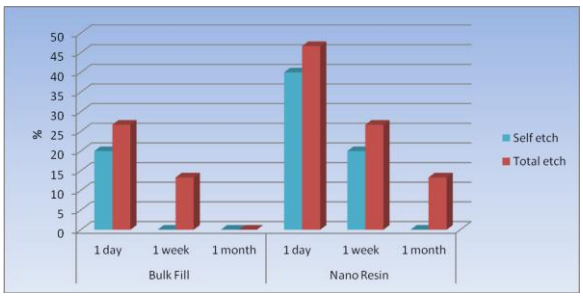

Figure 5: Bar chart representing the prevalence of hypersensitivity after using the two adhesive systems

Table 4 shows the frequencies, percentages and results of Cochran's Q test for comparison between the prevalence of hypersensitivity at different follow up times within each group.

Table 4: Frequencies, percentages and results of Cochran's Q test for comparison between the prevalence of hypersensitivity at different follow up times within each group

Group	1 day (n = 15)		1 week (n = 15)		1 month (n = 15)		P-value
	n	%	N	%	N	%	
Bulk Fill with Self etch	3	20.0 ^{A}	0	0.0 ^{B}	0	0.0 ^{B}	0.050*
Bulk Fill with Total etch	4	26.7 ^{A}	2	13.3 ^{B}	0	0.0 ^{C}	0.050*
Nano Resin with self-etch	6	40.0 ^{A}	3	20.0 ^{B}	0	0.0 ^{C}	0.011*
Nano Resin with Total etch	7	46.7 ^{A}	4	26.7 ^{B}	2	13.3^{C}	0.022*

*: Significant at P ≤ 0.05; Different superscripts in the same row are statistically significantly different.

Results showed that Bulk fills with self-etch, Bulk Fill with Total etch and Nano Resin composite with self-etch had no postoperative sensitivity after one month in all cases. Bulk Fill with Self etch had postoperative sensitivity in three patients at one day then subside from one week to one month. Bulk Fill with Total etch and Nano resin composite with self-etch had postoperative sensitivity from one day to one week then subside at one month. However, incremental Nano resin composite with Total etch showed postoperative sensitivity at one day in seven patients (46.7%) decreased to four patients at one week (26.7%) decreased to two patients at one month (13.3%).

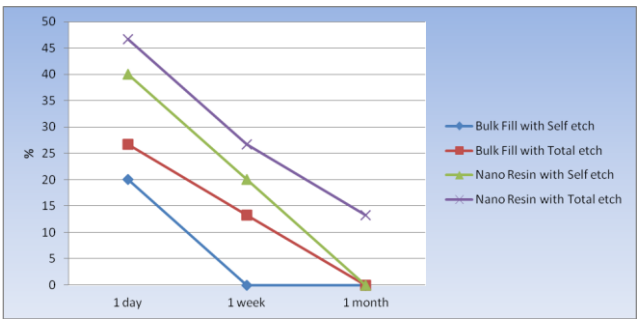

Figure 6: Line chart representing the prevalence of hypersensitivity at different follow up periods within each group

Discussion

Post-operative sensitivity, discoloured margins, recurrent caries and fractures of the restoration margins may be due to marginal leakage of saliva and its components. These clinical results are the major reasons for substitution of restorations and describe why polymerisation shrinkage is acknowledged as the major limitation of these materials. Dentin bonding agents and glass ionomer cement has been used to minimise contraction gap formation and the potential for bacterial leakage [12].

The formation of a hybrid layer that is infiltrated with adhesive resins results in effective dentin bonding. Nano-leakage occurred due to incomplete resin penetration in the hybrid layer permits to occur. Nano-leakage pathways may cause bond failure by stimulating hydrolysis of collagen fibrils and/or degradation of polymerised resins [13].

Therefore, the objective of this study was to assess and compare the post-operative sensitivity of Bulk fill composite placement and incremental Nano resin composite with different dentin adhesives strategy (total-etch or single-step self-etch).

In this randomised clinical trial, risk and intensity of postoperative sensitivity manifested when applying the bulk-fill technique and the conventional 2 mm incremental technique. An ideal resin composite that it can be cured in a single increment, promoting placing should be considered and may be referred to some effects of the bulk fill material which makes it very close to incrementally cured resin composite, except that higher depth of cure can reach [14], [15].

Higher translucency was found in the material used in this study (Tetric N-Ceram Bulk Fill) which can affect its superior depth of cure by increasing deeper blue-light penetration and minimise light scattering [16], [17].

To obtain a reliable adhesive-restoration interface over time, several new adhesive systems have been developed [18].

Knowing the success and longevity of various adhesives enables practitioners to choose the most appropriate material for clinical use. One-step self-etch adhesives systems that have become popular in restorative dentistry as they are easy to use and demonstrate low technique sensitivity with the simplified total-etch adhesives (one bottle) which are still popular among clinicians in routine clinical use. By using agents from the same manufacturer, certain chemical variations are eliminated, allowing a more controlled evaluation of the progression from etch-and-rinse through two-step agents and an assessment of any concomitant clinical advantage [19], [20].

A Method has been described to measure postoperative sensitivity; visual Analog Scale Score (VAS). The VAS is an instrument that measures subjective characteristics or attitudes that cannot be directly measured. When responding to a VAS item, respondents specify their level of agreement to a statement by indicating a position along a continuous line between two end-points. A 10 cm line with the anchor words “no sensitivity” at one end and “intolerable sensitivity” at the other end [21].

In the present study, comparing and evaluating incremental and bulk fill postoperative sensitivity in posterior composite restorations showed that on using the self-etch adhesive system; there was no statistically notable difference between the different resin composite types after 1 day as well as 1 week. After 1 month, all cases had no sensitivity. Using total-etch adhesive system; there was no statistically significant difference between the two resin composite types after 1 day, 1 week as well as 1 month.

The lack of postoperative sensitivity in the current study could be the result of the manufacturer’s instruction for adhesive application in addition to the low polymerisation shrinkage and polymerisation shrinkage stresses of both materials. These results were in agreement with Sancakli et al., who reported that outcome of post-operative sensitivity determined by both operator skill and experience [22].

Ashgar et al. attributed the low post-operative sensitivity to the lower post-gel shrinkage of bulk-fill composites. However, it was reported that post-operative sensitivity is a patient-related factor, such as pain experience and amount of discomfort that can vary between patients [23].

In the present study, comparing postoperative sensitivity of the different bonded adhesive strategy using Bulk Fill composite; there was no statistically notable difference between the different adhesive systems after 1 day as well as 1 week. After 1 month, all cases had no sensitivity. Using incremental Nano resin composite; there was no statistically significant difference between the two adhesive systems after 1 day, 1 week as well as 1 month. The outcome of the present study is in check with the latest systematic review of the Literature Reis A et al., that systematic review concluded that presence of postoperative sensitivity immediately after the restorative procedure does not influence by the type of adhesive strategy used in bonding procedures in posterior resin composite restorations [24].

Favour our results are those by Berkowtiz G et al., who found that postoperative sensitivity did not affect by the cavity depth. Browning WD et al., reported that immediate postoperative sensitivity was not affected by either the adhesive strategy (etch-and-rinse / self-etch) or the filling technique (incremental / bulk) and 20.3% was the overall risk of it, but related to other many factors during cavity preparations and restorations procedures [25], [26].

Agreement our results are those by Blanchard et al., who found that the type of dentin bonding agent used play an important role in greatest sensitivity associated with [27].

The results demonstrated that low post-operative sensitivity is due to the careful application of the treatment steps, the right use of adhesive materials by following the manufacturer's instructions, and clinical placement techniques that might depend on resin composite materials used.

Limitations: Further clinical researches are needed with extended follow-up periods to assess long-term postoperative sensitivity.

In conclusion, the post-operative hypersensitivity is related to many factors as the procedure of cavity preparation, adhesive approach, and type of resin composite used & placement technique of the composite. Etch & Rinse approach provides high bond strength with noticeable postoperative hypersensitivity. The self-etch approach proved the minimal post-operative hypersensitivity with simplified bonding steps. Bulk fill composite with bulk packing in 4mm thickens increment together with self-etch adhesive is considered as practical approach in class II cavity restorations regarding time saving, simplicity & least post-operative hypersensitivity.

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Dental Science - Case Report

Orthodontic Treatment of a Periodontally - Affected Adult Patient (Case Report)

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Abstract

BACKGROUND: The advanced periodontal disease is characterised by a strongly pronounced loss of attachment and reduction of the alveolar bone support, which leads to luxation, migration of the teeth, functional discomfort and poor facial aesthetics.

CASE PRESENTATION: The aim of this paper is to present the case of a 26-year-old female patient, registered at the Clinic of Periodontology with highly expressed gingivitis, unsatisfactory periodontal status, presence of diastemas between the frontal teeth and attachment loss of 5-6 millimetres in different areas. We conducted a thorough classic periodontal treatment, as well as training for proper maintenance of oral hygiene, with frequent professional oral-prophylactic sessions, complemented with orthodontic treatment. Fixed orthodontic appliances were installed, and mild forces were applied for gradual levelling of the teeth, with constant control of the periodontal status. After 20 months of treatment, the patient was in retention.

CONCLUSION: Orthodontic therapy of periodontally-affected teeth can begin only after exhaustive administration of a periodontal treatment. Orthodontic treatment as an addition to the periodontal restoration must be gradual with mild forces for an optimal dental response, thus helping to improve function, facial aesthetics and psychological confidence of adult patients.

Introduction

Dentofacial aesthetics is the primary motivational factor in adolescent and adult population for conducting orthodontic treatment. The number of adult patients undergoing orthodontic therapy has constantly been rising in the last 2-3 decades [1]. 20-25% of orthodontic patients are adults, and there is an increasing trend in the number of adult patients as a result of their increased awareness of the importance of their oral health and their need for a better aesthetic appearance [2]. The main driving factor in adults is to improve their dental and facial appearance [3], [4]. Twelve per cent of adults seeks orthodontic treatment to prevent occurrence or progression of periodontal disease [5].

Adult patients are divided into two different groups: 1st group-young adults (under 35 years of age, usually after their 20s) who were in need but could not receive orthodontic treatment during the adolescent period. The 2nd group consists of mature patients in their 40s-50s who have other dental problems and need orthodontic treatment as a part of a larger therapeutic plan that includes numerous dental disciplines [6].

Studies suggest that orthodontic therapy providing good dental aesthetics also has a strong impact on the psychosocial aspect of the patient's life [7]. It has been confirmed that almost 80% of patients accept treatment because of the aesthetic aspect

rather than dental health and function [8].

Today, orthodontic treatment can be justified as a part of periodontal therapy if it is used to reduce plaque accumulation, correct abnormal gingival and osseous forms, improve aesthetics and facilitate prosthetic replacement [9].

Age, per se, is not a contraindication to orthodontic treatment. Fact is that the tissue's response to orthodontic forces, cell mobilisation and conversion of collagen fibres is much slower in adults. Adult bone is less reactive to orthodontic force. There is a great risk of marginal bone loss and loss of attachment with mild gingival infection [2]. Dental response to orthodontic forces is slower in adults, but the teeth are moving in the same manner regardless of age.

A large number of adult patients have problems with malocclusion due to having neglected their periodontal health, leading to a loss of bone substrate around the teeth, resulting in pathological migration, rotations, tipping and extrusions of the teeth. Special attention should be given to the periodontal status of adult patients since most of them already suffer from periodontal disease. However, orthodontic treatment is no longer a contraindication in the therapy of advanced periodontal disease. This treatment can help rescue and restore the deteriorated dentition [10].

The advanced periodontal disease is characterised by a strongly pronounced loss of attachment, reduction of alveolar bone support, leading to tooth mobility, pathological migration, tooth extrusion, tipping, loss of contact point, presence of spacing between the teeth and marginal gingival recession. In many cases, this functional discomfort is accompanied by a pronounced poor aesthetic in the anterior dental region, which is reflected in the entire face [10].

The management of adult orthodontic patients with severe bone loss continues to present a challenge. Well-aligned dentition may be more conducive to periodontal health, than a crowded dentition and malocclusion. It has been widely believed that appropriately applied orthodontic forces do not damage the periodontium. On the contrary, they can support the periodontal tightness, but oral hygiene is obligatory.

Orthodontic therapy of the periodontally-affected teeth can begin only after a thoroughly performed periodontal treatment in multiple sessions when the periodontal inflammation would be eliminated. In a motivated patient who responds well to initial periodontal therapy, orthodontic treatment provides positive, satisfactory aesthetical and functional results, and a good long-term prognosis. Maintaining high-level oral hygiene at home, as well as frequent professional visits is very important (imperative) during and after the end of an active orthodontic therapy [11]. This can be supported by findings of Mattingly [12], Paolantonio [13], Sallum [14] and Perinetti [15], which confirm that long-term fixed appliances can contribute to unwanted, but predictable qualitative alterations in the subgingival bacterial biofilm that become progressively pathogen with time, if oral hygiene is not well. The combination of orthodontic intrusion and periodontal treatment in animals with good oral hygiene and healthy tissue showed an improvement in the periodontal condition [16]. A reduction of probing depth in bone defects following tooth extrusion can also be achieved [17]. General factors as morphology and deepness of defects, oral hygiene, plaque control and patient compliance, can strongly affect the predictability of periodontal regeneration [18].

The goal of the paper is to show the possibilities in the therapy of a periodontally-compromised adult patient, patient selection, preparations and stages of therapy, prerequisites for success and further recommended surgical procedures.

Case History

A 26 years old female patient visited the Clinic of Oral pathology and periodontology, complaining about the wide spaces between her teeth, strongly expressed gum bleeding and tooth luxation in the front region. She complained of poor self-esteem and bad social life. She was treated at our clinic for the first time when she was 17. After a long period of time without any therapy, she returned with those problems.

There was no significant medical history of any disease which may have contributed to periodontal disease. However, she noted that one of the parents had early teeth loss, and the two younger sisters had a problem with bleeding from the gingiva.

Figure 1: Presence of diastema between upper left central and lateral incisors

Upon clinical examination, we noticed that she had an asymmetrical face and a convex facial

profile. The lips were incompetent, and she was showing hyperactivity of the lower and upper lip while closing the lips. There were also generalised deposits of dental plaque and calculi due to poor oral hygiene. No active caries lesions were present. The pocket depth ranged from 3-6 mm in different areas of dentition. Her periodontal condition was poor, with gingival recession in many areas, especially in the lower incisor region, presence of wide spaces between the teeth, especially in the lower jaw as well as in the upper left central and lateral incisor (Figure 1, Figure 2 and Figure 3).

Figure 2: Upper jaw from the occlusal side

Before starting with the therapy, the patient was informed about the complications that could occur during the orthodontic treatment such as the possibility of root resorption, more bone loss around the teeth and worsening of periodontal disease, as well as the need to maintain oral hygiene at the highest level. Informed consent was obtained from her.

Figure 3: Presence of wide spaces between the teeth in the lower jaw and migration of the teeth

The periodontal treatment was started in September 2015. We proceeded with a thorough conservative periodontal treatment consisting of the complete elimination of dental calculus and biofilm. After that, scaling and root planning were conducted in all 4 quadrants during several sessions. In the initial phase of the therapy, due to the presence of a severe expressed gingival inflammation, antibiotic therapy was included as an addition to the conservative treatment. In the whole duration of the process, the patient was trained for proper maintenance of oral hygiene at home.

This process was ongoing for over a year, with frequent professional oral-prophylactic sessions every 3-4 months. Over a year of observation before the installation of orthodontic appliances helped us judge the patient's cooperation in oral hygiene maintenance until it was made sure that it was possible to start with orthodontic therapy. Ensuring that the movement of the teeth would occur in a healthy periodontal environment was of paramount importance before proceeding with the therapy. If this had not been done, orthodontically-applied forces could enhance the gingival inflammation and destruct the supporting tissues [19].

Figure 4: X-Ray before the start of the therapy

At the beginning of the periodontal treatment, an X-Ray was made for precise detection of periodontal status and osseous defects (Figure 4).

Figure 5: An upper fixed orthodontic appliance was placed

In January 2017, an upper fixed orthodontic appliance was applied (Figure 5). 022 slot SWA was used, alignment and levelling of the teeth were with light forces using NiTi wires. To avoid the incisor root desorption, we applied low intrusion forces (5-15 gr/tooth). In the second phase we used elastic bands

with long filaments to close the spaces and make good contacts.

Figure 6: Applied lower fixed orthodontic appliance

After six months, the lower fixed orthodontic appliance was applied (Figure 6) and 022 slot SWA was used, alignment and levelling were achieved with light forces using NiTi wires and elastic bands with long filaments.

Figure 7: Dental status at the end of the 1st year of orthodontic therapy

At the end of first year of orthodontic therapy, the oral situation was pleasant and as expected (Figure 7 and Figure 8).

Figure 8: Improvement of the overall oral situation

After 20 months of active treatment, the patient is in retention (Figure 9, 10, 11, and 12).

Figure 9: Dental status after 20 months of orthodontic therapy

Continuing monitoring of oral hygiene and administration of Gengigel (0.8% hyaluronic acid) to improve the attachment, was coordinated by the parodontologyst.

Figure 10: Satisfactory results after 20 months

Treatment results

After an active orthodontic phase of 20 months, the spaces between her upper and lower incisors were closed; the incisors were retracted to achieve acceptable overjet and overbite relation. Clinical examination revealed well-aligned arches, a harmonious occlusion and good periodontal health. Improved lip relationship, smile and facial esthetics were achieved. Patient's cooperation in oral hygiene maintenance was satisfactory. The patient was very satisfied with the treatment and had improved psychosocial confidence.

Figure 11: Satisfactory facial appearance

Orthodontic intrusion and levelling of periodontally-migrated teeth changed the topography of the original horizontal defects.

The therapeutic procedure at this patient will continue with surgical treatment of the deep periodontal defects in the frontal area and lateral regions of the upper jaw, as well as overlapping the recessions of the lower frontal teeth.

Figure 12: X-Ray at the end of the orthodontic treatment

Discussion

The number of adult patients in need of orthodontic treatment has increased in recent years. The patient must be evaluated for systemic diseases, perio-restorative problems, TMJ disorders and vulnerability to root resorption. The biomechanics must be customised for the individual treatment requirement. It has been found that the expectations of adult patients are usually high, and the limitations of orthodontic treatment must be explained at the beginning of treatment to arrive at realistic treatment objectives [2]. Thomson in his population-based longitudinal study found that periodontal attachment loss and gingival recession was not significantly different between the orthodontic treatment group and non-orthodontic treatment group [20]. However, Hye-Young Sim et al. investigated the association between orthodontic treatment and periodontitis in a nationally representative sample of the Korean population. The results indicated that orthodontic treatment was associated with decreased prevalence of periodontitis [21]. The importance of periodontal health has increased as the number of adult orthodontic patients has increased.

Orthodontics can serve as an adjunct to periodontal treatment procedures to improve oral health in a number of situations. Achieving esthetically acceptable results in periodontally-compromised patients requires various teeth movements, which can also help control the periodontal breakdown and restore good oral function [22]. The fixed appliance allows easy splinting of teeth to achieve stable anchorage [23], so force magnitude must be reduced to minimum. According to Deppa [24], teeth alignment can be achieved by orthodontic soft aligners in periodontally involved teeth.

A viable periodontal ligament is important for cell proliferation on the application of the orthodontic forces. There is reduction in periodontal ligament vascularity with ageing and insufficient source of preosteoblasts. It is obligatory to use lighter, controlled force levels in adults because the greater forces result in vascular compression and necrosis of blood vessels of periodontal ligament. There is a risk of iatrogenic damage to the periodontium with uncontrolled forces, and thus it is important to keep the periodontal status under control during treatment. Adults are more vulnerable to root resorption on application of orthodontic force. Light continuous force must be applied to minimise the risk of root resorption, and the patient must be informed of the potential risks before starting the treatment [1], [2], [9]. Tulloch [23] suggested that tooth movement can be undertaken 6 months after completion of active periodontal treatment if there is sufficient evidence of complete resolution of inflammation.

The most important factor in the initiation, progression and recurrence of periodontal problems is the presence of microbial plaque. Inadequate maintenance of oral hygiene during orthodontic treatment increases the risk of developing gingival inflammation. There is much evidence of increased count of Lactobacillus in saliva after orthodontic braces placement [25]. Many clinical studies have reported that plaque accumulation and gingivitis increased during orthodontic treatment [26]. The composition and types of oral bacteria were altered as a result of orthodontic treatment [27], [28]. Recent animal studies suggested that orthodontic tooth movement had a synergistic effect on the periodontium by increasing the presence of Il-1 β and TNF-α [29].

The surgical phase consists of techniques performed for pocket therapy and the correction of related morphological problems, namely, mucogingival defects. The purpose of surgical pocket therapy is to eliminate the pathological changes in the pocket walls, to create a stable, easily maintainable state, and if possible, to promote periodontal regeneration. A critical aspect of periodontal regeneration is the stimulation of a series of events and cascades, which can result in the coordination and completion of integrated tissue formation [30]. Many approaches have been used involving polypeptide growth and differentiation factors, extracellular matrix proteins and proteins involved in bone metabolism. These materials are largely physiological molecules or molecules released by cells which regulate processes in wound healing. These growth factors, primarily secreted by macrophages, endothelial cells, fibroblasts and platelets, include platelet-derived growth factor (PDGF), bone morphogenetic protein (BMP) and transforming growth factor (TGF). These biological mediators have been used to stimulate periodontal

wound healing, promoting migration and proliferation of fibroblasts (for periodontal ligament formation) or promote the differentiation of cell to become osteoblasts, thereby favouring bone formation [31]. Guided tissue regeneration (GTR), demineralised freeze-dried bone allograft, or a combination of these, are considered to be the most predictable regenerative procedures for achieving favourable treatment outcomes in periodontally-affected adult patients. These findings were further supported by many researchers who indicated that periodontal bone grafts consistently led to better bone fill of the defect, than the non-grafted controls. Histological analyses of cementum regeneration in animals demonstrated that regenerative treatment with bone grafting leads to some degree of regenerated cement, periodontal ligament and bone [32]. Regenerative procedures have a more predictable positive response in deep and narrow defects rather than shallow ones.

A multidisciplinary approach is always necessary to treat complex dental and periodontal problems, and there cannot be a better example than ortho-perio interaction. Periodontists should recognise the importance of orthodontic intervention in achieving results unattainable with periodontal therapy alone [33], [34], [35]. Adult orthodontic treatment can help prevent or improve periodontal problems, can help prevent and reduce further bone loss around teeth, improve the dentist's chances to restore missing teeth, adjust aesthetics to get a better smile and facial appearance, enhance function of teeth, increase self-confidence and self-esteem, and finally, improve overall oral health.

In conclusion, patient education, motivation, enhanced oral hygiene maintenance and regular periodontal care are essential during orthodontic treatment. Orthodontic therapy in periodontally-compromised patients requires extensive periodontal care, before, during and after the treatment. In some cases, periodontal restorative surgery may be required for sealing the pockets. In order to prevent relapse of the teeth to their previous state and ensure long-term results, the appliance of lingual bonded retainers is recommended. Interdisciplinary approach complemented by patient education, cooperation and good oral hygiene, will transform a patient with an unattractive dentition due to periodontal breakdown into a person with a good occlusion and a radiant smile. Adult patients must undergo regular oral hygiene procedures and periodontal maintenance to maintain healthy gingival tissue during active orthodontic therapy.

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Asthma and Chronic Obstructive Pulmonary Disease Associated With Occupational Exposure in Dairy Farmers - Importance of Job Exposure Matrices

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Abstract

AIM: To evaluate the prevalence of chronic respiratory symptoms, lung function impairment, and chronic obstructive respiratory diseases in dairy farmers. Our objective is to then examine their relation to exposure duration and to explore the usefulness of job exposure matrices as tools for exposure assessment, and predictors for respiratory health impairment.

METHODS: A cross-sectional study was performed, including 83 dairy farmers (mean age: 52.6 ± 8.7 years; mean exposure duration: 23.7 ± 7.6 years) and 80 office workers as a control group (mean age: 52.7 ± 8.2 years) matched for age, smoking habits, and socioeconomic status. Methods of evaluating examined subjects included a questionnaire on respiratory symptoms in the last 12 months, spirometry and histamine challenge, as well as the use of job exposure matrices (JEM).

RESULTS: Dairy farmers had a significantly higher prevalence of cough (38.5), phlegm (27.7%), and wheezing (21.7%), than controls ($p < 0.05$). All mean baseline spirometric parameters were lower in dairy farmers compared to the controls, but statistical significance was confirmed only for MEF25, MEF50, and MEF75% ($p = 0.010$, $p = 0.001$, and $p = 0.004$, respectively). The prevalence of bronchial hyperresponsiveness, asthma, and chronic obstructive pulmonary disease was higher in dairy farmers but without statistical significance. JEM were useful tools for exposure assessment and predictors of factors for asthma and COPD development.

CONCLUSION: The results suggest that occupational exposure among crop farmers is associated with a higher prevalence of respiratory symptoms, lung function impairment, and a higher prevalence of chronic respiratory diseases. JEM showed good potential for farming exposure evaluation and promoted their applicability within the diagnostic algorithm focused on respiratory health assessment.

Introduction

Lung diseases have been recognised among dairy farmers for decades. Studies of dairy farmers worldwide have shown increased rates of chronic bronchitis, chronic obstructive pulmonary disease (COPD), and asthma. Researchers have consistently reported the presence of chronic obstructive lung diseases, with decreased flows, bronchial hyperresponsiveness (BHR), and increased symptoms of wheezing, cough, and phlegm production [1], [2], [3], [4], [5], [6], [7].

Work on dairy farms has been associated with adverse respiratory symptoms, primary symptoms of bronchoconstriction, and decreased pulmonary function [4], [8], [9], [10], [11]. Dairy farmers may be at risk for lung inflammation due to the proximity of aerosol sources (e.g., cows) and exposure duration. Additionally, dairy farm workers often work long shifts for more than 5 days a week performing the same or similar tasks (e.g., milking) [12]. These aerosols may contain a mixture of manure, animal dander, hair, animal feed, gram-positive (muramic acid), and gram-negative (endotoxins) microbiological components [10].

Chronic airway diseases developing from exposure to large animal-feeding operations include a spectrum of upper and lower respiratory tract

disorders: rhinitis, mucous membrane inflammation syndrome, sinusitis, asthma, asthma-like syndrome, chronic bronchitis, COPD, hypersensitivity pneumonitis and organic dust toxic syndrome (ODTS) [13], [14]. These diseases commonly occur following exposure to large animal feeding operation farming environments, particularly swine confinement facilities and commercial cattle feedlots [15].

Asthma is associated with large animal farming exposures. It is now well-recognised that children raised on farms have less allergic, IgE-mediated asthma [16]. It was postulated that exposure to endotoxin or other bacterial components abundantly present in various farming environments leads to decreased IgE-mediated disease development [17], [18], which is also consistent with the hygiene hypothesis.

COPD is a major public health concern with increasing morbidity and mortality rates worldwide [19]. Although smoking is the main risk factor for the disease, 25 to 45% of COPD cases are non-smokers [20]. Occupational exposure could also be involved in the development of COPD [21]. Several studies have demonstrated that farmers are more likely to have respiratory symptoms than the general population, although fewer of them smoke [7]; in particular, FEV1 and FVC of dairy farmers decline faster than expected [1], [4].

One of the most important public health problems in farming is respiratory diseases. Having in mind that exposure to most of the respiratory hazards in this sector can be controlled and reduced work-related respiratory diseases subsequently in dairy farmers caused by these agents are potentially preventable [22], [23].

In the present study we have compared the prevalence of chronic respiratory symptoms, lung function impairment, and chronic obstructive respiratory diseases between dairy farmers and office workers, further examined its relation to exposure duration, and explored the usefulness of job exposure matrices as tools for exposure assessment in dairy farmers, and predictors for respiratory health impairment.

Subjects and Methods

Study design and setting

Cross-Sectional research was conducted in the Center for Respiratory Functional Diagnostics by the team from the Institute for Occupational Health, Skopje-WHO Collaborating Center for Occupational Health and GA2LEN Collaborating Center within the period September 2017 and February 2018.

Study Sample

The representative study sample was calculated by the software program PEPI 4.04, with 95% confidence level and confidence interval ± 5.

To achieve the necessary sample size (having in mind possible selection and response bias), we have taken a representative sample of 83 dairy farmers and 80 matched office controls in a large-scale agricultural enterprise.

Subjects

We have examined 83 subjects (mean age = 52.6 ± 8.7) employed as dairy farmers (mean duration of exposure 23.7 ± 7.6). They were engaged in dairy farming with main activities composed of preparation of fodder feeding and animal meals, milking, staying in the barn, preparation of straw, and haymaking, cattle raising, as well as taking care about milk hygiene and health of the animals. They were exposed to various respiratory agents: dust, inappropriate microclimate conditions, chemical hazards, vapours, gases, as well as to heavy manual work, animal contact, unfavourable body positions, and repetitive hand movements. Inclusion criteria for the examined group (EG): employed subjects with age range 18 to 64 years involved in dairy farming and exposed to at least one occupational respiratory hazard (dust, gases, fumes, and vapours).

Exclusion criteria for the examined group: subjects younger than 18 or older than 64 years, and subjects not engaged in dairy farming. To avoid selection bias and results' deviations, the study did not include subjects with exposure to respiratory hazards other than dairy farming.

Depending on the exposure duration, the examined subjects were divided into two subgroups: exposed less or more than 20 years.

Also, a similar group of 80 office workers (mean age = 52.7 ± 8.2) matched for age, duration of employment, daily smoking and socioeconomic status was studied as a control group (CG), with no data for occupational exposure to respiratory hazards.

The subjects in both groups who were diagnosed by a physician to have some chronic respiratory disorder (asthma, COPD, bronchiectasis, sarcoidosis, etc.), or treated with bronchodilators and corticosteroids were not included in the study. Also, both groups did not comprise any subjects in whom either spirometry or bronchodilator reversibility testing was contraindicated.

All study subjects were informed about the study and gave their written consent accordingly.

The Institute's ethics committee has approved the content of our study protocol, whereas each examined subject was informed and gave written consent before any involvement in the study.

Questionnaire

All study subjects were interviewed by a physician and completed the standardised questionnaire, including questions on work history, respiratory symptoms in the last 12 months, and smoking habit.

Chronic respiratory symptoms in the last 12 months (cough, phlegm, dyspnea, wheezing, and chest tightness) were obtained using the European Community for Coal and Steel questionnaire (ECCS-87), and the European Community Respiratory Health Survey (ECRHS) questionnaire [24], [25].

Classification of smoking status was done according to the World Health Organization (WHO) guidelines on definitions of smoking status [26].

Daily smoker was defined as a subject who smoked at the time of the field survey at least once a day, except on days of religious fasting. Among daily smokers, lifetime cigarette smoking and the daily mean of cigarettes smoked were also assessed. Pack-years smoked were calculated according to the actual recommendations [27].

Ex-smoker was defined as a formerly daily smoker, no longer smokes.

Passive smoking or exposure to environmental tobacco smoke (ETS) was defined as the exposure of a person to tobacco combustion products from smoking by others [28].

Baseline spirometry

All study subjects underwent spirometry testing, performed by spirometer Ganshorn SanoScope LF8 (Ganshorn Medizin Electronic GmbH, Germany), measuring forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), FEV_1/FVC ratio, and maximal expiratory flow at 50%, 75%, and 25-75% of FVC (MEF_{50}, MEF_{75}, and MEF_{25-75}, respectively), by recording the best result from three measurements of the values of FEV_1 within 5% of each other. The results were expressed as percentages of the predicted values according to the European Community for Coal and Steel (ECCS) norms. The spirometry results were given as percents of their predicted values due to the current European Respiratory Society (ERS) and American Thoracic Society (ATS) recommendations, including reproducibility and acceptability [29].

Histamine challenge

BHR was assessed by the histamine challenge test performed according to the actual European Respiratory Society (ERS)/American Thoracic Society (ATS) recommendations [30], [31]. Namely, concentrations of 0.5, 1, 2, 4, and 8 mg/mL histamine (Torlak, Serbia) were prepared by dilution with buffered saline. Afterwards, the doses of aerosol generated by Pari LC nebuliser with an output rate of 0.17 mL/min were inhaled by the mouthpiece. Subjects inhaled increasing concentrations of histamine using a tidal breathing method until FEV_1 fell by more than 20% of its base value (provocative concentration 20-PC_{20}) or until the highest concentration was reached.

According to the ATS recommendations, BHR was categorized as moderate to severe BHR (PC_{20} < 1.0 mg/mL), mild BHR (PC_{20} = 1.0 - 4.0 mg/mL) and borderline BHR (PC_{20} = 4.0 - 8.0 mg/mL) [31].

Job exposure matrices

To assess occupational exposure to respiratory agents among dairy farmers, we have used job exposure matrices recommended by the European Association of Schools of Occupational Medicine (EASOM), both qualitative matrix, and quantitative matrix with exposure intensity and exposure frequency [32].

Diagnostic criteria for asthma and COPD

According to the actual recommendations by Global Initiative for Asthma (GINA), asthma in subjects with normal spirometry findings is defined as symptomatic BHR with $PC_{20} \leq 4$ mg/mL, whereas in subject with respiratory impairment with positive bronchodilator test [33].

According to the actual recommendations by Global Initiative for Chronic Obstructive Lung Disease (GOLD), COPD is defined by post-bronchodilator FEV_1/FVC ratio lower than 0.70 in subjects with dyspnea, chronic cough and/or cough with phlegm [34].

Statistical analysis

We have analysed the data using Statistica for Windows version 7. Continuous variables were expressed as mean values with standard deviation and categorical variables as numbers and percentages. The chi-square test (or Fisher's exact test) was used for testing differences in the prevalence of respiratory symptoms, while the comparison of spirometric measurements was performed by independent-samples T-test.

A P-value of less than 0.05 was considered statistically significant. Logistic regression analysis was used to assess the risk for chronic respiratory symptoms, asthma and COPD development within job-exposure matrices, adjusted for age and smoking

habit. Study variables were checked for normality by Kolmogorov-Smirnov and Shapiro-Wilk's W test.

Results

Table 1 gives an overview of the overall and demographic characteristics of the study subjects.

Table 1: Demographics of the study subjects

Variable	Dairy farmers (n = 83)	Office workers (n = 80)
Gender / M/F ratio	2.6	2.7
Age range (years)	20 - 63	21 - 64
Age (years)	52.6 ± 8.7	52.7 ± 8.2
BMI (kg/m^2)	25.4 ± 3.6	26.2 ± 3.7
Duration of employment (years)	26.3 ± 10.1	25.3 ± 9.8
Duration of exposure	23.7 ± 7.6	/
Daily smokers	39 (46.9%)	39 (48.7%)
Life-time smoking (years)	18.9 ± 7.6	19.2 ± 7.8
Cigarettes / day	14.6 ± 6.8	14.8 ± 7.2
Pack-years smoked	12.5 ± 4.8	12.9 ± 4.9
Ex-smokers	9 (10.8%)	12 (15%)
Passive smokers	7 (14%)	8 (16%)

Numerical data are expressed as mean value with standard deviation; frequencies as number and percentage of study subjects with certain variable; BMI: body mass index; kg: kilogram; m: meter.

The subjects of examined and the control group reported neither diagnose of any chronic respiratory non-occupational disease (sarcoidosis, tuberculosis) established before the study nor treatment with oral corticosteroids, bronchodilators, antihistamines or any other medications that could potentially influence the functional and clinical findings.

The frequency of chronic respiratory symptoms in the last 12 months is higher in dairy farmers compared to office controls, with a significant difference for cough, cough with phlegm, and wheezing (Table 2). The association of respiratory symptoms and exposure duration among dairy farmers is shown in Table 2.

Table 2: Prevalence of respiratory symptoms in the last 12 months in both examined groups and prevalence of respiratory symptoms in the last 12 months in dairy farmers with a duration of workplace exposure more and less than 20 years

Respiratory symptoms in the last 12 months	Dairy farmers (n = 83)	Office workers (n = 80)	P-value*
Any respiratory symptom	34 (40.9%)	23 (21.2%)	0.102
Cough	32 (38.5%)	17 (24.3%)	0.016
Phlegm	23 (27.7%)	12 (15%)	0.048
Dyspnea	17 (20.5%)	8 (10%)	0.063
Wheezing	18 (21.7%)	8 (10%)	0.041
Chest tightness	8 (9.6%)	6 (7.5)	0.626
Dairy farmers			
Respiratory symptoms in the last 12 months	Exposed > 20 years (n = 59)	Exposed ≤ 20 years (n = 24)	P-value*
Any respiratory symptom	32 (54.2%)	6 (25%)	0.015
Cough	27 (45.7%)	5 (20.8%)	0.034
Phlegm	19 (32.2%)	4 (16.7%)	0.151
Dyspnea	14 (23.7%)	3 (12.5%)	0.370
Wheezing	14 (23.7%)	4 (16.7%)	0.479
Chest tightness	5 (10.1%)	2 (8.3%)	0.983

Data are expressed as number and percentage of study subjects with certain variable; * Tested by chi-square test or Fisher's exact test where appropriate.

The risk for development of chronic respiratory symptoms is about six-fold higher among dairy farmers exposed more than 20 years compared to those with shorter job exposure (OR = 5.93 (1.63-23.51) CI 95%).

The mean values of spirometric parameters are lower in dairy farmers compared to controls, but being significant only for MEF_{25}, MEF_{50}, and MEF_{75} (Table 3).

Table 3 shows the mean values of spirometric parameters in dairy farmers with an exposure duration of more than 20 years and those with less than 20 years.

Table 3: Mean values of spirometric parameters in examined groups and mean values of spirometric parameters in dairy farmers with a duration of workplace exposure more and less than 20 years

Spirometric parameter	Dairy farmers (n = 83)	Office workers (n = 80)	*P*-value*
FVC (% pred)	93.1 ± 9.6	94.3 ± 9.9	0.427
FEV_1 (% pred)	86.2 ± 8.8	87.2 ± 8.9	0.466
FEV_1/FVC%	73.4 ± 4.9	74.9 ± 5.1	0.054
MEF_{25} (% pred)	57.9 ± 7.1	60.7 ± 6.9	0.010
MEF_{50} (% pred)	58.2 ± 7.2	61.9 ± 7.1	0.001
MEF_{75} (% pred)	59.2 ± 6.7	62.3 ± 7.2	0.004
MEF_{25-75} (% pred)	61.9 ± 7.9	64.2 ± 8.3	0.068
Dairy farmers			
Spirometric parameter	Exposed > 20 years (n = 59)	Exposed ≤ 20 years (n = 24)	*P*-value*
FVC (% pred)	92.2 ± 9.4	94.1 ± 9.9	0.400
FEV_1 (% pred)	85.3 ± 8.4	86.7 ± 9.1	0.503
FEV_1/FVC%	72.2 ± 5.1	74.3 ± 4.8	0.087
MEF_{25} (% pred)	57.1 ± 6.9	58.2 ± 7.3	0.519
MEF_{50} (% pred)	56.5 ± 7.2	59.9 ± 6.7	0.050
MEF_{75} (% pred)	57.7 ± 6.9	61.1 ± 7.1	0.046
MEF_{25-75} (%pred)	60.3 ± 7.1	61.9 ± 7.9	0.370

Data are expressed as mean value with standard deviation. FVC: forced vital capacity; FEV_1: forced expiratory volume in 1 second; MEF_{50}, MEF_{75}, MEF_{25-75}: maximal expiratory flow at 50%, 75%, and 25-75% of FVC, respectively; % pred: % of predicted value; * Tested by independent-sample *T*-test.

The mean values of spirometric parameters among dairy farmers exposed more than 20 years are lower than in those with exposure less than 20 years with a significant difference for MEF_{50} and MEF_{75}.

Table 4 gives an overview of the overall status of the EG and CG subjects concerning the presence or absence of some chronic respiratory disease.

Table 4: Frequency of asthma and COPD among study subjects

	Dairy farmers (n = 83)	Office workers (n = 80)	P-value*
No disease n (%)	56 (67.5%)	67 (83.7%)	P > 0.05
COPD n (%)	7 (8.4%)	3 (3.8%)	P > 0.05
Asthma n (%)	6 (7.2%)	4 (5%)	P > 0.05
Chronic bronchitis n (%)	14 (16.9%)	6 (7.5%)	P > 0.05

Data are given as a number and per cent of study subjects with a certain variable.

The effect of job exposure, among EG subjects, is assessed by their exposure to a certain respiratory hazard, and by their daily work activities. It is assessed as exposure to every hazard individually, but also as a combination of exposure to several respiratory hazards simultaneously. Furthermore, 83 dairy farmers are responsible for work in barns and other confinement spaces, working as cow breeders. Their daily activities include milking, animal feeding, mechanisation use, indoor and outdoor cleaning etc.

During daily activities, they are exposed to a wide spectrum of respiratory hazards such as dust, temperature variations, fodder, gases, vapours, moisture, fumes etc.

Distribution of chronic respiratory symptoms associated with exposure to respiratory hazards, analysed by the qualitative job-exposure matrix in dairy farmers is shown in Table 5 through the odds ratios, after adjustment for age, gender, and smoking habit.

Table 5: Frequency of chronic respiratory symptoms associated with exposure to respiratory agents in dairy farmers (Prevalence ORs (95% CI)*)

Data from job exposure matrices	Cough	Cough with phlegm	Chronic bronchitis	Dyspnea	Wheezing	Chest tightness
Qualitative matrix						
Exposure to dust	2.45 * (0.56-5.04)	1.41 (0.12-3.25)	3.21 * (0.67-9.14)	1.54 (0.33-3.36)	2.37 * (0.28-5.09)	1.78 (0.32-3.76)
Exposure to gases/fumes/vapors	1.42 (0.24-3.47)	3.04 * (0.47-7.12)	2.25 * (0.42-5.09)	2.73 * (0.42-4.48)	1.76 (0.21-3.87)	1.43 (0.17-3.51)

Data are given as odds ratios (ORs) with 95% confidence interval (95% CI); * P < 0.05; OR = odds ratio; CI: confidence interval; * Tested by logistic regression after adjustment for age, gender, and smoking habit.

According to the table exposure to dust significantly increases the risk for cough, chronic bronchitis, and wheezing, while exposure to gases/fumes/vapours has a significant influence on the cough with phlegm, chronic bronchitis, and dyspnea among dairy farmers.

Table 6 gives an overview of exposure to respiratory hazards according to the job exposure matrices among dairy farmers, related to the registered chronic respiratory diseases (asthma, COPD, and chronic bronchitis).

Table 6: Exposure to respiratory hazards according to job exposure matrices related to chronic respiratory diseases in dairy farmers

	No disease N (%)	COPD N (%)	Asthma N (%)	Chronic bronchitis N (%)	P-value*
Subjects n (%)	56 (67.5%)	7 (8.4%)	6 (7.2%)	14 (16.9%)	
Qualitative job-exposure matrix					
Dust	37 (66.1%)	6 (85.7%)	5 (83.3%)	12 (85.7%)	P > 0.05
Gases/fumes/vapors	33 (58.9%)	5 (71.4%)	5 (83.3%)	11 (78.6%)	P > 0.05
Matrix with exposure intensity					
Dust exposure					
Low	26 (70.3%)	0	0	2 (16.7%)	/
Intermediate	7 (18.9%)	2 (33,3%)	1 (20%)	3 (25%)	P > 0.05
High	4 (10.8%)	4 (66,7%)	4 (80%)	7 (58.3%)	P < 0.05*
Gases/fumes/vapors exposure					
Low	24 (72.7%)	1 (20%)	0	2 (18.2%)	/
Intermediate	6 (18.2%)	1 (20%)	1 (20%)	2 (18.2%)	P > 0.05
High	3 (9.1%)	3 (60%)	4 (80%)	7 (63.6%)	P < 0.05*
Matrix with exposure frequency					
Dust exposure					
Rare	25 (67.6%)	0	0	3 (25%)	/
Sporadic	8 (21.6%)	3 (42,9%)	1 (20%)	2 (16.7%)	P > 0.05
Regular	4 (10.8%)	4 (57,1%)	4 (80%)	7 (58.3%)	P < 0.05*
Gases/fumes/vapors exposure					
Rare	26 (78.8%)	0	1 (20%)	3 (27.3%)	/
Sporadic	5 (15.2%)	2 (40%)	1 (20%)	1 (9.1%)	P < 0.05*
Regular	2 (6%)	3 (60%)	3 (60%)	7 (63.6%)	P < 0.05*

Level of statistical significance: *P < 0,05; * Tested by chi-square test or Fisher's exact test for trend.

According to the table, development of asthma, COPD and chronic bronchitis in dairy farmers is significantly associated with regular exposure to dust with a high level of intensity, and sporadic and regular exposure to gases, fumes and vapours with high exposure intensity.

Association of asthma and COPD with exposure to respiratory hazards verified by job exposure matrices among dairy farmers, and expressed through odds ratios after adjustment for age, gender, and smoking habit, is shown in Table 7.

Table 7: Risk for development of asthma and COPD due to occupational exposure to respiratory agents according to the matrices for job exposure among dairy farmers

	OR (95% CI)	
	Asthma	COPD
Qualitative job-exposure matrix		
Dust	1.83 (0.40-3.71)	1,91 (0,43-3,90)
Gases/fumes/vapors	1.68 (0.32-3.64)	1,74 (0,27-3,81)
Matrix with exposure intensity		
Dust exposure		
Low	1.57 (0,19-3,63)	1,68 (0,30-3,73)
Intermediate	1.69 (0,22-3,87)	2,07 * (1,03-4,15)
High	2.28 * (1,21-4,36)	3,12 * (1,45-6,35)
Gases/fumes/vapors exposure		
Low	1.59 (0,45-3,52)	1,61 (0,42-3,79)
Intermediate	1.82 (0,63-3,77)	1,81 (0,53-3,92)
High	2.67 * (1,23-5,12)	3,14 * (1,75-6,25)
Matrix with exposure frequency		
Dust exposure		
Rare	1.67 (0.44-3.12)	1.63 (0.43-3.12)
Sporadic	1.81 (0.61-3.56)	1.83 (0.49-3.88)
Regular	3.03 * (1.33-5.98)	2.47 * (1.26-5.29)
Gases/fumes/vapors exposure		
Rare	1.54 (0.27-3.02)	1.67 (0.39-3.12)
Sporadic	1,71 (0,44-3,12)	1.85 (0.48-3.33)
Regular	2,18 * (1,04-4,05)	2.46 * (1.25-5.17)

Data are given as odds ratios (ORs) with 95% confidence interval (95% CIs). * P < 0.05; OR = odds ratio; CI: confidence interval; * Tested by logistic regression after adjustment for age, and smoking habit.

According to the results in the table, a high level of dust and gases/fumes exposure regularly significantly increases the risk for asthma development among dairy farmers. On the other hand, intermediate and high level of regular dust exposure in dairy farmers significantly increases the risk for COPD development. Having in mind exposure to gases/fumes/vapours, the risk for COPD development is significantly associated with a high level of exposure regularly.

According to data obtained by job-exposure matrices, asthma, COPD and chronic bronchitis in dairy farmers are significantly related to the high intensity of dust exposure regularly, as well as high intensity of exposure to gases, fumes and vapours both on sporadic and regular basis.

Discussion

Chronic respiratory symptoms, functional lung impairment and respiratory disorders remain important clinical and public health issues for farmers worldwide [35].

The actual study compares the prevalence of chronic respiratory symptoms, lung function impairment and chronic obstructive respiratory diseases between dairy farmers and office workers, focusing on job exposure matrices as an effective tool for exposure assessment. The prevalence of chronic respiratory symptoms among dairy farmers in the actual study is 40.9%, and 65% of them report their work-relatedness, while office workers report the

frequency of 21.2% and no workplace association. The prevalence is higher among exposed workers, and significant for cough, phlegm, and wheezing.

Several publications report on two cohorts of dairy farmers established in the Doubs region of France [1], [2], [36]. Gainet et al., reevaluated the original 1986 cohort after 12 years with 157 dairy farmers and 159 controls [37], whereas the original cohort included 250 dairy farmers and 250 controls [1]. Accelerated declines in FVC and FEV_1 were associated with age, smoking, and male gender. The authors concluded that dairy farming was associated with increased risk of lung disorders and that a relationship exists between cumulative exposure to organic dust and a decrease in blood oxygen saturation and respiratory function [37]. In 1999, Chaudemanche et al. reevaluated a Doubs cohort from 1994 and compared 215 dairy farmers with 110 controls [4]. Current FEV_1 was lower among dairy farmers than controls, and dairy farming was associated with an accelerated decline of FEV_1 and FEV_1/VC over time. Mounchetrou et al. conducted a 12-year follow-up of the 1994 cohort, reevaluating 219 dairy farmers [38]. The key findings of this study were that those working on "traditional" dairy farms stopped working on the farm earlier than those who worked on "modern" farms. Other predictors of early cessation of work were the presence of asthma or impaired lung function, and age at inclusion. In 2006, Thaon et al., performed another follow-up on this same cohort, including 219 dairy farmers, 130 non-dairy farmers, and 99 controls [39]. The increased decline in FEV_1 and FEV_1 / FVC was associated with handling animal feed and years of exposure [39]. These studies show a consistent excess of chronic bronchitis among dairy farmers, with a continuing decline in pulmonary function in this cohort over more than a decade. Rask-Andersen conducted a 12-year follow-up among 380 Swedish farmers, mostly dairy farmers, focusing on asthma [40]. Greater declines were seen in farmers with asthma and chronic bronchitis [40]. Eduard et al.'s study of Norwegian farmers included personal exposure assessment of participants who had undergone clinical evaluations [7] with 12% of participants being dairy farmers and showed that FEV_1 was significantly reduced for livestock farmers. Several studies measured pulmonary function with inhalation exposure assessment. COPD was associated with higher exposures to dust and endotoxin in the study by Monsó et al., about the European farmers [14]. The duration of feeding (foddering) was identified as a significant risk factor in the occurrence of farming-induced COPD [41].

Several researchers have confirmed an increased prevalence of self-reported adult-onset asthma among US dairy workers compared with rural controls [42]. Similar, but less-pronounced findings were observed in a population-based study of 2903 dairy workers from New Zealand [43]. In a 12-year follow-up study among 380 Swedish (mostly dairy) farmers, an increase in asthma prevalence was found that was considerably greater for dairy farmers (from 2% to 9%) compared with the general Swedish population (from 3% to 6%) [40]. A nested case-control study among 2000 farming apprentice's and 400 rural controls showed that the new onset of asthma was associated with dairy production (OR = 2.5) [44]. In another study reevaluating symptoms in the French Doubs cohort of 219 dairy farmers, 130 other agricultural workers, and 99 controls, they observed an increased OR for indices of asthma (OR = 1.5-2.5, not significant) among dairy farmers compared with controls [39]. Results from the same cohort suggest the early cessation of work to be associated with the presence of asthma [38].

In a cross-sectional study of 4735 Norwegian farmers, dairy farmers were more likely to have COPD (OR = 1.30), and reduced FEV_1 compared with crop farmers [7]. The prevalence of COPD among dairy farmers was 13.5%. Farmers with atopy were more susceptible to developing COPD [7]. No significant difference in lung function was found in a cross-sectional analysis of farmers and non-farmers among 150 subjects from the USA with COPD [45]. Monsó et al. conducted a cross-sectional study of COPD among 105 European farmers working in animal confinement buildings [14]. Lung function was measured before and after work, and symptoms documented using questionnaires based on the European Community Respiratory Health Survey (ECHRS) [46]. Eighteen of the farmers (17%) had COPD (7 mild, 8 moderate, 3 severe), and 20 (19%) had a variability of over 10% during the work shift. The ECRHS reported that the highest risk of developing occupational asthma (OA) was registered in farmers (OR 2.6, CI 1.3 to 5.4) followed by agricultural workers (OR 1.8, CI 1.0 to 3.2) [7], [47]. In adult farmers, asthma symptoms are more likely regarded as work exacerbated asthma (WEA) as opposed to OA [7]. Subjects with moderate or severe asthma and/or subjects not receiving optimal treatment of their asthma may develop WEA when exposed to potential irritants such as dust, fumes and sprays. OA in 90% of the time is IgE-mediated to components, which include animal dander, storage mites, and cockroach [48], while only a small portion of cases is irritant-induced OA. Farmers and agricultural workers have increased risk of respiratory morbidity and mortality from chronic bronchitis and COPD. Eduard et al., [7] reviewed multiple studies from European farmers and found that Danish swine farmers had the highest prevalence of chronic bronchitis at 32%, compared to 28% in farmers that had swine and cattle. Farmers that did not raise any livestock displayed the lowest prevalence at 18.6% [15]. Also, livestock farmers and dairy farmers demonstrated significantly increased risk of developing COPD (livestock farmers, OR 1.4, CI: 1.1 to 1.7; dairy farmers, O.R.1.3, CI: 1.0 to 1.7) [7]. Moreover, raising more than one type of livestock enhanced the risk of farmers developing chronic bronchitis and COPD as compared to crop farmers.

The livestock farmers also showed the lowest FEV_1, consistent with the pattern of lung function decline [7].

Agricultural workers inhalation exposure to dust measured over the work-shift has been reported from 0.8 to 20 mg per cubic meter (mg/m^3) [15], [10], [49]. As dairy production has increased in size due to the raising of economy, task-specialisation has increased [50]. However, little information is available on the characterisation of task-based exposures among dairy workers. Previous studies of inhalation exposure have combined exposure measurements across several tasks in dairy production (e.g., milking and feeding); consequently, limiting the application of the industrial hygiene hierarchy of exposure controls [51].

In the absence of detailed questionnaires, ambient monitoring or expert's evaluation, the exposure assessment using job exposure matrices can provide useful information within epidemiological studies [52].

Within the EG, the exposure to occupational respiratory hazards (dusts, gases, fumes, vapors) in each subject, besides through self-reported Questionnaire for occupational exposure to respiratory hazards, in the current research has also been estimated according to the data obtained from job exposure matrices to respiratory hazards (qualitative, matrix with exposure intensity, and matrix with exposure frequency). In this way, exposure to certain occupational respiratory hazards is also consistent with the specific work activities of the farmers, and it is possible to determine the predictive factors (qualitative or quantitative exposure to respiratory hazards) for the occurrence and development of chronic respiratory symptoms, as well as ventilatory impairment among the EG.

The results of our previous study recognised the role of job exposure matrices in farming exposure assessment and characterisation, their potential to be a predictive factor in the development of respiratory diseases, and promote their applicability within the diagnostic algorithm for respiratory health assessment among crop farmers [53].

Exposure to dust significantly increases the risk of cough, chronic bronchitis and wheezing, exposure to gases/fumes/vapours significantly affects the risk of cough with phlegm, chronic bronchitis and dyspnea, while exposure to gases/fumes/vapours significantly impacts the occurrence of dyspnea in the EG. The exposure intensity matrix gives an overview of the influence of the exposure degree to respiratory hazards (low, medium, high) on the occurrence of chronic respiratory symptoms, as well as the spirometric parameters in the EG subjects, while the exposure frequency matrix indicates it for the frequency of exposure to respiratory hazards (rarely, occasionally, regularly).

An Italian study dedicated to the exposure of chemical hazards in agricultural workers shows that with the help of matrices, it is possible to make a quantitative assessment of the cumulative exposure of the subjects, as well as to propose measures for preventing and early detection of respiratory disorders and workplace promotion of health workplace among agricultural workers [54]. The British matrix was applied in a study by Zutphen et al., [55] to explore the relationships between specific hazards and chronic non-specific lung disease, without associating with the lung function parameters. In the French study PAARC (Pollution Atmospheric et Affections Respiratoires Chroniques), an association was found between dust, gases, and fumes, and respiratory symptoms in both genders, and the FEV_1/FVC ratio in men. Examining the relationship between occupational exposures and pulmonary function, the study discovers a significant association of known risk factors and the FEV1 decline as evidence for the validity of matrices [56].

The asthma prevalence is associated with workplace exposure to dust, gases and fumes, estimated as self-reported or through an external job-exposure matrix [57]. A matrix specific for asthma was developed in the French epidemiological study dedicated to the association between genetic factors and the environment in asthma (EGEA) [58]. The study shows that the asthma risk associated with occupational exposure to specific high molecular weight agents can be identified using an asthma-specific job-exposure matrix.

According to the data from job exposure matrices in the current research, it was concluded that the occurrence of asthma, COPD and chronic bronchitis in EG subjects is significantly related only to regular and high-intensity exposure to dust, as well as occasional and regular exposure to gases, fumes and vapours with high exposure intensity.

Epidemiological studies in France, the Netherlands and Norway provide data on an individual basis for occupational exposure to respiratory hazards and lung function. Significant associations have been found between occupational exposure assessed by a specific job exposure matrix and pulmonary function in research in rural settlements in France and the Netherlands, but there is no significant relationship with self-reported exposure to respiratory hazards using a questionnaire [59].

Taking into account the matrices with the intensity and frequency of exposure in the current research, it can be concluded that the high level of exposure to dust and gases/fumes/vapours on a regular basis, significantly increases the risk of asthma in subjects from EG, and the medium and the high degree of regular exposure to dust significantly increases the risk of COPD. In terms of exposure to gases/fumes/vapours, the risk of developing COPD is significantly related to the high level of exposure regularly.

In our previous study about COPD in never-

smoking dairy farmers, the results have shown that dairy farmers had a significant association between COPD and employment duration of over 20 years, but also between COPD and work-related chronic respiratory symptoms. The study findings are in line with the results from other similar studies about the cause-effect association between job exposure to respiratory hazards among dairy farmers and the development of persistent airway obstruction among dairy farmers [60].

In a study on the performance of the job-exposure matrix in detection of risk factors for the onset of COPD, Le Moual et al., [61] indicate the association between the estimated occupational exposure through the specific population matrix and impaired pulmonary function, unlike the so-called, self-reported exposure that has not revealed such a relationship in men or women in the French study, nor in a study in Denmark's rural areas. The study shows that job exposure matrices are relatively easy to design, their application is not limited to the number and categories of workers involved, and have much better performance than the self-reported method using questionnaires, especially when it comes to larger groups of respondents with similar work activities [61].

The job-exposure matrices for respiratory hazards in farmers provide an opportunity to evaluate occupational exposure by cross-summarizing the results for the types of activities and exposure to various hazards through several indicators (presence, intensity, frequency and/or probability), combining with data from work history and assessment of exposure during the total exposure duration period.

The matrices are widely used for assessing occupational exposure and for generating hypotheses in large groups of respondents, especially in the absence of specific questionnaires for occupational exposure. Despite the expected disadvantages, the matrices offer great opportunities and deserve a special place in the assessment of exposure to occupational respiratory hazards. Further research is needed to improve its performance and predictive value [56].

This study has certain limitations, namely, the relatively small number of subjects in the study groups, and an absence of ambient monitoring, which could aggravate a clear relationship between occupational exposure and respiratory impairment in dairy farmers.

In conclusion, our data revealed a higher prevalence of respiratory symptoms, significantly lower values of small airways indices, and a higher prevalence of asthma and COPD in dairy farmers compared to controls, also associated to exposure duration. The results recognised the role of job exposure matrices in assessment and characterisation of farming exposure, confirmed their potential to be a predictive factor in the development of respiratory diseases, and promote their applicability within the diagnostic algorithm focused on respiratory health assessment.

This knowledge should further contribute in the detection of critical points for action, but also indicate the need for reduction of adverse occupational exposures through adequate preventive measures, obligatory use of respiratory protective equipment, and implementation of engineering controls.

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Factors Influencing Health Promoting Behaviours in Women of Reproductive Age in Iran: Based on Pender's Health Promotion Model

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Abstract

INTRODUCTION: Health promotion behaviours are considered as preventives of non-communicable diseases and key determinants of maintaining and improving the health status.

AIM: This study aimed to investigate and identify effective factors on health-promoting behaviours based on Pender model in women of reproductive age from February to April 2017, in Savojbolagh, Iran.

METHODS: This cross-sectional study is conducted on 240 women aged between 15 to 49 years in Savojbolagh, Iran, in 2017. The questionnaire consisted of several items, including socio-demographic characteristics, health-promoting lifestyle profile-II (HPLP-II), self-efficacy, social support and constructs of Pender's health promotion model. SPSS-18 software has been applied for statistical analysis.

RESULTS: The mean age of the women was 31.10 ± 7.29 years. Total HPLP-II score was 106.64 ± 11.93. The highest and the lowest mean in the subscales were belonged to nutrition and physical activity, respectively. According to the bivariate analysis, the total HPLP-II score is significantly related to prior health-related behaviour ($p = 0.000$). There was a statistically significant relationship between stress management and the variables including perceived benefits, perceived barriers, prior health-related behaviour, situational influences, commitment to a plan of action ($p < 0.05$). Also, health responsibility had a statistically significant relationship with self-efficacy ($p < 0.05$).

CONCLUSION: According to our results, it can be inferred that there is a problem with the HPBs of women. Considering that health-promoting behaviours like physical activity had a low score, it is a necessity to plan and perform interventions for improving health promotion behaviours.

Introduction

Health-promoting behaviours (HPBs) refer to general activities that improve self-realisation and a sense of well-being, that include acts that assist persons in maintaining and promoting healthy lifestyles [1]. HPBs are categorised in six dimensions based on Pender's health promotion model as follows: physical activity, nutrition, stress management, health responsibility, interpersonal relations and spiritual growth [2]. Furthermore, health promotion behaviours are considered as preventives of non-communicable diseases (NCDs) and key determinants of maintaining and improving the health status [3]. NCDs are known as the leading causes of morbidity and mortality in most low- and middle-income countries [4].

Currently, 63% of annual global deaths (over 36 million people) belongs to NCDs, which most of them are preventable [5]. The estimated worldwide cost of NCDs was $ 6.3 trillion (US dollars) for 2010, and it is projected to be increased to $ 13 trillion by 2030 [6]. It is anticipated that these diseases will be causing seventy per cent of deaths in developing countries by 2020 [7].

The majority of the Iranian female population are in their reproductive age [8]. Because of the health of women of reproductive age impacts their long-term health and that of their family members, particularly their children, it is necessary to promote women's health [9]. Nearly 80% of 18 to 55-year-old women had multiple lifestyle risk behaviours reported by Sanchez et al., [10]. Kontis et al. estimated the effects of attaining targets for six risk factors (tobacco

and alcohol use, salt intake, obesity, and raised blood pressure and glucose) on NCD mortality between 2010 and 2025. They concluded that the probability of dying from the four main NCDs in women of 30 to 70 years age group would be decreased by 19% between 2010 and 2025 if targets of risk factors are achieved [11]. There are as several factors affecting health-promoting behaviours reported by previous studies such as age, education level, gender, employment status, family income, perceived social support, self-esteem, self-efficacy, previous health-related behaviors, perceived benefit, health knowledge and marital status [9], [12], [13], [14], [15], [16]. In this study, Pender's health promotion model has been applied to identify effective factors on HPBs. According to Pender's health promotion model, HPBs can be influenced by following variables: individual characteristics and experiences; behaviour-specific cognitions (perceived benefits of action, perceived barriers of action, perceived self-efficacy, situational influences and social support); and adherence to specific plans of action [17].

Finally, this study aimed to investigate and identify effective factors on HPBs in women of reproductive age in Savojbolagh, Iran.

Material and Methods

This cross-sectional study is conducted in Savojbolagh, Iran, from February to April 2017. The study population included women aged 15 to 49 years. The ethics committee at Tehran University of Medical Sciences provided ethics approval for the study, which is part of a PhD thesis in the field of health education and promotion, with the code IR.TUMS.VCR.REC.1395.57. All women were informed about the objectives of the study, and written consent was obtained from them. To select the sample size, 240 applicants were determined by considering the 95% confidence interval, the power of 80% and a 10% attrition rate.

The inclusion criteria for this study were as follows: being willing to participate in the research; residing in Savojbolagh county; within the 15 to 49 year age group; not pregnant, and not having experienced unpleasant events during the past month (such as the death of a family member or divorce).

The following tools have been utilised to collect data:

Self-efficacy: A five-point scale, developed by Sherer and Maddux, is used to measure self-efficacy in general situations by seventeen items. Total possible scores ranged from 17 to 85, which higher scores imply a deeper belief in one's ability to succeed in performing duties [18]. Cronbach's α for the scale was 0.76 in this study.

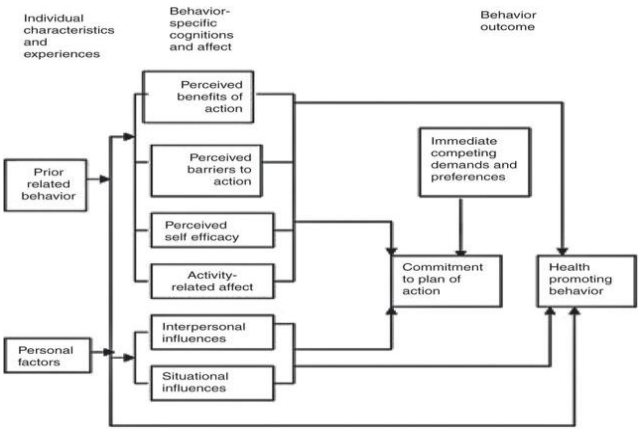

Figure 1: Pender's Health Promotion Model

Social support: This questionnaire is designed and developed by Vaux et al. in 1986. The questionnaire had 23 questions with three domains of family, friends and others for evaluation the social support. Family, friends and other people subscales have 8, 7 and 8 questions, respectively. A zero-one grading system is utilised for this study. Zero was minimum, and 23 was maximum grades for the participants, which higher grades indicate the larger scale of social support [19]. Cronbach's α for the scale was 0.82 in this study.

Health promotion lifestyle profile Scale-II: The HPLP-II, developed by Walker, Sechris, and Pender (1987), is applied for the purpose of determining the healthy lifestyle behaviours. The HPLP-II questionnaire consisted of 52 items with the six aspects of health-promoting behaviours including nutrition (9 items), physical activity (8 items), spiritual growth (9 items), health responsibility (9 items), stress management (8 items) and interpersonal relations (9 items). All items are scored from 1 to 4 using the Likert scale (1 = never, 2 = sometimes, 3 = often, 4 = routinely). The lowest and highest possible score for the entire scale were 52 and 208, respectively [2]. In this study, the alpha coefficient was 0.88 for the total scale and 0.67 to 0.90 for the subscales.

Constructs of Pender's health promotion model: The structures of Pender's health promotion model have been measured through questions including perceived benefits (6 items), perceived barriers (6 items), prior related behaviour (6 items), situational influences (6 items) and commitment to a plan of action (6 items). All the items are scored based on a five-point Likert-type scale. The content validity of the instrument was CVI = 0.86 and CVR = 0.80, with the Cronbach's alpha coefficient of 0.79 for the total scale and 0.74 to 0.86 for the subscales.

Data have been analysed with descriptive statistics (mean, standard deviation, frequency, percentage, etc.) and analytical tests (Pearson correlation test, t-test and ANOVA) by using SPSS 18 software.

Results

The mean age of the women was 31.10 ± 7.29 years. Almost half of the women (51.7%) were aged between 25 and 34 years. The majority of women (91.2%) were married. 57.5 % of women were overweight. Table 1 presents the demographic characteristics of the studied participants.

Table 1: Demographic characteristics of the participants (n = 240)

Demographic variables		Frequency	Percentage
Age	15-24	41	17.1
	25-34	124	51.7
	35 or older	75	31.2
Education	Primary School	29	12.1
	Guidance School	104	43.3
	High School	66	27.5
	Bachelor and higher	41	17.1
Marital status	Single	21	8.8
	Married	219	91.2
Occupation	Employed	41	17.1
	Housekeeper	199	82.9
BMI	less than 18.5	0	0
	18.5-24.9	37	15.4
	25-29.9	138	57.5
	30 and more	65	27.1

The mean total HPLP-II score of women was 106.64 ± 11.93. The highest and lowest mean in the subscales were for nutrition (19.29 ± 4.04) and physical activity (17.33 ± 3.89), respectively. The mean item score for each subscale is presented in Table 2.

Table 2: Mean and standard deviation for health promotion lifestyle profile

Scale/Subscale	Possible Range	Observed Range	Mean ± SD
HPLP II total	52-208	71-151	106.64 ± 11.93
Health responsibility	9-36	9-28	17.50 ± 3.49
Physical activity	8-32	9-29	17.33 ± 3.89
Nutrition	9-36	11-31	19.29 ± 4.04
Spiritual growth	9-36	10-28	17.67 ± 3.01
Interpersonal relationship	9-36	9-28	17.45 ± 3.37
Stress management	8-32	9-29	17.40 ± 3.68

Possible and observed ranges, mean and standard deviations for constructs of Pender's health promotion model among studied women are shown in Table 3. The mean score of perceived social support was 10.58 ± 1.56. Also, the mean score of self-efficacy was 45.38 ± 8.25.

Table 3: Mean and standard deviation for constructs of Pender's health promotion model

Constructs	Possible Range	Observed Range	Mean ± SD
Perceived Benefits	6-30	8-25	15.84 ± 2.50
Perceived Barriers	6-30	11-27	17.45 ± 2.66
Prior Related Behavior	6-30	8-25	16.91 ± 2.82
Situational influences	6-30	8-20	14.72 ± 2.10
Commitment to a plan of action	6-30	7-24	14.35 ± 2.52
Self-efficacy	17-85	28-69	45.38 ± 8.25
Social support	0-23	7-15	10.58 ± 1.56

According to bivariate analysis, the total HPLP-II score was significantly related to prior related behaviour (r = 0.242, p = 0.000). A statistically significant relationship is found between stress management and the variables such as perceived benefits, perceived barriers, the prior related behaviour, situational influences, commitment to a plan of action. There was also a statistically significant relationship between health responsibility and self-efficacy (Table 4).

Table 4: Relationships between studied variables and health-promoting behaviours

Variables	HPLP II total	Health responsibility	Physical activity	Nutrition	Spiritual growth	Interpersonal relationship	Stress management
Age	r = 0.096	r = - 0.002	r = 0.117	r = 0.123	r = 0.049	r = 0.008	r = 0.007
	p = 0.137	p = 0.979	p = 0.071	p = 0.056	p = 0.449	p = 0.907	p = 0.908
Education	F = 0.400	F = 1.346	F = 0.804	F = 0.569	F = 0.406	F = 2.940	F = 0.677
	p = 0.753	p = 0.260	p = 0.492	p = 0.636	p = 0.749	p = 0.034	p = 0.567
Marital status	t = -1.062	t = -0.092	t = -1.829	t = -1.137	t = -1.144	t = 0.510	t = 0.285
	p = 0.289	p = 0.927	p = 0.069	p = 0.257	p = 0.254	p = 0.611	p = 0.776
Occupation	t = -0.182	t = 0.949	t = -0.279	t = -0.849	t = -1.396	t = 0.885	t = 0.065
	p = 0.856	p = 0.344	p = 0.781	p = 0.397	p = 0.164	p = 0.377	p = 0.948
BMI	F = 0.174	F = 0.399	F = 0.135	F = 0.068	F = 0.958	F = 0.415	F = 1.217
	p = 0.840	p = 0.671	p = 0.874	p = 0.934	p = 0.385	p = 0.661	p = 0.298
Perceived Benefits	r = 0.122	r = -0.036	r = 0.044	r = 0.019	r = -0.010	r = -0.078	r = 0.581
	p = 0.058	p = 0.582	p = 0.500	p = 0.768	p = 0.879	p = 0.227	p = 0.000
Perceived Barriers	r = -0.065	r = 0.104	r = -0.053	r = -0.013	r = -0.083	r = 0.104	r = -0.266
	p = 0.315	p = 0.108	p = 0.412	p = 0.842	p = 0.198	p = 0.109	p = 0.000
Prior Related Behavior	r = 0.242	r = 0.052	r = 0.060	r = 0.050	r = 0.052	r = -0.007	r = 0.581
	p = 0.000	p = 0.424	p = 0.355	p = 0.437	p = 0.422	p = 0.919	p = 0.000
Situational influences	r = 0.036	r = -0.057	r = -0.013	r = -0.069	r = 0.053	r = 0.054	r = 0.166
	p = 0.581	p = 0.381	p = 0.840	p = 0.287	p = 0.411	p = 0.409	p = 0.010
Commitment to a plan of action	r = -0.008	r = -0.047	r = -0.022	r = -0.096	r = 0.036	r = -0.088	r = 0.199
	p = 0.903	p = 0.464	p = 0.735	p = 0.137	p = 0.575	p = 0.175	p = 0.002
Self-efficacy	r = 0.091	r = 0.198	r = 0.031	r = 0.016	r = -0.015	r = 0.074	r = 0.002
	p = 0.159	p = 0.002	p = 0.634	p = 0.811	p = 0.815	p = 0.253	p = 0.971
Social support	r = 0.027	r = -0.026	r = 0.021	r = 0.015	r = 0.055	r = 0.026	r = 0.007
	p = 0.672	p = 0.684	p = 0.747	p = 0.821	p = 0.395	p = 0.687	p = 0.920

Discussion

In this study, the total score of health behaviours was 106.64 ± 11.93, which was lower value in comparison with other studies conducted on urban Chinese women (20), middle-aged women in Iran [21] and pregnant women in Turkey [22]. In our study, the highest mean score was observed in the nutrition subgroup scale, which was inconsistent with previous studies [23], [24], [25]. However, it should be noted that other studies did not report the same results [15], [16], [26]. The high score of nutrition's sub-scale is obtained because of several parameters such as environmental characteristics of the Savojbolagh county and convenient accessibility to inexpensive fruits, vegetables and dairy products.

Women scored the lowest value for physical activity. Inactivity in these women caused overweight or obesity (mean BMI = 28.53). Our results were in agreement with most studies in different age groups [9], [13], [15], [26], [27], [28], [29]. As mentioned in these studies, an inactive lifestyle was a challenge for most countries as a major risk factor for most non-communicable diseases. Regarding the undeniable impact of exercise on peoples' health, it is required to study the reasons for the low physical activity in women by conducting qualitative studies.

In this study, HPBs were meaningfully and positively associated with prior health-related behaviours. This result was in line with that of previous researches [12], [30]. Pender proposed that prior related behaviour had direct and indirect influences on existing HPBs as they could lead to changes in present health-related behaviours and inspire habitual participation in HPBs, even without

attention to individual behaviours [1].

There was not any statistically significant relationship between health-promoting behaviours and variables including age, marital status, occupation, education and BMI (P > 0.05). In our study, a statistically significant relationship is observed between self-efficacy and health responsibility. Self-efficacy, defined as an individual's belief that he or she can successfully execute a given behaviour, is required to produce the desired outcome. Self-efficacy influences the adoption of HPBs, the cessation of unhealthy behaviors, and the maintenance of behavior modification when faced with difficulty [31]. It is worth mentioning that participants with a higher value of perceived self-efficacy applied greater exertion in practising healthy behaviours to improve their health, and they were more likely to accomplish health-promoting behaviour [32]. Lee et al. stated that self-efficacy positively correlated with health behaviours in mothers with infants and toddlers [33]. Also, Shin et al. showed that perceived self-efficacy had direct effects on HPBs in elderly Korean women [30].

The method of self-report for collecting data in this study, as a study limitation, possibly effected the tendency of participants to over or underestimate their health promotion behaviours. Another limitation was the fact that the study was cross-sectional. Therefore, the relationships observed between the HPBs and the related factors cannot be interpreted as causal. It is recommended that the importance of HPBs and their evaluation in other age groups should be considered. Finally, it is also necessary to conduct further and qualitative researches on the effect of other factors to explain women's opinion and experience of HPBs.

In our study, the HPBs have been evaluated for Iranian women in reproductive age in Savojbolagh County. According to our results, it can be inferred that there is a problem with the HPBs of women. Considering that health-promoting behaviours like physical activity had a low score, it is a necessity to plan and perform interventions for improving health promotion behaviours.

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Effectiveness of the Elderly Caring Model as an Intervention to Prevent the Neglect of the Elderly in the Family

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Abstract

BACKGROUND: The increasing number of older people is racing against diseases and problems that accompany the elderly, so it is very important to check the care of the elderly. Family concern as a caregiver is needed in carrying out care for the elderly to ensure that the elderly are not neglected.

AIM: The study aims to determine the effectiveness of the elderly caring model as an intervention to prevent the neglect of the elderly in the family.

MATERIAL AND METHODS: The quasi-experimental design with the pre-control group non-equivalent test post-test was the provision of training in the elderly caring model by comparing 2 groups namely the intervention group using the module and control group without using the module. The sample is a family that has an elderly (age ≥ 60 years) who are the main caregivers of the elderly with a total of 50 people each for each group taken by multistage cluster sampling. Data collection through questionnaires to determine the variables of family older people about family support, family health assignments, social relations, and elderly social activities and preventive behavior of elderly neglect. Data analysis used the independent sample t-test and general linear model report measure (GLM-RM) test for repeated measurements.

RESULTS: The results showed that there was an influence of the caring elderly model on increasing family support in the elderly, increasing family health duties on the elderly towards increasing social relations and social activities in the elderly and neglecting the neglect behaviour of the elderly in the family (p-value = 0,000). Improve the behaviour of preventing neglect of the elderly in the family compared to groups that do not use modules where the value of p = 0,000.

CONCLUSION: It can be concluded that the elderly caring model effectively prevents my employees from neglecting the elderly in the family.

Introduction

Entering the 2000 era, the elderly population in the world has increased by an average of 795,000 per month [1]. This figure is expected to increase steadily and reach double in 2030, including the population of Indonesia. The phenomenon of ageing in both developed and developing countries raises concerns, so demographers pay more attention to the issue of ageing. Meanwhile, the increasing mobility of productive age workers has made the care of the elderly in families more difficult. Similarly, the shift in family structure from extended families to small families has an impact on the reduction or loss of certain functions in the family such as care functions for the elderly, family support and the participation of the elderly to engage in social activities.

Given that the elderly are a group of vulnerable people who experience various changes due to ageing processes such as a decrease in physical, economic, social and psychological changes, the family as the biggest insurance for the elderly needs to be empowered so that the elderly are not neglected at home [2], [3]. Abandonment is an act of failure or negligence by the caregiver in carrying out obligations to the elderly to provide the fulfilment of physical, mental social needs so that it threatens the danger and welfare of the elderly. Neglect on the elderly is 3 aspects that are not separate, namely

physical, psychological and financial neglect. This is in line with the research conducted by Sijuwade [4] and Roobert [5] that the low quality of care for the elderly causes the elderly to be neglected by the family both physically, economically, and emotionally. The study done by Sijuwade [4] found 48% physical neglect, 20% economy neglect. The study done by Miko [7] found that elderly who entered the institution because the children felt burdened with economy 69.03%, family were often angry with the elderly 14.64% Then, the study done by Saadah [8] found that there are still many about 5.51%, elderly people who are under the poverty line and live in uninhabitable residences.

According to Miller [9], families who care for the elderly need to run a training program first. Families need to take part in activities in support groups and training education programs. The results of Wangmo [6] study found that neglect in the elderly is mostly done by caregivers who lack experience, lack of good education and training, and lack of individual to think critically who cannot understand what right to do. Families who care for the elderly need an initial understanding of the condition of the elderly with the right response [10]. Through training, it is expected that family knowledge and skills in caring for the elderly can be applied so that the elderly who have been repaired are cared for by the family. The purpose of the study, in general, is to determine the effectiveness of the elderly caring model to prevent the neglect of the elderly behaviour in the family. While the specific purpose of the study was to determine the effect of the caring elderly model on increasing family support, increasing family health tasks, increasing relationships and social activities of the elderly, and preventing the neglect of the elderly in the family.

Methods

Research Design

The study was quasi-experiment with a pre-test post-test group design approach. The intervention group is the group that gets the module and the control group without using the module. The module is a guide for changing behaviour, namely the elderly caring module [9]. Measurements of family support, family health assignments, and family social engagement and prevention behaviour for neglecting the elderly in the family were carried out before and after training (1 month and 3 months). To assess the effectiveness of the caring elderly model in the family in monitoring the evaluation of researchers assisted by health cadres in the community as a sustainable model, where researchers meet with cadres to ask about the progress and obstacles found in monitoring families, namely the ability of families to carry out support, health assignments, social engagement, and prevention behaviour for neglecting the elderly.

Population and Samples

The population in the study was all families caring for the elderly (> 60 years). Calculation of sample size using hypothesis testing is the average difference in two independent groups using formula [11]:

$$n1 = n2 = \frac{Z\sigma^2 (Z_1 - \alpha/_{2+} Z_1 - \beta)^2}{(\mu_1 - \mu)^2}$$

The sampling strategy is a multistage cluster, which is random sample selection in groups of individuals in naturally occurring populations by region. From the selected sub-districts obtained randomly one sub-district as the research area, from 7 sub-districts in the subdistrict, the new West Labuh sub-district was obtained as the intervention group (RW 10) and the Bandar Raya village (RW 3) as the control group. Based on the use of the formula by entering numbers into the formula, a sample for each group in each village of 50 caregivers was obtained.

The family criteria as the primary caregivers and responsible to the elderly, the family lives with the elderly or is elderly with the elderly but still in one city, families with elderly who are not lying alone are willing to be respondents during the study. The population in the study was all families had elderly (60 years) with the criteria of the family as the primary caregivers and responsible to the elderly family lives with the elderly or separated from the elderly but still in one city families with elderly people who are not bedridden willing to be a respondent during the research. The sampling strategy is multistage cluster sampling.

Data collection using a questionnaire

The questionnaire used is the development of caring theory (caring behaviour inventory for elders) by Watson [12]. Data on family characteristics consist of age, sex of caregiver, education, ethnicity, elderly who are treated (biological parents/in-laws). Specific data is the behaviour of preventing neglect of the elderly (physical, psychological, and financial neglect). Family support data (information support, award support, instrumental support, and emotional support). Family health assignment data and data on the participation of the elderly to engage in social activities (social engagement). Data were collected through questionnaires. Data validity and reliability tests were tried on 25 elderly people using direct interview instruments consisting of 4 parts, namely: 1) questionnaire about neglect of the elderly consisting of physical neglect with validity value 0.588-0.910 and reliability 0.936; psychological neglect with a validity value 0.699-0.88 and reliability 0.912; financial waiver

with validity value 0.668-0.88 and reliability 0.912; 2) questionnaire about family health duties with validity value 0.674-0.9959 and reliability 0.958; 3) questionnaire about family support consisting of information support with validity values 0.848-0.944 and reliability 0.988; award support with validity value from 0.727 to 0.966; emotional support with validity values 0.851-0.920 and reliability 0.964; instrumental support with validity value 0.755-0.951 and reliability 0.964; 4) questionnaire about social relations with validity value 0.685-0.936 and reliability 0.969.

Data analysis

For bivariate analysis knowing the effectiveness of the intervention between the intervention group and the control group using an independent test analysis using independent sample t-test. Multivariate analysis was used to determine behaviour change through repeated measurements (1 month and 3 months) using the General Linear Model Repeated Measure (GLM-RM). The purpose of the data analysis was to determine the difference in the increase in the mean score between the intervention group and the control group before and after 1 month and 3 months of the training intervention.

Results

Characteristics of respondents

For the age of caregiver, both the intervention group and the control group were more in the age range of 25-35 years, for the sex of the nurses there were more women both intervention groups and control groups, more high school family education, mostly Malay tribes, elderly who were treated by most biological parents.

Family Support, Family Health Task, Elderly Social Relationship, Elderly Neglect Between Intervention Groups and Control Groups

From Table 1, there is no difference in mean or score of family health task between the intervention group and the control group at the time before the intervention, where the value of p = 0.399 with the difference in the difference between the two groups is 0.40%. But there were differences in mean values after 1 month of intervention (p = 0.000 and difference in differences of 4.39%) and after 3 months of intervention (p = 0.000 and difference in the difference of 8.21%) between the intervention group and the control group. The multivariate results based on GLM-RM analysis found that there were differences in the increase in the mean value between the intervention group and the control group before intervention, 1 month and 3 months after the intervention.

Table 1: Value of Average Family Support Between Prior Intervention and Control Groups, after 1 Month, and After 3 Months Awarded Elderly Caring Model Training (n = 100)

Time	Number	Group	n	Mean	Sd	Minimum Maximum	Δ (%)	*P t-test*	*P* Multivariate
Pre	1.	Intervention	50	55.49	1.703	53-62	0.40	0.399	
	2.	Control	50	55.10	2.626	50-61			
Post 1	1.	Intervention	50	70.02	2.323	66-75	4.39	0.000	0.000
Month	2.	Control	50	64.14	2.545	61-69			
Post 3	1.	Intervention	50	78.01	2.303	74-83	8.21	0.000	
Months	2.	Control	50	66.16	2.151	64-78			

From Table 2, there is no difference in mean or score of family health task between the intervention group and the control group at the time before the intervention, where the value of p = 0.551 with the difference in the difference between the two groups is 0.15%. But there were differences in mean values after 1 month of intervention (p = 0.000 and difference in differences of 7.11%) and after 3 months of intervention (p = 0.000 and difference in the difference of 13.81%) between the intervention group and the control group. The multivariate results based on GLM-RM analysis found that there were differences in the increase in the mean value between the intervention group and the control group before intervention, 1 month and 3 months after the intervention.

Table 2: Value of the Family Health Task Mean Between the Intervention Group and the Control Group Before, after 1 Month, and After 3 Months Awarded Elderly Caring Model Training (n = 100)

Time	Number	Group	n	Mean	Sd	Minimum Maximum	Δ (%)	*P t-test*	*P* Multivariate
Pre	1.	Intervention	50	19.90	1.713	17-22	0.15	0.551	
	2.	Control	50	19.96	1.616	17-22			
Post 1	1.	Intervention	50	24.08	2.308	20-29	7.11	0.000	0.000
Month	2.	Control	50	20.88	1.547	18-24			
Post 3	1.	Intervention	50	29.00	2.372	25-34	13.81	0.000	
Months	2.	Control	50	21.96	1.795	19-29			

Table 3 shows that there is no difference in mean or score of the average social relations and social activities of the elderly between the intervention group and the control group at the time before the intervention, where the p-value is 0.147 with the difference between the two groups of 1.11%. But there were differences in mean values after 1 month of intervention (p = 0,000 and difference in differences of 5.15%) and after 3 months of intervention (p-value = 0,000 and difference in difference of 10.07%) between the intervention group and the control group.

Table 3: Value of Average Social Relations and Elderly Social Activities Between Control and Intervention Groups Before, after 1 Month, and After 3 Months Awarded Elderly Caring Model Training (n = 100)

Time	Number	Group	n	Mean	Sd	Minimum Maximum	Δ (%)	*P t-test*	*P* Multivariate
Pre	1.	Intervention	50	23.00	1.245	21-25	1.11	0.147	
	2.	Control	50	23.52	1.111	21-25			
Post 1	1.	Intervention	50	28.00	2.279	24-33	5.15	0.000	0.000
Month	2.	Control	50	25.26	2.193	22-30			
Post 3	1.	Intervention	50	32.00	2.279	28-37	10.07	0.000	
Months	2.	Control	50	26.14	2.450	22-32			

The multivariate results based on GLM-RM

analysis found that there were differences in the increase in the mean value between the intervention group and the control group before intervention, 1 month and 3 months after the intervention.

From Table 4 it was found that there was no difference in the mean or average score of prevention behaviour for neglecting the elderly in the family between the intervention group and the control group at the time before the intervention, where the p-value was 0.465 with the difference between the two groups 0.68%. But there were differences in mean values after 1 month of intervention (p-value = 0.000 and difference in difference of 5.87%) and after 3 months of intervention (p-value = 0.000 and difference in difference of 13.79%) between the intervention group and the control group. The multivariate results based on GLM-RM analysis found that there were differences in the increase in the mean value between the intervention group and the control group before intervention, 1 month and 3 months after the intervention.

Table 4: Mean Prevention of Elderly Neglect between Intervention and Control Groups Before, after 1 Month, and after 3 Months Awarded Elderly Caring Model Training (n = 100)

Time	Number	Group	n	Mean	Sd	Minimum Maximum	Δ (%)	*P t-test*	*P* Multivariate
Pre	1.	Intervention	50	55.50	1.821	53-60	0.68	0.465	
	2.	Control	50	55.00	0.782	54-55			
Post 1	1.	Intervention	50	65.30	2.023	62-70	5.87	0.000	0.000
Month	2.	Control	50	58.06	2.683	53-65			
Post 3	1.	Intervention	50	78.28	2.382	74-83	13.79	0.000	
Months	2.	Control	50	59.30	3.321	55-78			

Discussion

Abandonment is a decrease in the quality of care provided by the family to the elderly in fulfilling physical, emotional, and economic needs. Alavi's [14] research on the relationship between adult children, parents, and grandparents is getting weaker because of the generation gap that causes conflict and tension in the family that can bring unhealthy consequences so that the elderly is no longer productive and neglected. Research conducted by Raphael et al., [15] on the impact of educational training on family caregivers of elderly living with dementia to assess the level of care in maintaining the well-being of recipients at home in getting results that educational interventions through elderly caregivers at home were obtained results were significant differences between the intervention groups (self-care assistance to trained caregivers) and untrained family caregivers after 1 month and 3 months of training. The results showed that nursing education interventions in the form of self-care assistance and training to families as caregivers could improve the process of caregiving in elderly care at home. The results of a similar study conducted by Miller et al., [9] was found that family training programs as carers were the right way for health practitioners and researchers who wanted to educate families who care for elderly where there was an increase in knowledge and changes in participants' skills before and 3 months after training. The family training program is the right way for researchers who want to educate families who care for the elderly.

The results of the model effectiveness test have shown that the application of an effective elderly caring model can change family behaviour in preventing neglect of the elderly in the family. This can be seen from the significant difference in the behavior of preventing the neglect of the elderly before and after being given training between the intervention group and the control group.

The elderly caring model effective in increasing family support for the elderly

Social support is a condition, the availability of care from reliable people who respect and love individuals. Social support can come from partners, family, and friends. The results of the Desiningrum study [16] showed that the most dominant social support affecting the psychology of parents was emotional support from the family then followed by award support from the family, support for information and finally instrumental support. The results of the study of Kaur & Venkateashan [17] showed parents who received support from family members had a better quality of life than those who received support from a partner or did not get any support

Family support is a process of relationship between a family and its social environment. Family support is also an attitude, action, and family acceptance towards its members. There are 3 dimensions of family support: reciprocity, advice or feedback, and emotional involvement in social relations [16]. Reciprocity is someone's response or action to us from what we have given. In this study, reciprocity is a response from the family to the elderly for what has been given, e.g. child reciprocity to his/her parents. In the past, parents took care of their children from childhood to adulthood; then, when parents step on the elderly, the children should have been able to treat them properly. Feedback is the effect of how parents have treated their children. Emotional involvement is the presence of harmonious learning and positive social relations between an individual and others. Family relations and social support are significantly related to the quality of life of the elderly, where problems of adjustment to health, economy, and social have a long-term impact on the quality of life of the elderly, meaning that older people who have no problems have better quality of life because healthy parents do daily activities independently. The ability of the elderly to implement ADL is influenced by the role of the family through the support provided parents are expected to remain

useful in their old age such as the ability to adapt, accept all changes and setbacks experienced, as well as appreciation and fair treatment from the environment and family so that the elderly are far from lonely [18].

The elderly caring model effective in increasing the task of family health in the elderly

Family health tasks are needed to improve the health status of the elderly by family functions like health care for the elderly. According to Friedman [13] families have 5 tasks in the health sector that need to be understood and carried out, namely: the ability to recognize problems, be able to make the right decisions, be able to carry out simple treatments when the elderly are sick, able to maintain a home environment that supports the health of the elderly, and can utilize health services in the neighbourhood. The family is the most important source of assistance for its members which can influence lifestyle or change the lifestyle of its health-oriented members. Families are groups that can cause, prevent, ignore or correct health problems in their groups.

Research conducted by Griffin [19] found that families as a care giver in providing health care interventions effectively improved the results of health status in the elderly who experienced memory and cancer disorders. Likewise, the results of Dobrzyn's [20] study that the health aspects of the elderly varied depending on the form of care provided where the lowest health status was found in the elderly with limitations for ADL who were treated at home related to loneliness due to being left alone by the family. Similarly, the results of Yulianti's [21] study were that there was an influence on family health duties before and after family nursing care was carried out on the health status of the elderly. In research conducted on the implementation of family health tasks to the elderly is a process that must be known and carried out by the family when the elderly experience changes in health conditions and a series of activities that must be carried out so that changes in the condition of the elderly are quickly handled. Family health assignments describe the ability of the family to recognize health problems that occur in the elderly and respond quickly to care when the elderly is sick [22].

The elderly caring model effectively impreves social relations and social activities of the elderly

Aging in the elderly can cause various problems both physical, mental, and changes in socio-economic conditions that can lead to a decrease in social roles. This has resulted in the elderly slowly withdrawing from relations with surrounding communities so that it can influence social interaction [24]. Research shows that social involvement and the maintenance of various social relationships have a positive effect on the emotional well-being and physical health of the elderly and are predicted to reduce the risk of death [25]. Research conducted by Glass [26] through a 13-year cohort study on 5573 elderly people aged 65 years to elderly social engagement activities, namely: 1) Social activities undertaken (religious attendance, visits to cinemas, restaurants, sports events, playing cards, participation in social groups); 2) Fitness (swimming, walking, physical exercise), and 3) Productivity (gardening, preparing food, work, community). The results obtained by social activities and productivity with little or no increase in fitness can reduce the risk of causing death, improve cardiopulmonary fitness and musculoskeletal strength and benefit in survival through psychosocial relationships. It can be understood that the elderly who carry out social activities and relationships will avoid feeling lonely so that they are more confident and independent because the memory is still honed and can exchange information and share experiences with the surrounding environment [24].

In conclusion, the effective elderly caring model can prevent the neglect of the elderly in the family, so that the caring model is suitable for the family to prevent neglected elderly people. The caring model that is applied is the provision of family support to the elderly, carrying out family health tasks, and the participation of the elderly to engage in social activities.

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Awareness of Breast and Cervical Cancer among Women in the Informal Sector in Nigeria

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Abstract

BACKGROUND: There are no general consensuses on why the lifetime risk of dying from breast and cervical cancer in African is several times higher than that of developed countries. However, a notable window of opportunity for prevention and treatment are often wasted where there are little or no awareness and low level of screening.

AIM: To specifically highlight the awareness, knowledge and determinants of participation in the screening exercise for possible prevention or control of the diseases. To also provide insights on the development of contextual and relevant timely detection for effective early interventions.

METHODS: A cross-sectional study was conducted in southern and northern geopolitical zones of Nigeria. The respondents, which consisted of 1,023 women aged 15-49 years, completed a 116-item questionnaire assessing the correlation between cancer awareness and participation in screening. Nineteen, In-depth interviews were also conducted for this study. The outcome measured included awareness of breast and cervical cancer, how it can be prevented or detected, and the availability and actual participation in screening exercises.

RESULTS: While women are familiar with breast cancer, little is known about cervical cancer, and the awareness of the former is not correlated with participation in screening. The lack of enthusiasm to seek screening exercise is rooted in three key factors: lack of specific information, belief, economic and inadequate screening facilities.

CONCLUSION: The study thus recommends that policies and programs aimed at the breast and cervical cancers awareness and early intervention should address the underlying problems.

Introduction

The most important cause of premature mortality among women in the world is breast and cervical cancer [1], [2], [3] making both cancers a major health problem for the women and the nations. The incidence, mortality, and survival rates for breast and cervical cancers vary across the world [4]. Globally, the incidence and mortality of breast cancer have increased over the past three decades, with an estimated annual rate of 3.1% and 1.8% respectively, and an estimated annual rate of 0.6% and 0.46% respectively over the same period, respectively [5].

In Nigeria, cervical and breast cancer are major public health challenges. Incidence of cancer in Nigeria from population-based cancer registries covering 2 years 2009-2010 reported a standardised incidence rate of breast cancer (SIR) in Ibadan, a population-based cancer registry (IBCR) of 52.0 per 100,000 and a population-based cancer registry (ABCR) of 64.6 per 100,000. The IBCR also reported a standardised incident rate of cervical cancer age (SIR) of 36.0 per 100,000 and 30.3 per 100,000 in the ABCR [3], [5].

Research has shown that the incidence of both breast and cervical cancer in developed countries with lower mortality rates is higher compared to lower death rates in developing countries [6], [7], [8]. This is due, as stated by [6], [9] to the availability of early cancer screening programs that detect early invasive cancer, some of which would have progressed to the late stage of the disease, reducing cancer mortality in those countries. This means women have poor overall outcomes in developing countries compared to their counterpart in

developed countries due to late detection and diagnosis [6], [7], [8]. This is one of the major reasons while out of 78,897 women estimated diagnoses of cervical cancer in Africa, the risk of death from cancer in African women is 2 times higher than in developed countries, and 61,671 dies from the disease annually [10].

One report suggested that the incidence of cervical cancer remained largely stable over time, possibly due to poor screening coverage in Nigeria [4]. The presentation of the advanced stage of the disease by most women when cure is unrealizable pose a significant management problem for the gynaecologists and may be responsible for higher incidence and lower survival rate in both cancers [3], [9], [11]. Low screening participation has been attributed to many factors, including lack of knowledge about the benefits of early detection, and the screening uptake will increase with improved knowledge. Other factors are; low socioeconomic status, the barrier to effective strategies for reaching informal sector women [7], [12]. All of these are partly attributable to the lack of established national breast and cervical screening programmers and the lack of culturally sensitive, customised health promotion campaigns, [13], [14].

For instance, cervical screening has been highly successful in the developed world. Since the introduction of organised cervical screening in the United States in the 1960s, in 2007, cervical cancer was ranked 12th in women's cancer deaths, which was the number one killer of women. While cervical cancer accounts for 7% of all malignancies in the U.S. and most developed countries, it accounts for 24% of all such cancers in developing countries, with 78% of all cases in resource-poor countries worldwide [11], [15]. This disparity was attributed primarily to the lack of screening and treatment of pre-cancer lesions [11], [15].

There is a risk that every woman will develop breast and cervical cancer. There are various risk factors that can affect the susceptibility of each woman. Early menarche, late menopause, late childbirth, oral contraceptives and hormonal therapy for menopause increase the risk of breast cancer, alcohol intake; also, research has indicated the impact of diet and environmental factors. Also, the main risk factors contributory to Human Papillomavirus (HPV) that causes cervical cancer in Nigeria are; being unmarried, illiterate, being positive for anti-Herpes Simplex Virus (HSV) antibodies, tobacco use, parity, multiple sex partners of women and their spouses' extramarital affairs [16]. Early participation in screening services is a major intervention for prompt and appropriate management of women with abnormalities [17].

Many factors have been identified from various studies as a barrier to screening in a variety of populations. Not much has been done to study awareness and practice of women in the informal sector, although the generalisation may be available. It would be assumed that women in the informal sector are less able to compete on the labour, capital and product markets because they have relatively low levels of education and skills, and socio-cultural, political and economic factors restrict women's time and mobility. Therefore, this study investigates Breast and cervical cancers awareness and practice in a sample of women in the informal sector in Nigeria. Women in the informal sector involve women operating a business without binding official regulations, as well as those operating under official regulations that do not compel official returns on their operations or production process. Activities are ranging from petty trading and personal service providers [8].

Material and Methods

Study Population

A qualitative cross-sectional study was conducted between January and February 2015 in southern and northern geopolitical zones of Nigeria to examine cancer awareness and practice among women in the informal sector in Nigeria to promote strategies to reduce the incidence of cancer. The study population included a total number of 1,023 women age 15-49 living in southern and northern geopolitical zones of Nigeria. The study targeted women in the informal sector in both rural and urban setting. The participants were examined based on prepared questions on demographics, cervical and breast cancer awareness, attitudes related to cancer's risk factors, awareness and actual practising of Breast examination and pap smear test access to the screening exercise. The research adopted both quantitative and qualitative approaches. The quantitative aspect employed a structured face-to-face interview. Regarding the sampling method, the six-geopolitical zones of Nigeria were divided into two (Southern and Northern) excluding the Federal Capital Territory. Two states from each region were randomly chosen, and in each state, two local government areas were selected.

The qualitative segment feature in-depth interview. These involved cancer survivors and medical practitioners. The assistance of medical and paramedical personnel was sought in locating and seeking of permission of some of their patients who survive cancer challenges. In the beginning, we recruited field assistants in each state to facilitate efficient and effectiveness of the fieldwork. Workshops were organised to inform and train the field assistance about the objectives, the content of the questionnaire and the procedure. Also, the respondents were informed about the purpose of the study, and their

consent was obtained before proceeding to interview them.

Measure

This study adopted the UK Cancer Awareness Measure (CAM) developed to reliably assess awareness of cancers [18]. The questionnaire was administered in the language understood by each respondent.

Information assessing socio-demographic characteristics of the respondents includes age, religious affiliation, formal education, their marital status, occupation, and whether they have children. Participants were also asked whether they have heard about cancer generally and specifically, awareness of breast and cervical cancer. Also, participants were asked how confident are they likely to identifying the breast and cervical cancer's symptom, whether they are aware that they are preventable and how they can be prevented. Information about practice included Self Breast examination, awareness of mammogram and breast screening using mammogram. Cervical cancer practice was measured by the vaccine for prevention Pap smear screening exercise and whether they have participated in PAP smear screening exercise.

Statistical analyses

Sample characterisation was based on frequencies and percentages. Descriptive analyses of demographic characteristics and awareness of cancer's separately for breast, and cervical cancers were conducted. Awareness of screening programmes for both types of cancer about actual participation in the screening exercise and whether the respondents will be able to notice cancer symptoms was also examined. Finally, the chi-square test was used to examine the possibility of awareness leading to screening exercise participation. Using the interview guide, a thorough interview was conducted. All interviews recorded and transcribed in English electronically. The aspect covered in the interview included the beliefs and perceptions of the respondents about cancer, as well as barriers to participation in screening exercise and actual cancer experience. The themes and categories emerging from the data were investigated. In some instances, responses to relevant issues and themes were cited verbatim to illustrate responses.

Results

Table 1 displays selected socio-demographic characteristics of the sample respondents. The computed mean age of the respondents is 33.6 years. The age distribution represents a normal curve distribution starting at 8.8% (age group < 20), reached the peak at age group 30-39 (33.7%) and finally declined at age group 40 and above (29.6%). Most participants (59.7%) reside in urban centres. The proportion ever is more than three-quarters of the population. This is distributed as married and living with a partner were (62.3%), separated/divorced (6.3%), the divorced (3.7%) while the singles were 27.8% (Table 1). The parity level is very high with only 2.5% at zero parity while the rest have had at least a child. The proportion of women who have had up to five children and above is 12.9% (Table 1).

Table 1: Background information about the Respondents

Locations	Frequency	Per cent	Children ever born (CEB)	Frequency	Per cent
Kwara State	259	25.3	Zero Parity	18	2.5
Ogun State	764	74.7	1-2 children	281	38.6
Total	1023	100.0	3-4 Children	335	46.0
Place of Residence			5 Children & above	94	12.9
Rural	412	40.3	Total	728	100.0
Urban	611	59.7	Educational Attainment		
Total	1023	100.0	No Schooling	80	7.8
Age Group			Primary Education	347	33.9
Less than 20 years	90	8.8	Secondary School	432	42.2
20-29 years	285	27.9	Tertiary institution	164	16.0
30-39 years	345	33.7	Total	1023	100.0
40 years & above	303	29.6			
Total	1023	100.0	Working Status		
Mean age = 33.6 years			Employees	204	19.9
Marital Status			Self-Employed	596	58.3
Single/Never Married	284	27.8	Unemployed	175	17.1
Married/LWP	637	62.3	Full-Time Housewife	48	4.7
Separated/Divorced	64	6.3	Total	1023	100.0
Widowed	38	3.7	Occupation		
Total	1023	100.0	Manufacturing	14	1.4
Religious Affiliation			Trading/Distribution	443	43.3
Christianity	634	62.0	Farming	271	26.5
Islam	356	34.8	Education	89	8.7
Others	33	3.2	Services	206	20.1
Total	1023	100.0	Total	1023	100.0

Source: Field Survey, 2015.

Breast and Cervical Cancer Awareness and Practices

The level of awareness of both breast and cervical cancers can be assumed to be generally high among the studied population. While 90.9% indicated awareness on breast cancer, relatively low value was obtained for cervical cancer (32.7%) as shown in Table 2. In the case of breast cancer, 55.4% of the respondents do not practice breast examination, and 79.6% were not aware of the mammogram test (Table 2). Those who have ever participated in mammogram were estimated to be 5.8%. Cervical cancer screening and vaccination were less popular among women. Awareness of Pap smear test was (11.7%), and vaccination against cervical cancer was (11.8%), participation in pap smear screening and ever vaccinated were just (7.8%) and (3.2%) respectively (Table 2).

The Chi-square analysis shows a positive association between awareness of breast cancer and participation in mammogram test (sig. = 0 .014). Notwithstanding that the level of awareness is very low (6.4%), the result indicated that every woman who had participated in mammogram had pre-knowledge

of the disease, as shown in Table 3. However, 93.6% of those who have heard about the disease have not participated in the test (Table 3). This could account for the weal level of contingency coefficient (0.078).

Table 2: Breast and Cervical Cancer Awareness and Practices

Breast Cancer Awareness			Cervical Cancer Awareness		
Ever Heard of Cancer	Frequency	Per cent	Ever Heard about Cervical Cancer	Frequency	Per cent
Yes	942	92.1	Yes	334	32.7
No	81	7.9	No	686	67.3
Total	1023	100.0	Total	1020	100.0
Ever Heard about Breast Cancer			Heard about Pap Smear Screening		
Yes	930	90.9	Yes	114	11.7
No	93	9.1	No	861	88.3
Total	1023	100.0	Total	975	100.0
Ever Done Self Breast Examination			Ever Participated in Pap Smear Screening		
Yes	452	44.6	Yes	72	7.8
No	561	55.4	No	857	92.2
Total	1013	100.0	Total	929	100.0
Know Mammogram			Aware of Vaccination against Cervical Cancer		
Yes	206	20.4	Yes	112	11.8
No	802	79.6	No	841	88.2
Total	1008	100.0	Total	953	100.0
Ever Done Mammogram			Ever Vaccinated		
Yes	57	5.8	Yes	30	3.2
No	930	94.2	No	902	96.8
Total	987	100.0	Total	932	100.0
Confident against Cervical Cancer Infection	Frequency	Percent	Confident against Breast Cancer Infection	Frequency	Percent
Very Confident	90	8.8	Very Confident	122	11.9
Fairly Confident	131	12.8			
			Fairly Confident	146	14.3
No Confidence	802	78.4	No Confidence	802	78.4
Total	1023	100.0	Total	1023	100.0

Source: 2015 National Breast and Cervical Cancer Survey.

The correlations though weak, but with more awareness and information, the association can be improved. This is significant in the sense that study [9] argues that knowledge and certain demographic variables can serve as important modifying factors that would awaken awareness of the life threat posed by cancer and facilitate the role that perception can play in influencing the likelihood of screening.

Table 3: Relationship between awareness of Breast Cancer and participation in Mammogram Test

	Ever Done Mammogram		
Ever Heard about Breast Cancer	Yes	No	Total
Yes	57 (6.4%)	840 (93.6%)	897 (100.0%)
No	-	90 (94.2%)	90 (1000.0%)
Total	57 (5.8%)	930 (94.2%)	987 (100.0%)
Pearson Chi-Square = 6.070			
Contingency Coefficient = 0.078		Approx. Sig =0 .014	
Pearson's R = 0.078		Approx. Sig= 0.014	

Source: 2015 National Breast and Cervical Cancer Survey.

A similar analysis was conducted to confirm the relationship that exists between knowledge about cervical cancer and participation in Pap smear screening. The result obtained from the Chi-square analysis shows a positive association between awareness of cervical cancer and participation in the screening with both correlations statistics showing a similar result (0.233). Though the level of significance is high (Approx. Sig = 0 .000), the contingency coefficient is only 22.7%, indicating a weaker relationship (Table 4).

Table 4: Relationship between knowledge about Cervical Cancer and participation in Pap Smear Screening

	Ever Participated in Pap Smear Screening		
Ever Heard about Cervical Cancer	Yes	No	Total
Yes	52 (16.5%)	264 (83.5%)	316 (100.0%)
No	20 (3.3%)	591 (96.7%)	611 (100.0%)
Total	72 (7.8)	855 (92.2)	927 (100.0)
Pearson Chi-Square = 50.524		Approx. Sig = 0 .000	
Contingency Coefficient = 0.227		Approx. Sig = 0 .000	
Pearson's R = 0.233		Approx. Sig = 0 .000	
Spearman Correlation = 0.233		Approx. Sig = 0 .000	

Source: 2015 National Breast and Cervical Cancer Survey.

Discussion

Breast and cervical cancer awareness among women in informal sectors were investigated. This section highlights the findings from this study. Variables associated with awareness and practice in breast and cervical cancers were considered: awareness of breast and cervical cancer, breast self-examination, mammogram screening, awareness of Pap smear screening and vaccine against cervical cancer.

This study found that, while women are familiar with breast cancer, little is known about cervical cancer. However, awareness with breast cancer does not lead to being mindful of it as to know what to do or how to go about the screening exercise. As shown by the excerpt from the in-depth interview with the breast cancer survivor, below, some that have participated in the screening exercise for breast or cervical cancer, did that by chance, not as planned exercise. A 43 older woman from Abeokuta in Ogun State Nigeria narrated her experiences with breast cancer as thus:

"I had a quarrel with my husband because of his infidelity, and he stopped providing for the home keep neither for the children school fees. This is what led me to want to do family planning. It was during the necessary routine checkup that the nurse discovered an abnormal lump in my breast, she then recommended me for further examination, and that was how I started battling for survival".

The findings of this present study agree with earlier findings [9] also talk about the low level of awareness and screening among women in pokie Ogun state. Authors found that women in Egypt would not god to doctor unless they were ill as a barrier to cancer screening [19]. The lack of enthusiasm to seek screening exercise is rooted in three key factors: lack of specific information, belief, economic and inadequate screening facilities.

Women lack specific information on breast and cervical cancer. Seventy-eight per cent (78%) of

the respondent cannot say confidently the likely causes and symptoms of both breast and cervical cancer. In their study of Arab women, [12] reported that only 5% of their respondent had a good general knowledge of breast cancer. As stated by [9], either the respondents are unaware of the symptoms because they do not have the condition or because they do, but are unable to link the symptoms with the condition. Achieving a decision to seek medical care or prevention starts with the ability to recognise the symptoms and signs correctly. Failure to recognise the symptoms correctly and promptly could act as a barrier to screening exercise or as a source of delay [1]. Some of the participants were asking: "what do I do, how can I be screened? Where ….?

Some authors believe that socio-cultural elements shape the behavioural characteristics of individuals within their environment [12]. And as the Health belief model suggested, variations in utilisation behaviour can be accounted for by beliefs about the individual's view of their vulnerability to disease. Religion and traditional beliefs can be a challenge to the screening exercise. For instance, participant see cancer as a death sentence, and when the investigator asked if they are aware of the screening exercises and whether they have participated in one, immediately, they screamed 'God forbid, it can never happen to me'. Some beliefs that it is not normal and not good for one to go for screening, as described by A 35-year-old mother of 2 in Kwara State:

"it is not good for one to be looking for what is not lost. By the time one begins to subject oneself to screening, that is when the thing will happen to the person. Is better to the belief that it cannot happen and not do what can make it happen".

Economic reality influences women's attitude, value and behaviour disposition to diseases preventive measures. Given the limitations on women's income in informal employment and their complete exclusion from the cash economy in some cases, the extent to which poor women, especially those in charge of households, can afford expenditure (related to health care) such as taking preventive measures such as cancer screening. Women's health decisions are increasingly influenced by the unprecedented harsh economic climate. This is a major risk factor observed during this study for the trend of responses to screening questions. Cancer does not present symptoms until a later stage, especially cervical cancer, yet women in the informal sector do not see a reason to go and waste little resources they have in the hospital because of what that cannot feel. From the in-depth interview, one of the breast cancer survivors has this to say:

"S*ometimes when we hear about cancer on the radio, we talk about it in the market, and we say a prayer that God will not allow us to encounter sickliness that will be more than what we can handle.*

Going for screening is out of it, because it will involve money, and most of us don't have no budget for screening when one is not sick".

Women (age 21-35 years)

Inadequate screening facilities are another challenge to the uptake of screening. Respondents' belief that the availability of the facility will encourage their participation in screening exercises. As a respondent explained:

" as a woman, one needs to protect oneself, but most of the clinic around don't conduct the screening, they normally direct people to the general hospital where there are many people and also too far". Several studies, such as the study of factors associated with women taking cervical cancer screening in Portland, Jamaica [20], identify a negative association between not knowing where to go for cervical screening and having Pap smear. The considerable literature on the association of breast and cervical cancer outcomes with early screening exercise has necessitated awareness and practices are very important factors in prevention and reduction of mortality. Cancer can easily be halt with the timely detection and early intervention. Consequently, women sensitivity and participation in screening exercises are non-negotiable forces in reducing the rate of breast and cervical cancer in Nigeria.

The hindrance to participation in screening breast and cervical screening exercises among women in the informal sector has been highlighted. Borrowing from the studies by [6], [19], [21], this study, therefore, recommends ascertaining individual, social and structural predictors of breast and cervical among women in the informal sector. These factors are especially necessary for identifying population-specific barriers and to design, evaluate and present a targeted population-based breast and cervical cancer control interventions and programs for different category of women.

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Exploring the Experiences of Iranian Women Regarding Obesity Self-Management: A Qualitative Study

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Abstract

BACKGROUND: Despite the high and growing prevalence of obesity in Iran and a variety of interventions by the healthcare providers control the problem, and it is still a prevalent health problem among Iranian women.

AIM: The aim of this study is to explore the perspective of obese Iranian women in the process of self-management regarding the facilitating factor in self-management of obesity.

METHODS: In a qualitative study, the participants were selected through purposeful sampling, and the data were collected using semi-structured interviews and focus group (n = 25) between July 2017 and September 2018. All the interviews were transcribed verbatim and the data were analysed using constant comparative method.

RESULTS: Supporting the umbrella was the main category found in the present study. The participating obese individuals found "support" as the main factor with an outstanding effect on motivating, incentivising and keeping diet in long-term. This category contains subcategories: self-help; family, friends, and peers' support; and medical team's support.

CONCLUSION: The findings suggested the critical role of support in obesity self-management process. This critical factor improves our perception of the multi-aspect and complicated nature of obesity self-management. Moreover, policymakers and providers of health services can utilise this finding in the design of care plans with higher chance of success.

Introduction

According to the World Health Organization (WHO), chronic and non-communicable diseases will be the cause of 75% of mortalities in the world by 2020 [1], [2]. Obesity is a chronic disease and a major health concern in developing and even developed countries [3], [4], [5]. There is a strong relationship between obesity and type 2 diabetes, cardiovascular diseases, hypertension, liver diseases, gallbladder disease, sleep apneas, higher risk of cancer, and joints disorders. Every year, around 147 billion dollars is spent on health and therapeutic services for obesity [6], [7], [8], [9]. The problems caused by obesity are highly complicated and not limited to physical ones and may affect one's chance to have a dynamic life. The problems also degrade the chance of having an active and fruitful live [10]. According to the WHO, global prevalence of overweight and obesity in adult women in 2012 were 65-80% and 35-50% respectively; these figures in Iran were 60% and 30% respectively. Moreover, obesity in women is more prevalent than men [11].

Although the aetiology of obesity is not fully determined yet, the interactive effect of genetic, metabolic, psychological, and environmental (social, economic, cultural, physical) factors plays a determinant role [12], [13]. While the majority of obese individuals are aware of the importance of lifestyle modification for the management of their condition, they fail to continue the interventions for different reasons. Also, they might take measures to achieve the ideal weight; however, the therapeutic program they adopt is not effective in long-term, so that the disappointments convince them to stop the program. Studies have shown that only about 20% of overweight and obese individuals manage to lose

weight in long-term [14].

Most of the successful approaches to chronic diseases (including obesity) treatment are patient-centred management programs, which are efficient to achieve weight control and create behavioural changes. Self-management in chronic diseases contains three fields of medical management (adherence to special nutrition and treatment regimen), role management (following and keeping proper behaviors, altering unhealthy behaviors, and altering roles in life), and emotional management as a tool to change attitudes towards future (managing emotions like fear, hopelessness, depression, weight stigma, and shame). These fields were introduced by Corbin and Straus. Nurses play a key role in active engagement of individuals in managing their condition and avoiding progress, recurrence, or side-effects of their medical condition [15], [16], [17], [18].

Since efficient obesity management is a top priority of public health, a deeper insight into the experience and perception of obese individuals of self-management facilitators may lead to a higher quality of life in this group of care seekers. Several quantitative studies in Iran and other countries have surveyed the effects of specific interventions on obesity. Despite the importance of managing obesity and the side-effects (e.g. physical, mental, economic, social and family) [19], [20], [21], the problem has not received enough attention it merits in Iran. The multi-aspect nature of the problem and the side-effects, the fact that it is more prevalent in women than men and the gap is growing, and the high prevalence in young women infertility age and the infertility problems caused by the disorder all highlight the need for studying and surveying the problem. The aim of this study was to explore the experiences of Iranian women regarding their obesity self-management.

Methods

The study was carried out as a qualitative study based on a grounded theory approach using constant comparative analysis method. The participants were obese individuals who were selected through purposeful sampling. Inclusion criteria were Iranian nationality, being from different cultural and ethnic groups of Iran, Farsi speaker, desire to share information, ability to share an obese life experience, a history of obesity self-management, having enough time to attend interview sessions, and not having specific disease (self-statement). Participants' selection ensured the highest diversity in terms of self-management, social, demographics, economic, ethnic, and education levels. The interviews were continued until data saturation and were completed by focus group. The study was carried out from July 2017 to September 2018 in public spaces like body fitness clubs, parks, and obesity clinics.

Semi-structured interviews were conducted by a doctoral student in nursing trained in interviewing techniques, including qualitative interviews. The interviews were voice recorded and took between 27 and 56 min (37.93 min on average).

The interviews were handled using open-ended questions like the below one:

- How do you manage your obesity?

- Can you explain about your obesity management?

- What helps you for better obesity control?

Also, the participants were asked to share their experience with the facilitators of obesity self-management. Using probing questions, the interviewer tried to explore deeper into the subject and find more details. Moreover, a focus group was conducted with seven obese individuals visited an obesity clinic.

All interviews were transcribed verbatim immediately, and the transcriptions were read by the authors several times to achieve a common understanding of the participants' experiences. Analyses were conducted using constant comparative analysis to extract the codes and categorise them into specific categories. Data management, encoding and organising, were done in MAXQDA (v.10). Lincoln and Guba's criteria were used to ensure trustworthiness, credibility, transferability, dependability, and confirmability. The criteria were followed and implemented throughout a 15-month interaction period with the participants and attending the research setting, giving thick description of the participants' life experiences, peer debriefing, member checking, auditing data analysis, interview recording, and immediate transcription.

Ethical Considerations

The study was approved by the Ethics Committee, Iran University of Medical Sciences (IR.IUMS.REC.1395.9223493201). Participation was completely voluntary, and all the participants signed a written letter of consent for interviewing and audio recording.

Results

In total, 18 interviews and a focus group of seven obese persons visiting obesity clinic (28-50 years old with a mean age of 38.56 years) were performed to achieve data saturation (n = 25) (Table 1 and Table 2).

Table 1: Demographic characteristics of participants in the interview

No.	Age	Marital status	Ethnicity (Province)	Previous weight	Current weight	Previous BMI	Current BMI
1	39	Married	Mazandaran	127	112	48	43
2	50	Married	Mazandaran	86	69	34	27
3	40	Married	Mazandaran	90	70	37	28
4	50	Married	Azerbaijan	75	50	30	20
5	35	Unmarried	Azerbaijan	75	59	30	22
6	47	Married	Azerbaijan	75	64	30	24
7	37	Married	Tehran	85	65	32	24
8	35	Unmarried	Tehran	92	70	33	25
9	28	married	Hormozgan	105	80	40	30
10	33	married	Azerbaijan	85	70	33	25
11	40	married	Golestan	93	70	34	26
12	31	married	Mazandaran	97	74	30	26
13	49	married	Tehran	90	74	39	32
14	42	Unmarried	Kurdistan	-	-	-	-.
15	30	married	Mazandaran	-	-	-	-
16	31	Unmarried	Mazandaran	-	-	-	-
17	27	married	Mazandaran	-	-	-	-
18	50	married	Tehran	-	-	-	At overweight range

Supporting umbrella (as the main category) was the main facilitator for obesity self-management. The subcategories included self-help, family and significant ones' support, and medical team's support (Table 3).

Table 2: Demographic characteristics of the participants in the focus group

No.	Age	Marital status	Ethnicity (Province)	Previous weight	Current weight	Previous BMI	Current BMI
19	32	Married	Gilan	168	134	59	47
20	44	Married	Azerbaijan	135	112	49.7	45.7
21	51	Married	Azerbaijan	102	98	42.3	42.1
22	27	Unmarried	Kurdistan	132	133	49	49.1
23	47	Married	Tehran	93	79	40	37.2
24	31	Married	Azerbaijan	83	85	33	33.2
25	50	Married	Tehran	94	81	38	34

Support was one of the main issues that were frequently mentioned and emphasised by the participants. Obese individuals found it a major factor with extraordinary effects on motivating and incentivising individuals to start or keep a regimen.

Table 3: Obesity self-management facilitators

Category	Subcategories
Supporting umbrella	Self-help
	Family, friends, and peers' support
	Medical team's support

Comprehensive and continuous supports make people keep their regimen with more energy and motivation so that the chance of keeping the regimen in these individuals is higher. A participant noted: "*I think this is a brilliant teamwork that brings people together (nutritionist, sports medicine specialist, psychologist). All of these experts work to help us lose weight without any side effect*" (focus group). Subcategories of supporting umbrella, the following will be explained in detail:

Self-help

One of the participants commented on the necessity of self-help by saying that:

"*If I see an obese person, I will tell him or her to start right now because tomorrow is too late. No one can help you like yourself*" (participant No.11).

A nutritionist said: "*My first question of my clients is that 'why they came to me?' and 'what made them to decide to lose weight?' You know, these are important. This is even more important for younger people. The fact that one decides to lose weight based on one's own will and desire or by others' pressure plays a key role in the outcome. Others' pressure works for a while but not too long. However, things are much easier with an internal desire in the patient who has come to realise that losing weight is good. Desire and will are very important*" (participant N. 14).

Achieving a level of self-awareness to make the right decision and start a therapeutic regimen was another instance of self-help revealed in the interviews. A participant noted: "*an individual starts a diet with a reasonable level of persistence, when they achieve that level of awareness and insight to realise that losing weight is good for them*" (participant N.4). It is notable that by looking for help, obese individuals try to help themselves to solve their obesity and the negative consequence.

"*Frustration" was the word used by the participants so that they look for help everywhere. A participant said: "I came to the conclusion that I need to help myself… so I asked myself, "what are you doing with yourself?*'" (Participant N.4).

Having great goals was another point that was highlighted by the participants. "*When you set a goal for yourself and fight for, you need to remember that all the hardships that you experience will lead to great success. When you accept this, no temptation can misguide you. When frustrated, you should concentrate on your goal and ideal. This surely gives you strength and energy*" (Participant N. 8).

Also, the fact that weight management takes too long to achieve the desired result brings in several notable problems, and people may look for help everywhere. The participants noted that they help themselves to keep their regimen by adopting different solutions like making family along with yourself, taking measures to attenuate others' sensitivity, keeping oneself busy by spending time outside the home, rewarding oneself for losing weight, exercising in open space, joining obesity clinics, sharing experience with peers, and compensation in the case of failure to stick to the regimen and punishing oneself for failure.

One of the participants said: "*I try not to stay at home and spend more time at work because I tend to eat less when I am outside…*" (Participant N.6).

Of other instances of self-help was to observe the positive physical and mental outcomes of losing weight, which was a motivator for continuing obesity management. A participant stated: "*When I saw the positive physical and mental outcomes of losing weight, I felt a strong motivation to keep my regimen*" (participant N.7).

A huge majority of the participants emphasised the mental outcomes of losing weight and noted that those outcomes were great motivation to keep the therapeutic regimen. A participant mentioned: "*The first thing after starting the regimen was the recovery of the lost self-confidence and I felt being younger. I also felt being a more useful person who can help herself, which was a very valuable achievement for me…*" (Participant N.7).

Achieving a high level of ability and independence was another instance of self-help, which is achieved gradually and through hard work. Gaining the power to control the situation is a critical factor in continuing the regimen. A participant said: "*Even if I am tempted to eat in a party and do so, I will compensate the next day by doing more physical activity…also, keeping the regimen had a negative effect on my mental and spiritual situation in early days…now I know that it was because of the wrong implementation of the regimen; now I am cautious about my blood sugar level, and this is a sort of self-management for me*" (participant No 5).

Family, friends and peers' support

Family, friends and peers' support was another subcategory of supporting umbrella. This factor is a two-edged blade that may lead the individual towards gaining or losing control. A participant commented in this regard: "*Family's support is very important or it would be great if you could convince them to do group exercises. Some insist on going to gym clubs, but I think you can bring the gym to your house and do the exercising with the family*" (participant N.5).

Helping was another instance of support by others. Using others' experience was one of the items mentioned by participants. One noted: "*Group exercising was very helpful; it was good for motivation knowing that you were doing group work. For one thing, the fee that you have to pay convinces you to continue and for another seeing how others work hard keeps you motivated. Also, watching others' good job and concern about a healthy life makes you think more about your way of life. All these lead you towards your goal*" (participant N.10).

On the other hand, a participant highlighted the undeniable role of using obesity clinics services and the desire to visit such places: "*I like it here as I can see here how others lose weight and that newcomers arrive and ask for comment on how to lose weight and what to eat. Sharing information and knowledge is very good*" (focus group). Indeed, using the experience of successful peers in losing weight through sharing experience is a great help to boost one's motivation to continue their path.

Medical team's support

Since obesity is a complicated and multifactor phenomenon, its management and treatment need multidisciplinary cooperation. The active support for obesity management by a diverse medical team, including nutritionist, physician, psychologist, psychiatrist, sports specialist, and nurse who work in good harmony guarantees successful management for the patients. A participant commented on the undeniable supportive role of medical team members and nutritionist in particular: "*There are many challenges and most thoughts and worries that keep your mind occupied and distract you from the diet. You cannot deny them; however, and despite all these, an expert's support and motivation can be very effective on outcome*" (Participant N.13).

An active group of experts in the field of obesity management are nutrition and diet therapy experts who are in charge of designing food plans and make obesity management possible through working with other groups of experts as a medical team. Having a reasonable and well-thought plan and persistence are of the strong facilitators of therapeutic regimens so that without them, failed experiences become the main factor in leaving the regimen and losing trust in the nutritionist in next attempts. A participant said: "*Keeping the diet was tough only for the early days because it was not a normal thing for me. However, since the regimen was based on an expert's knowledge, it was easy to adapt to…*" (Participant N.8).

Motivating supports and feedback were of other instances of medical team's support. Supports and empathy of an expert send the signal to the care seeker that they are not alone, and it functions as a notable facilitator in the weight control process. A participant noted: "*my nutritionist was like a consultant and gave me hope and motivation to a great extent…*" (Participant N.10). Positive feedbacks by the gym coach was also highlighted as a motivating factor as one of the participants noted: "*My experienced coach would give me the right instruction and positive feedbacks, which were very helpful and kept me motivated to continue exercising with more energy and vitality*" (Participant N.2).

Financial load of participation in the therapeutic programs was another factor to be taken into account. Many issues that keep the mind busy on one hand and social and economic problems that people are faced with, on the other hand, put more emphasis on the role of support. A participant commented: "*things that keep your mind occupied, stressors, and worries make you distracted from focusing on the diet… however, having someone to keep you company in this path is very helpful. Having someone to motivate and support you makes it easier to overcome challenges in the way*" (Participant N.13).

Discussion

According to the results of this study, support was the main category with three subcategories, including self-help, family/significant ones/peers' support, and medical team's support. In a study on Swedish women participating in a regimen intervention, two categories of facilitators including attempts to achieve self-decision making (having clear goals with motivation and avoiding food) and receiving support (from friends, family, and the inspiring project) were emerged [22], which is consistent with the results of our study.

Our participants noted that when the supports are continuous, they can overcome the barriers with more energy and motivation. It has been shown that facilitators of obesity self-management were high social status, social support, high self-efficiency, and good mental state [23]. Another study concluded that using the environmental-ecological-social model to induce behavioural changes in individuals and motivate jogging was a facilitator for losing weight. This facilitator consisted of elements including intra-personal (knowledge, skill, attitude, and behaviour), inter-personal (family and friends' effect), and public and organisational policies (national and local plans) [24].

Achieving self-awareness and a sense of responsiveness to oneself and others were instances of self-help; which is an effective facilitator that prepares individuals to start the treatment and keep following it. Shay et al. showed that the lack of awareness about a healthy lifestyle and the social/cultural elements affecting the lifestyle was an undeniable effective factor. People who achieve self-awareness tend to be more persistent in keeping their regimen as keeping it is their own decision as a way to help themselves. According to self-decision-making theory, the choice must fit with the decision maker's interests and purposeful at the same time to make a choice improves motivation, performance, persistence, and productivity. Therefore, to improve weight-loss performance, choosing by the individual seems essential [25].

Health concerns and problems and receiving advice from health professions are among the factors that create motivation [26], make individuals help themselves, and motivate to make every attempt they can to achieve the goal. This was the point mentioned by the participants in this study. Positive outcomes such as successful weight loss was another facilitator for obesity self-management. The participants referred to this concept in different ways and argued that experiencing positive outcomes was a strong facilitator of motivation for self-help. Many studies reported that individuals describe losing weight with terms like being reborn, a chance to start a new life, finding a meaning for life, happiness, lightness, and emotional sooth [27]; transferring from bad habits to an intentional change [28]; and awareness and clear mentality about choosing food [29]. Thomason maintained that self-management approaches to lose weight rely heavily on a combination of behavioural, mental, anthropometric, and metabolic outcomes [30]. Carrard showed that self-weighing was one of the approaches of controlling weight that led to positive mental outcomes including remaining calm and returning to normal way of life. The behavioural outcomes in return were a life with awareness and compensation in the case of failure to keep the diet [31]. Grave et al. showed that losing weight was related to a decrease in mental stresses and desire to eat. The psychological outcomes of losing weight were better mood, self-acceptance, and self-satisfaction gave that obese individual usually experience a sort of discrimination and less-attractiveness. They tend to blame their behavioural problems like laziness and lust for eating for their overweight and stigmatise themselves [32].

Exercising was another facilitator that was mentioned by the participants and other studies as well [29]. It has been shown that if the college environment supports and promotes physical activity, students will be motivated to do physical activity [33]. Self-motivation is one of the motivating factors highlighted in another study. People tend to use different approaches to motivate and help themselves and keep themselves incentivised [29].

Over the years, people achieve a level of capability that leads to control over one's behaviour in terms of weight-management, role management, and emotional management, which constitute obesity self-management. At this level, people can control their weight without others' help, and it is achievable only when the treatment process is implemented in a rational and reasonable manner. Sand et al. mentioned the facilitating role of prior successful attempts and feeling the ability to manage one's weight [34]. Family support and motivation and continuous supports of this kind are highly important and adopting a new behavioural habit need family support and positive feedback by the therapist, which lead to self-monitoring in turn [35], [36]. These are consistent with the findings of the present study about the necessity of comprehensive support.

Another finding was the peers' experience as a motivation to keep the diet, exercise, and do physical activity by using their valuable experiences. This same idea has been mentioned by different studies that have emphasised on involvement and participation in weight-loss behaviours [34], [37], [38]. Having a supportive environment and social support and implementation of public policies to keep people motivated have been mentioned by several studies as an external facilitator [37], [39], [40], [41]. Being responsiveness towards others and oneself, having a clear plan, and nutritional education based on facts were other facilitators mentioned in other studies consistent with present study [25], [29]. Chugh and

Diaz noted in their study that adopting an empathic and friendly approach to weight loss by the medical team without prejudgment and disrespect and promoting self-motivation to lose weight were among the essential factors. The patients emphasised the expected consultation and examination from health care personnel [38], [42]. Healthcare providers play a key role in obesity management by screening, consulting, and supporting individuals to lose weight. Among the key services provided by nurses are motivating interviews, providing information sources, distributing informative brochures, giving recommendations about weight-loss diets with details, measuring waist circumference, implementing continuous supportive programs, and behavioural therapy to create long-lasting changes, all of which are done taking special condition of each patient into account [43]. These findings are consistent with the present study. According to the participants, the mere act of losing weight is not hard, but the hardest part is to remain in shape afterwards. This needs continuous follow-up and motivation.

The present study was not free of limitation; for instance, the responses might be deviated by social acceptance concerns; although, the authors made their best to minimise such concerns throughout the interviews.

The findings suggested the critical importance of support in the process of obesity self-management. This critical factor improves our perception of the multi-aspect and complicated nature of obesity self-management. Moreover, policymakers and providers of health services can utilise this finding in the design of care plans with higher chance of success.

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Academic Staff Satisfaction with their Work: A Cross-Sectional Study in a Medical University

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Abstract

BACKGROUND: Employees' work satisfaction, combined with democratic management, are important predictors of future productivity in any organisation.

AIM: The aim of this study is to investigate job satisfaction in academic staff as well as the associated working environment factors, using an original self-administered questionnaire.

METHODS: A cross-sectional survey was conducted using an original standardised questionnaire. It involved 370 academic staff members at one of the five medical universities in Bulgaria. The questionnaire consists of 17 items (including occupational hazards, management style, conflict solving and demographic characteristics) rated on a 5-point Likert scale.

RESULTS: The results revealed that the majority of academic staff (71.7%) works in a risky environment. Employees indicate that “mental strain”, “work with chemical agents and dust” and “work with biological hazards” are the most common risk factors. Democratic leadership and cooperation are most commonly applied management styles.

CONCLUSION: The instrument for the measurement of job satisfaction revealed high values of psychometric characteristics for reliability and validity. The study found a high level of satisfaction of academics with their working conditions. It is necessary to conduct similar studies periodically to detect more precisely the decrease in academic staff work satisfaction and take timely and adequate measures to improve it.

Introduction

The work environment consisted of multiple factors, including a company's workplace culture, management styles, hierarchies and motivation for participation in the decision-making process of the organisation, good relations with co-workers, job security, autonomy given to employees and wages [1]. These factors influence job satisfaction and are the key to developing a high-performance workforce [2].

In the literature on organisational behaviour and organisational psychology, job satisfaction is considered the most extensively researched area [3], [4]. Most investigations have been performed among university and hospital employees [5], [6], [7]. Few types of research have been conducted among medical academic staff members as an occupational group characterised by overcrowded classrooms, the presence of unhealthy factors, time pressures and increased workload [8], [9], [10]. Job satisfaction is regarded as an essential component of employee’s motivation among academic staff and is believed to be their basic inner feeling regarding their job as it reflects the degree to which employees feel personally fulfilled and content in their job roles [10].

Some researchers have found out that internal motivators, support from supervisors and

authority play a greater role in academic staff job satisfaction than wages and working conditions [11], [12]. Similarly, other study documents that personal relationships play a more dominant role in the overall job satisfaction compared to payment [1]. Further, a Polish study revealed that income did not influence the professional satisfaction of the dentists [9]. Therefore, novel management skills, time and energy are necessary to improve the overall work performance. Abugre J. indicates that academics were most satisfied with work nature, supervision and communication, and job security [11]. Recent researches reveal that academics find the nature of work, supervision, communication, and job security more satisfying [13], [14].

No single conceptual model can completely and accurately portray the construct between working environment and job satisfaction. Establishing accurate tools to monitor and improve job satisfaction should be adopted by the university as its main organisational policy [15]. For Bulgaria, the problem is comparatively new as all research in this area was carried out following the country's economic transition. Therefore, there is a need for such studies to provide more public knowledge of this issue, train both employees' and employers in this area, aid and encourage both parts to increase work satisfaction. Similar studies in Bulgaria are few, even fewer have been conducted among academic staff. The continuous efforts of the Medical University, Plovdiv to improve the working environment and the employee's satisfaction along with the management system standards of ISO 9001:2015 provided the grounds for conducting the present research.

The aim of this study is to investigate academics' job satisfaction and working environment factors associated with it using an original self-administered questionnaire.

Material and Methods

Design

A cross-sectional survey was conducted among the academic staff (full professor, associate and assistant professor) at the Medical University in Plovdiv (one of the five Medical Universities in Bulgaria). The sample is representative with regards to the Medical University, Plovdiv. Our standardised questionnaire was specially prepared to achieve our goal in studying different aspects of satisfaction with the working environment. This study was carried out with the co-operation of the Committee on Working Conditions and an Occupational Health and Safety expert. It was approved by the Vice-Rector for Quality and Accreditation of the University

Participants and procedures in the pilot study

A pilot survey was conducted among 20 academic employees to assess the reliability and validity of the prepared tools before the main study. A convenience type sampling was used with equal gender representation. Following instruction briefing, the participants in the pilot survey filled out the questionnaire twice over two weeks. In the process of repeated filling, participants had no access to the original completed forms.

Participants and procedures in the main study

The questionnaires were distributed among 370 academic staff members out of a total of 738 colleagues from six departments at the Medical University in Plovdiv. The Committee on Working Conditions and an expert in Occupational Health and Safety (OHS) also co-operated in our research. The questionnaire consisted of 22 specific questions. Seventeen of the items evaluated the academic staff satisfaction with the working conditions in four aspects including: management and ensuring health and safety working conditions (job safety, presence of System for Quality Control and continuous monitoring of work environment conditions) superior-subordinate communication (free horizontal and vertical communication, receiving feedback for the introduced changes), teamwork (support and respect for each other) and work organization (working hours and rest balance, intensity of the daily work, interchangeability of the staff and daily workload). Additionally, several occupational hazards (physical, chemical, biological, ergonomic and psychological) were investigated as well as the management style, the manner of conflict solving and demographics.

The independent variable in this research is the working environment in which the employees work within an organisation. The dependent variable is the employee job satisfaction with the working environment.

A 5-point Likert scale was used ranging from "complete disagreement" to "complete agreement". A scale of 1 to 5 was used to evaluate different aspects of job satisfaction. The value of index 1 or 2 corresponds to dissatisfaction; the value of index 3 shows neutral value of satisfaction and the value of index 4 or 5 indicates satisfaction of respondents.

The questionnaire included questions on workplace, presence and type of risk factors, management styles and how conflicts are handled in the working place. Information on the sex and age of respondents is also present. To determine the impact of the above factors on the overall respondent satisfaction, the items related to the satisfaction of the received remuneration were excluded.

The study was conducted from December

2015 to March 2016 at the Medical University in Plovdiv.

Reliability and construct validity of the questionnaire

Internal consistency of the questionnaire was evaluated through Cronbach's Alpha (α). To evaluate reliability, we used the split-half-reliability model and calculated the Spearman-Brown coefficient (r_{sb}) for each item. The Wilcoxon Signed Ranks Test was applied to compare the two related samples (in this study – to compare the results between the two moments of evaluation).

Exploratory factor analysis with principal axis factoring extraction was used to assess the underlying structure of the items as well as orthogonal rotation, using the Varimax method. Initially, sampling adequacy was assessed by the Keiser-Meyer Olkin test (KMO) and Bartlett's test of sphericity. Severely violated the assumption of multivariate normality distribution of the data excludes the application of confirmatory factor analysis.

Assessment of academic staff's job satisfaction

Job satisfaction among staff was assessed by descriptive analysis, analysis of variance and analysis for hypothesis testing and dependencies. Criterion χ^2 was used for the comparison of the results in two-dimensional distributions and Spearmen rank correlation to measure the degree of association between two variables. The level of significance for the null hypothesis was $P < 0.05$. Data were processed with the help of the statistical product SPSS version 22.0.

Results

Assessment of the reliability of the questionnaire

The pilot survey consisted of 9 (45.0%) males and 11 females (55.0%), aged from 27 to 68 years (mean age 48.58 ± 11.60).

Table 1 presents the mean values for each scale for the first and second measuring, the values for the Wilcoxon test and Spearman-Brown (r_{sb}) coefficient for each item. The obtained high values for r_{sb} (> 0.6) and Cronbach's α for the whole panel (0.749) show that the questionnaire has very good reliability.

Table 1: Results from the test re-test of the questionnaire among lecturers (n = 20)

	Mean score of I-st measurement	Mean score of I I-nd measurement	Wilcoxon test*	r_{sb}
Q1. The management of the unit you work in feels responsible for complying with health and safety working conditions.	4.45	4.45	0.00*	0.64
Q2. The system of quality management assists in solving problems related to health and safety at your workplace.	4.00	3.80	1.63*	0.92
Q3. Health and safety working conditions are observed in your unit.	4.25	4.20	0.27*	0.58
Q4. When there is a problem/negligence related to the health and safety of the employees, it is discussed directly in close co-operation with the superior.	4.40	4.35	1.00*	0.95
Q5. Each employee can offer proposals for improving the working conditions in the unit.	4.32	4.16	1.34*	0.86
Q6. After alterations for improving the working conditions are applied, you receive feedback from the management on their efficiency.	3.95	3.70	1.67*	0.82
Q7. When a problem/ negligence is signalled, there is a feeling that the person is criticised and not the causes of the problem.	2.70	3.05	1.84*	0.85
Q8. The staff does not feel uneasy to discuss openly the acts of people at a higher hierarchical level at the organisation	3.45	3.10	1.44*	0.69
Q9. The employees inform and consult their superior when they have a problem.	4.45	4.05	1.84*	0.55
Q10. You receive feedback on the results of your working activity when you finish a certain task or a project.	4.20	4.00	1.41*	0.74
Q11. You are satisfied with the hours for the beginning and end of the working day.	4.30	4.25	0.33*	0.75
Q12. You are satisfied with the distribution of work and rest within the working day.	3.90	3.85	0.28*	0.83
Q13. You are satisfied with the intensity of the assigned work.	4.00	3.85	0.79*	0.49
Q14. You are satisfied with the system for substitution at the department (interchangeability).	3.95	3.40	2.23	0.82
Q15. You are satisfied with the number of staff to cope with the daily workload.	3.90	3.45	2.33	0.77
Q16. People support each other.	3.55	3.45	0.52*	0.70
Q17. People treat each other respectfully.	3.85	3.60	1.41*	0.59

*P > 0.05.

Construct validity of the questionnaire

To confirm the construct validity of the questionnaire, exploratory factor analysis (EFA) was performed (Table 2). Result analysis was performed based on the 365 respondents who had answered all the 15 questions included in the EFA (out of a total of 370 validly completed questionnaires). The respondents represent 48.4% of the academic staff of the university.

Based on principal axis factoring and extraction with listwise deletion of missing values, exploratory factor analysis revealed evidence for a 4-factor structure related to perceived employees' satisfaction (Table 2). The KMO test and Bartlett's test of sphericity showed that the data were adequate for factorial analysis (KMO = 0.878 and Bartlett's test P = 0.000).

Two questions from Table 1, (Q7) "When a problem/negligence is signalled, there is a feeling of personal criticism or devaluation, not that the causes of the problem are addressed" and (Q8), "The staff does not feel uneasy to discuss openly the acts of people at a higher hierarchical level at the organization", were deleted from the factor matrix.

Table 2: Factor analysis (Method: Principal Axis Factoring) with factors and factor loadings (sorted by weight of coefficients)

	1 (organisation of the working activity)	2 (superior-subordinate communication)	3 (teamwork)	4 (healthy and safe working conditions)
Q (13)	0.766			
Q (12)	0.721			
Q (11)	0.628			
Q (15)	0.529			
Q (14)	0.431			
Q5		0.730		
Q4		0.640		
Q (9)		0.563		
Q6		0.550		
Q (10)		0.543		
Q (17)			0.834	
Q (16)			0.819	
Q1				0.641
Q3				0.538
Q2				0,537
% of Variance after Rotation: Varimax with Kaiser Normalization	17.10	15.40	13.93	11.27

This was due to low Extraction commonalities, which indicate that these variables do not have a direct correlation to the remaining panel of questions. All other items were organised into four sub-scales including, ensuring health and safety working conditions (3 items), superior-subordinate communication (5 items), teamwork (2 items) and organisation of the working activity (5 items). The level of factor-loadings for all items was > 0.4. The relative weight of the four factors is evenly distributed. Using the Varimax rotation method, we demonstrate that these factors account for 57.7% of the studied dependent variable, "Satisfaction of lecturers with the working conditions".

Demographic characteristics of respondents

The response rate was 50.1%. Demographic data of the respondents are presented in Table 3. Comparison between the sample structure and all academics at the Medical University in Plovdiv revealed no statistically significant differences (χ^2 = 0.534, P = 0.602).

Table 3: Demographic characteristics of respondents (n = 370)

Age	n (%)
Under 30	39 (10.5)
31-40	74 (20)
41-50	85 (23)
51-60	81 (21.9)
Over 61	35 (9.5)
Mean age (SD)	46.13 (11.68)
Total	314 (84.9)
Missing	56 (15.1)
Gender	
Male	85 (24)
Female	269 (76)
Department	
Faculty of Dental Medicine	81 (21.9)
Faculty of Medicine	96 (25.9)
Faculty of Pharmacy	71 (19.2)
Faculty of Public Health	56 (15.1)
Medical College	40 (10.8)
Department of Languages and Specialized Training	26 (7.0)

Academic teachers' opinion of work environment factors and their general satisfaction with the working conditions

The answers to respondent's show that a considerable number of them-263 (71.7%) work in a risky work environment. Out of 15 listed risk factors, the respondents have indicated mental strain in the first place-146 (39.5%), followed by 'work with chemical agents and dust'-140 (37.8%), and 'work with biological hazards-133 (35.9%). The nonparametric analysis confirmed the relationship between the working environment risk factors and the workplace of the employees. Staff working at the Pharmacy and Dental Faculties are most frequently exposed to chemical factors (χ^2 = 61.389, P = 0.00); regarding exposure to biological hazards-employees from the Faculties of Dental Medicine and Medicine are at greater risk (χ^2 = 83.916, P = 0.00).

Academic staff at the University receives SNAP benefits as main compensation for working in an unsafe environment (41, 11.1%). The opinion of respondents regarding their general satisfaction with the working conditions, assessed based on guaranteed OHS, superior-subordinate communication, teamwork, and working process organisation at the workplace is presented in Table 4.

Table 4: Descriptive statistics and percentages of respondents' ratings regarding their job satisfaction (n = 370). Responses to subscales are provided

Questions	Mean of responses	Totally disagree [1] n (%)	Disagree-[2] n (%)	Agree/ disagree [3] n (%)	Agree [4] n (%)	Totally agree [5] n (%)
Questions related to the satisfaction with the OHS management system						
Q1 The management of the unit, where you work is responsible for compliance with the health and safety working conditions.	4.32	5 (1.4)	8 (2.2)	31 (8.4)	145 (39.2)	181 (48.9)
Q2 The system of quality management assists in solving problems related to health and safety at your workplace.	3.95	12 (3.2)	21 (5.7)	49 (13.2)	179 (48.4)	109 (29.5)
Q3 In your unit, the health and safety of the working conditions are observed.	4.27	4 (1.1)	13 (3.5)	26 (7.0)	164 (44.3)	163 (44.1)
Questions related to the satisfaction with superior-subordinate communication						
Q4 When there is a problem/negligence related to health and safety of the employees, it is discussed directly in close co-operation with the superior.	4.25	4 (1.1)	12 (3.2)	34 (9.2)	156 (42.2)	164 (44.3)
Q5 Each employee can offer proposals on improving the working conditions in the unit.	4.18	5 (1.4)	18 (4.9)	36 (9.7)	156 (42.2)	155 (41.8)
Q6 After alterations for improving the working conditions are applied, you receive feedback from the management on their efficiency.	3.88	8 (2.2)	32 (8.6)	68 (18.4)	148 (40)	114 (30.8)
Q9 The employees inform and consult their superior when they have a problem.	4.14	8 (2.2)	19 (5.1)	32 (8.6)	165 (44.6)	146 (39.5)
Q 10 You receive feedback on the results of your working activity when you finish a certain task or a project.	3.95	8 (2.2)	22 (5.9)	64 (17.3)	164 (44.3)	112 (30.3)
Questions related to the satisfaction with working activity organisation						
Q11 You are satisfied with the hours for the beginning and end of the working day.	4.20	10 (2.7)	19 (5.1)	15 (4.1)	171 (46.2)	155 (41.9)
Q 12 You are satisfied with the distribution of work and rest within the working day.	4.14	11 (3.0)	20 (5.4)	25 (6.7)	165 (44.6)	149 (40.3)
Q13 You are satisfied with the intensity of the assigned work.	3.98	13 (3.5)	30 (8.1)	32 (8.6)	170 (45.9)	125 (33.9)
Q14 You are satisfied with the system for substitution at the department (interchangeability).	3.91	19 (5.1)	28 (7.6)	44 (11.9)	157 (42.4)	122 (33.0)
Q15 You are satisfied with the number of staff to cope with the daily workload.	3.66	32 (8.6)	39 (10.5)	55 (14.9)	141 (38.1)	103 (27.8)
Questions related to satisfaction with teamwork						
Q16 People support each other.	3.78	20 (5.4)	40 (10.8)	58 (15.7)	134 (36.2)	118 (31.9)
Q17 People treat each other respectfully.	3.86	18 (4.9)	34 (9.2)	49 (13.2)	148 (40.0)	121 (32.7)

The results ascertained relatively high respondent evaluations of satisfaction with working activity organisation, including work and rest balance, working day duration, work intensity, communication and teamwork (Table 4). The respondents' most common answer is 'agree' regarding questions, related to satisfaction with working activity organisation (Table 4). A relation between satisfaction with assigned work intensity and satisfaction with number of staff at the departments was ascertained (r_s = 0.529, P = 0.00).

The respondents' opinion on the management style and on the manner of solving conflicts at the departments is presented in Figure 1. It compares the theoretical background for the applied management

style and methods of conflict management with the results of the study [16].

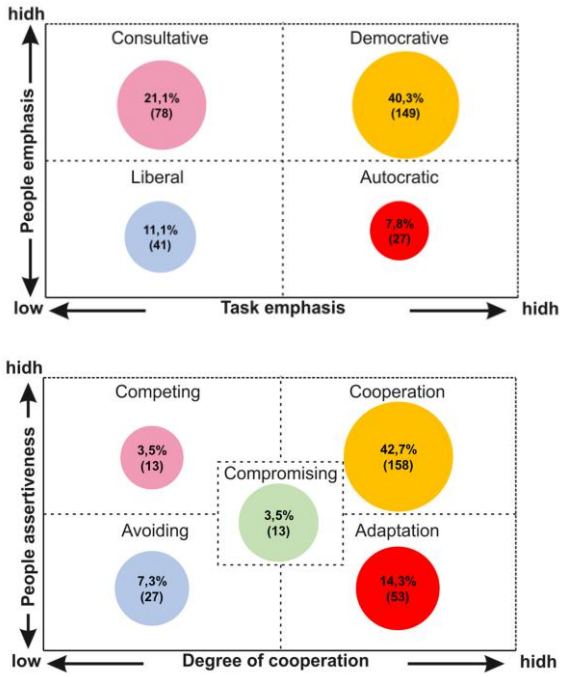

Figure 1: Percentage distribution of answers in terms of applied management style and methods of conflict management at the departments(*Adapted from Robert Blake and Jane Mouton in The Managerial Grid (Houston: Gulf Publishing, 1964, 1994)) "Conflict and Conflict Management" by Kenneth Thomas in The Handbook of Industrial and Organizational Psychology, edited by Marvin Dunnette (Chicago: Rand McNally, 1976); Thomas, K. W., and Kilmann, R. H. "An Overview of the Thomas-Kilmann Conflict Mode Instrument (TKI)."Kilmann Diagnostics Website (2009), available on http://www.kilmanndiagnostics.com/overview-thomas-kilmann-conflict-mode-instrument-tki; and Rahim, M. A. (1983). A measure of styles of handling interpersonal conflict. Academy of Management Journal, 26(2), 368-376.*

Result analysis indicates that democratic leadership is the most commonly applied management style at the workplace (40.3%, n = 149) and cooperation is the most common approach to handle conflicts (42.7%, n = 158) according to the answers of respondents (Figure 1). Nonparametric analysis confirms that the management style (P = 0.000) and the methods for conflict solving at the workplace (P = 0.000) exert influence on satisfaction with feedback from working activity results; satisfaction with the system of substitution (P = 0.000); and satisfaction with teamwork (P = 0.000).

Discussion

Main findings

The analysis of our results showed that the studied working environment is relatively free of risk factors except for excessive mental strain, exposure to biological agents, chemical hazards and dust. An important aspect to be considered in the environment domain is the mentally demanding nature of the work. There are few studies on the psychology of work nature of university teachers. One of them was conducted with university teachers in China. In this study, a larger number of participants (22.3%) reported experiencing occupational stress [12].

Similarly, our results are comparable to those of other authors. They documented that occupational stress is considered a major hazard for employees [14], [17], [18]. The most stressful characteristics of clinical teacher's work, related to the psychologically demanding nature of their job, are intense concentration on the same task for an extended period, excessive workload, time pressure, conflicts with work colleagues and students.

Furthermore, patients refusing to consent, insufficient resources and lack of "teacher-friendly" clinical environment also contribute to stress. Foreign researchers argue that supervisory support and co-worker's involvement have a positive impact on work stress [19]. The present study establishes that positive college environments produce important positive outcomes and a high level of faculty's job satisfaction.

The factor analysis of our data confirmed that working activity organisation, superior-subordinate communication and healthy environment are the most significant factors for work satisfaction. It is worth mentioning, the respondents' trust in the certified System of Quality Management of Medical University Plovdiv, which assists in solving problems related to the health and safety working conditions at the workplace. Another study confirmed that job safety (as elements of the working environment) have an impact job satisfaction [20]. Interestingly as in other studies, important values for the academic staff are autonomy, academic freedom and flexible working hours [14], [21].

In case, employees feel dissatisfied and underestimated in their jobs; their attitude towards the job and their performance are adversely affected. Therefore, it is beneficial for an organisation to provide a flexible working environment for employees and ensure that their opinions are respected. It is important for employees to feel that they play a part in the decision-making process of the organisation [22]. Other authors noted that employees are willing to be a part of the decision-making process, especially regarding issues that affect them directly. It contributes to their sense of belonging [23]. This results in congenial work environment, where both the management and the workers will voluntarily contribute to a healthy occupational atmosphere. Also, the present study found lower satisfaction with the system of substitution and with the number of staff in the departments. This could be explained with the nature of the teacher's work.

Our results showed that top management

support is positively related to job satisfaction. Teamwork was found to be of crucial importance in evaluating respondents' satisfaction. Mutual respect, trust, and support are essential for teamwork, shared sense of community, and empathy. It has been demonstrated that academic staff members receive greatest satisfaction from their relationship with their supervisor. The supervisor satisfaction factor was the one, among 14 other factors examined in another Bulgarian study [24].

The findings of our study provide further evidence to the thesis that job satisfaction is more dependent on internal traits (for instance: superior-subordinate communication) than on the external environment (for instance: healthy and safe working conditions) [14], [25]. Overall, our study shows that surveyed academics are satisfied with their job (positive average ratings are seen in Table 2) even though the majority of the interviewed consider that they work under significant mental strain. It is worth noting that another study revealed that occupational stress of faculty members at a tertiary education institution in Cyprus had a negative impact on the degree of satisfaction with their achievements, value and growth [26]. These researchers also reveal dissatisfaction of faculty with the organisational design, structure and processes (communication, change implementation, motivation, supervision style, participation in decision-making) [26].

Similar to our results, other studies have also proven that friendly relationships between the manager and other staff members are important reflections of job performance, regardless of the need to perform under pressure and overloaded work schedule [14], [27].

The relevance to company culture, elements involving conflict handling and predominant communication styles were also addressed in our study. Based on the respondents' opinion, the most commonly applied management style is democratic leadership. The results of the current study revealed that democratic management style and good effective supervision results in high employee satisfaction level. The management style in the departments reflects the characteristic behaviour and attitude of the immediate manager towards his or her subordinates.

In the process of decision-making and exerting authority, an indirect measure of working activity satisfaction is presented. Other researches have also proved that the management style in an organisation is an important feature which affects job-related stress in employees and thus jobs satisfaction [26]. Fletcher [27] in his study on how the presence of staff development management system program affects staff performance, commented on the development of a management system program for providing feedback to the employees in areas that needed improvement.

Moreover, further training for the staff was suggested, as an attempt to handle the improvement and development criteria systematically. Also, the author asserted that the manager's expectations in terms of the work performance by his or her subordinates should be unambiguous and communicated to the subordinates. Employees should be made familiar with what is expected from them [27].

Limitations: The nature of the cross-sectional design of our research is subject to certain limitations. The study depicted the situation only at a specific point in time. As a result, data were collected only from present workers and excluded those that were absent for health reasons. Another limitation was the lack of information about remuneration satisfaction. We did not ask our respondents about pay satisfaction since we speculated that they, being of higher social standing, would be more concerned about other factors such as communication with their superiors, peers and workload, i.e. with satisfying their higher-level needs as defined in Herzberg Two Factor theory. Furthermore, the study was based on a single institution. Hence, results that were seen may not be representative for all academic medical staff in Bulgaria.

Similarly designed studies should be conducted in other universities to clarify whether the collected data from various universities will present a different scenario.

In conclusion, the tool used to measure job satisfaction revealed high values of psychometric characteristics for reliability and validity. The questionnaire allows us to explore job satisfaction of academic medical staff and could be readily used by the Committee on Working Conditions and Occupational Health to establish and maintain acceptable working conditions and suitable work atmosphere.

The study found a high level of satisfaction of university teachers with their working conditions. To improve continuously employee satisfaction, it is necessary to conduct similar studies periodically to detect decrease in academic staff work satisfaction and take timely and adequate measures to improve it.

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Review Article

IL-6 Activities in the Tumour Microenvironment. Part 1

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Abstract

The predominant role of IL-6 in cancer is its key promotion of tumour growth. IL-6 binds IL-6 receptor (IL-6R) and the membrane-bound glycoprotein gp130. The complex I-6/IL-6R/gp130 starts the Janus kinases (JAKs) and signal transducer and activator of transcription 3 (STAT3) or JAK/STAT3 pathway. IL-6R exits in two forms: a membrane-bound IL-6Rα subunit (mIL-6R) that participates in classic signalling pathway and soluble IL-6R subunit (sIL-6R) engaged in trans-signalling. The pro-tumour functions of IL-6 are associated with STAT3, a major oncogenic transcription factor that triggers up-regulation of target genes responsible for tumour cell survival. IL-6 combined with TGF-β induces proliferation of pathogenic Th17 cells. The anti-tumour function of IL-6 is the promotion of anti-tumour immunity. IL-6 trans-signaling contributed to transmigration of lymphocytes in high endothelial venules (HEV). Dendritic cell (DC) secreted IL-6 in the lymph node influences the activation, distribution and polarisation of the immune response. Elevated serum levels of IL-6 and increased expression of IL-6 in tumour tissue are negative prognostic marker for patients' survival.

Introduction

The cytokine interleukin-6 (IL-6) is a member of a group of cytokines that possess a four-helical structure [1]. It was described first as a B cell differentiation factor in 1986 [2], [3], [4]. IL-6 has various biological activities such as stimulation of the growth of tumour cells of murine plasmacytoma and human myeloma [5]. IL-6 also has an inhibitory effect on the antiviral antibody response [6]. Moreover, IL-6 is produced by several types of cells such as monocytes, macrophages, Kupffer cells [7], keratinocytes, endothelial cells, B cells and T cells [1].

The intracellular signaling is induced when the complex of IL-6 and IL-6 receptor (an 80-kDa ligand-binding chain IL-6Rα, CD126) binds the membrane glycoprotein 130 (gp 130) (a signal-transducing chain, IL-6Rb, CD130) [8], [9] that initiates the Janus kinases (JAKs) and signal transducer and activator of transcription (STAT) or JAKs / STAT pathway [8]. IL-6R is found in two forms, a transmembrane form mIL-6Rα, and a soluble form sIL-6R. IL-6 binds to both of these forms and subsequently interacts with the gp 130 to trigger downstream signal transduction and gene expression [7]. The gp130 lacks an intrinsic kinase domain, and therefore the members of the JAKs family, like JAK1, JAK2 and tyrosine kinase 2 (Tyk2), are linked to gp130 [5]. The complex of IL-6, IL-6R and gp130 phosphorylates the afore-mentioned kinases and later activates the cytoplasmic transcriptional factors as STAT1 and STAT3 [10]. Therefore, IL-6 activates transcriptional factors through IL-6R/gp130 complexes with following downstream effects [5].

The membrane-bound IL-6Rα subunit is located on the membrane of target cells. The second receptor subunit is the gp130 associated with mIL-6Rα/IL-6 that subsequently activates the "classic signaling pathway" [11]. The complex IL-6 / mIL-6Rα

leads to dimerisation of gp130 and subsequent activation and phosphorylation of STAT3 via JAK. The classic signalling is realised during the early immune responses and activates acute-phase proteins like C-reactive protein (CRP) [9]. This “classic signalling” is accomplished on cells, expressing both the mIL-6R subunit and gp130 subunit. The latter is widely expressed, but the former is found only on hepatocytes, leukocytes and megakaryocytes [5], [9].

The second mechanism of induction of intracellular reaction is when IL-6 associates with soluble IL-6R (sIL-6R) and binds gp130 on cellular membranes that do not express mIL-6Rα. That process is defined as “trans-signalling” an alternative of classic signalling [12]. The presence of sIL-6R in the serum is a result of shedding of the mIL-6R from the cellular membranes induced by apoptosis and realised through a dis-integrin and a metalloproteinase 10 (ADAM10 or ADAM17) [13]. A second way of achieving sIL-6R is via differential splicing of IL-6 mRNA [12]. The shedding of the IL-6R is also initiated by CRP [14,15], or bacterial toxins [16]. The shedding of IL-6R is released from neutrophils at the beginning of the inflammatory process [9]. The presence of sIL-6R and IL-6 induces Th17 cells and is responsible for the balance between Th17 and T regulatory cells (Tregs) [17]. Therefore, IL-6 trans-signaling modulates the T cell response [18]. IL-6 trans-signalling is observed in many cell types such as epithelial cells, neutrophils, macrophages and T cells [9] and that the complex of IL-6 with the sIL-6R is associated with the cellular membrane gp130 [19] (Figure 1).

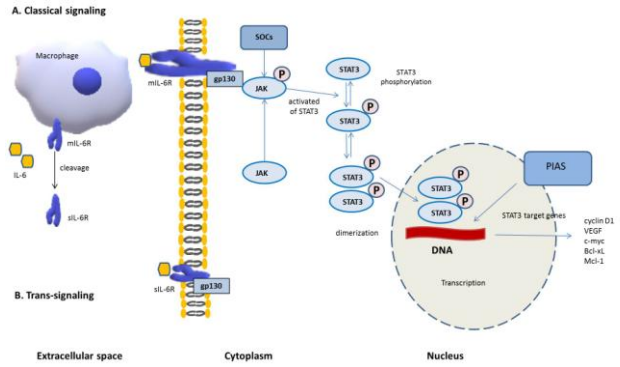

Figure 1: IL-6/JAK/STAT3 signaling; A) Classical-signaling: IL-6 binds to mIL-6 R, and interplays with membrane gp 130; B) Trans-signaling: sIL-6R cleaved from macrophage membranes binds to IL-6 and then the complex interplays with membrane gp130; Then the complex IL-6 / IL-6R / gp130 triggers the activation of JAK, and meanwhile the suppressor of cytokine signalling (SOCS) acts on JAKs and stops phosphorylation of gp130, STATs and the JAKs themselves. STAT3 (an oncogenic transcriptional factor) is activated by JAKs, phosphorylated and formed dimers (pSTAT3-pSTAT3). The dimerised pSTAT3 complex moves to nucleus and pSTAT3 complex trigger transcription of STAT3 target genes (cyclin D1, VEGF, c-myc, etc) through interaction with DNA. Cancer promotion is initiated. The protein inhibitors of activated STATs (PIAS) can suppress the transcription of STAT3 target genes

IL-6 up-regulates several acute-phase proteins such as CRP, fibrinogen, etc. [15], and IL-6 has both anti- and pro-inflammatory activities [9]. The anti-inflammatory functions are realized by the complex IL-6/mIL-6R and include activation of STAT3, followed by intestinal cell proliferation, inhibition of epithelial cell apoptosis and release of acute-phase proteins [19], [20]. The pro-inflammatory activities are realized by the complex IL-6 / sIL-6R and include activation of the immune system through recruitment of mononuclear cells (myeloid-derived suppressor cells – MDSC and macrophages), inhibition of T cell apoptosis and down-regulation of Treg differentiation [17], [21] (Figure 2).

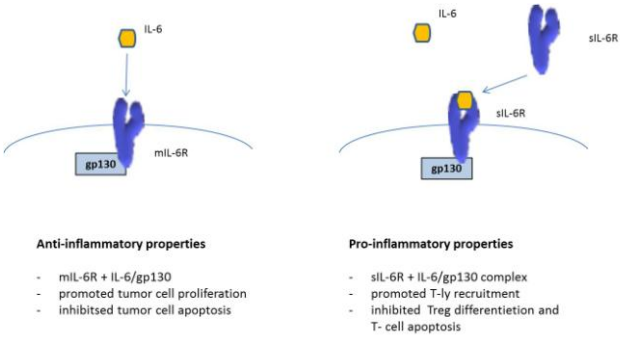

Figure 2: The dual role of IL-6 signalling: IL-6 classical signalling with anti-inflammatory properties and IL-6 trans-signalling with pro-inflammatory properties

During chronic inflammation, IL-6 induces proliferation of Th17 cells and inhibits the differentiation of Tregs [11]. The predominant cell type that secreted IL-6 during acute inflammation is monocyte/macrophage and in chronic inflammation – T lymphocyte [22]. IL-6 is also produced by endothelial cells, B cells, T cells, fibroblasts and some tumour cells [1]. IL-6 can be secreted by stromal fibroblasts in a mouse model of gastric cancer [23].

Colon tumours usually have a decreased expression of membrane-bound IL-6R in comparison to normal epithelial colon tissue. Nevertheless, the expression of ADAM17, associated with shedding of the IL-6R, is increased in tumours, and therefore the “trans-signalling” pathway is involved in colon carcinogenesis [24], [25].

Interleukin-6 and cancer development

Pro-tumour functions of IL-6

The predominant role of IL-6 in cancer is the promotion of tumour growth. The interaction of IL-6 and its receptor-activated JAKs with following induction/activation of STAT3 through tyrosine phosphorylation and subsequent transcription of target genes [9] is vital in cancer formation. In turn, IL-6 induces IL-6-dependent STAT3 activation, resulting in up-regulation of genes that promote the survival of cancer cells [26]. The target genes responsible for

tumor cell survival (Bcl-2, survivin, Mcl-1), [27] proliferation (c-Myc, Cyclin D1, Cyclin B) [28], angiogenesis (VEGF) [29], metastasis (MMP2, MMP9) [30], [31], cell adhesion (ICAM-1, TWIST1), inflammation (IL-6, IL-17, IL-23, Cox2), and others [32]are influenced by IL-6 activities.

STAT3 is a major oncogenic transcription factor that is activated by the binding of IL-6 to the IL-6 receptor [25]. The first event is the binding of IL-6 to mIL-6R followed by gp130 dimerisation and trans-phosphorylation of STAT3 through tyrosine phosphorylation. Subsequently, STAT3 trans-locates to the nucleus in epithelial tumour cells, where STAT3 dimers bind DNA and modulate the expression of some target genes [22], [33]. Additionally, the IL-6 / STAT3 pathway blocks the maturation of dendritic cells (DCs), inhibits T cell activation [34] and maintains immunosuppression through MDSC and macrophages (tumour-associated macrophages – TAMs) [35].

IL-6 is also involved in the differentiation of monocytes to macrophages, downregulates apoptosis of T lymphocytes, and the production of Th2 cytokines [3], [36], [37].

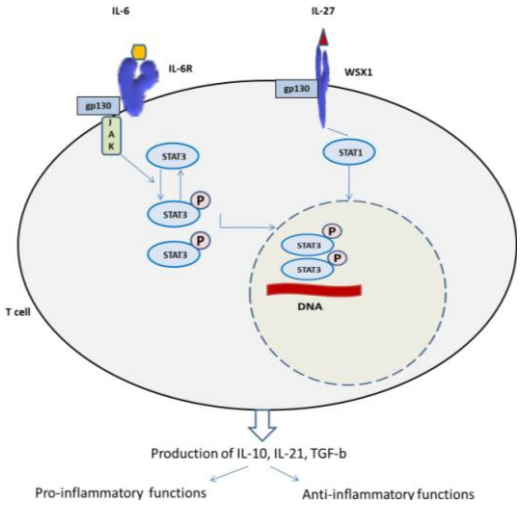

Figure 3: IL-6 and IL-27 trigger a common signal transduction pathway in T cell

Several molecules secreted by tumour cells, including IL-1β, TNF-α, IL-6 and TGF-β are considered to be promoters of Th17 differentiation from naïve CD4+ T cells. There exists evidence that Th17 cells increase in number in the tumour microenvironment (TME) [38]. In contrast, IFN-γ and IL-4, the main cytokines involved in Th1 and Th2 polarisation, respectively, negatively regulate Th17 differentiation [25], [38]. The pro-inflammatory cytokines, IL-6 and TNF-α, are produced in *TME* mostly by hematopoietic cells and also by tumour cells. They are tumour-promoting and further enhance nuclear factor kappa B (NF-kB) and STAT3 activation [39,40]. Moreover, IL-6 and IL-27 mediate signal transduction through STAT3 and STAT1 activation of Th17 and Treg differentiation [48]. IL-6 combined with TGF-β3 or TGF-β1 induce proliferation of pathogenic Th17 cells [42]. IL-6 and IL-27 both can initiate common signal transduction pathways in T cells [41] (Figure 3).

STAT3 is an essential activator for Th17 cell proliferation [43], and on the other hand IL-6, a STAT3 activator, together with TGF-β increased the expression of main transcription factors RORα (human) and RORγt (mouse) for Th17 cell induction and IL-17 production [44], [45]. In contrast to STAT3 activation, STAT1 activation inhibits the development of Th17 cells [46]. Cytokines like IL-27 and IFN-γ are involved in the inhibition of Th17 development in a STAT1-dependent manner [5], [46]. Another cytokine that inhibits Th17 cells development is IL-2 in a STAT5 manner [47]. Therefore, the STAT family transcription factors, via the action of various cytokines, exert positive or negative influences on Th17 development. Interferon-regulatory factor 4 (IRF-4) exerts positive effect on Th17 cell appearance [48] and T-bet negatively influence the development of Th17 cells [49]. Treg helpers are mainly naturally occurring thymus-derived Tregs (nTregs) and TGF-β-induced Tregs (iTregs) [17]. Also, iTregs generate from naïve T cells in the periphery, after stimulation with TGF-β [50]. There are other T cells with regulatory functions including the CD8+ Tregs, Tr1 cells, and Th3 cells [51]. The balance between Th17 cells and Tregs is controlled by IL-6 that maintains immune homeostasis. TGF-β is important for Th17 and Treg cells differentiation, and it induces both Foxp3 and RORγt expression [52]. Therefore, IL-6 is considered to be a pro-inflammatory cytokine that promoted Th17 cell differentiation and inhibits Tregs development. The cytokine IL-17 has dual roles in TME, having pro- and anti-tumour activities [53] (Figure 4).

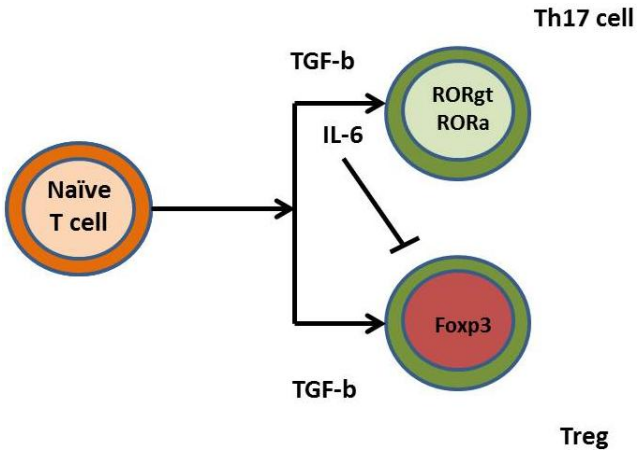

Figure 4: IL-6 maintains Th17/Treg balance. IL-6, together with TGF-β triggers Th17 differentiation from naïve T cells. On the other hand, IL-6 alone inhibits Treg differentiation triggered by TGF-β itself

IL-6 is a growth factor for human colon cancer cells, and inhibition of IL-6 signalling interferes with the growth of tumour cells [24]. In TME tumour-infiltrating lymphocytes (TILs) produce elevated levels

of pro-tumorigenic cytokines such as IL-17A, IL-17F, IL-21, IL-22, TNF-α and IL-6 [40]. Some of the cytokines like IL-6 and TNF-α are also produced by tumour cells [20]. Colorectal cancer (CRC) cell lines - DLD-1 and HT-29, are affected by IL-6, TNF-α and IL-17 cytokines, and result in enhanced NF-kB and STAT3, which induce colorectal cancer cell growth [40].

IL-6 plays a major role in promoting proliferation of tumour cells and in inhibiting apoptosis via binding to IL-6Rα to the gp130. Following activation of JAK / STAT signalling pathway [54] namely of STAT1 and STAT3 [55] cancer initiation and proliferation occurs. Similarly, to TNF-α, IL-6 supports tumour development by induction of normal epithelial cells to convert into cancer stem-like cells [56]. STAT3 can mediate nuclear translocation of β-catenin. The nuclear co-expression of pSTAT3 and β-catenin is associated with poor survival of colon cancer patients [57]. IL-6 initiates tumorigenesis by hypermethylation of tumour suppressor genes or by hypomethylation of retrotransposon long interspersed nuclear element-1 (LINE-1) in oral squamous cell cancer [58]. IL-6 is a powerful (relevant) angiogenic factor, and its high levels correlate with that of VEGF in colorectal cancer [59,60]. Moreover, IL-6 initiates VEGF action in gastric cancer [61]. The secretion of IL-6 and subsequent STAT3 phosphorylation up-regulate some angiogenic mediators such as VEGF, VEGFR2 and neuropilin 2 [62].

In conclusion, IL-6 in the TME supports tumour development, metastasis and evasion from the effective anti-tumour immune response.

Anti-tumour functions of IL-6

The main anti-tumour function of IL-6 is the promotion of anti-tumour immunity [63], [64]. The analysis of many specimens of human tumours reveals that the immune contexture, defined by the type of immune cells, their activity, and distribution mainly in the invasive front, is a better prognostic factor as compared to histological staging and grading [63]. There is evidence that IL-6 trans-signalling is important in the initiation of T cell immune responses [65], [66]. Using trans-signalling IL-6 is a key cytokine in the modulation of anti-tumor immune response [67]. IL-6 maintains anti-tumour immunity at two main sites: first in the lymph nodes where lymphocyte priming takes place and second in tumour nests where IL-6 promotes the recruitment of effector T cells in TME [68].

In lymph nodes, dendritic cells (DCs) encounter tumour antigens. Also, naïve T cells and memory T cells enter lymph nodes through high endothelial venules (HEV). The polarisation interacts with the naïve T cells and initiates T cell polarisation [69], [70]. DCs secrete IL-6 in the lymph node that influences the activation, distribution and polarisation of the immune response [71].

In HEV, IL-6 trans-signaling acts on T lymphocytes to initiate tethering and rolling on the endothelial surface of HEV. Later the interaction between CCL21 on endothelial cells and CCR7 chemokine receptor on T lymphocytes initiates the chemokine activation that helps firm adhesion. The lymphocyte firm adhesion molecule 1 (LFA-1) binds to intercellular adhesion molecule 1 or 2 (ICAM-1 or ICAM-2) on endothelial cells and lymphocyte trans-endothelial migration in HEVs in lymph nodes or the tumour site [70], [72], [73]. IL-6 trans-signaling contributes to L-selectin-mediated and transmigration of lymphocytes to HEV [74]. Usually, tumour vessels had tortuous structure and express low levels of trafficking molecules such as ICAM-[66], [67]. Endothelial cells of tumour vessels and cancer-associated fibroblasts are the main producers of IL-6 at tumour sites [74]. Thus, injection of H-IL-6 induces high IL-6/sIL-6Rα concentration in TME. IL-6 trans-signaling increases CD8+ T cells trafficking into tumours and supports adoptive T cell transfer in adoptive cell therapy [67]. In mouse models the administration of H-IL-6 or application of systemic thermal therapy before adoptive CD8+ T cell transfer leads to enhanced tumour cell apoptosis and delay of tumour cell growth [67], [74].

The anti-tumour activities of IL-6 trans-signalling are used as basis for anti-tumour therapy. Thermal therapy is based on enhanced lymphocyte recruitment as response to febrile temperatures about 39.50 for periods up to 6 hours [66], [75]. The thermal stress leads to transient decrease in lymphocyte count with following increase of it in cancer patients with subsequent tumour restriction [76], [77].

Thermal therapy up-regulates gp130 on the endothelial cells in tumour microvessels [78] and thus supports IL-6/sIL-6Rα activity with following CD8+ T cell trafficking and recruitment into the tumour site [74], [78]. Taken together, the administration of H-IL-6 or thermal therapy could restrain cancer development when combined with adoptive CD8+ T cell vaccination [67], [78], [79].

Elevated levels of IL-6 and other serum biomarkers in cancer patients

Various biomarkers for the initiation and development of cancer exist. These biomarkers are associated mainly with inflammation and obesity [15], [80]. Chronic inflammation is related to colon carcinogenesis [68], [81]. It has been reported that cancer-associated inflammation determined disease progression and survival in CRC [82].

The existing meta-analysis shows that serum CRP and IL-6 levels could be associated with the risk of CRC development [33], [83], [84] but this is not useful for identifying colorectal adenomas [85]. TNF-α serum levels were studied in the risk of CRC

development [86]. Another investigation report increased mRNA level of IL-6 that is predictive for colorectal cancer development with distant metastases [87]. Several CRC case-control studies show increased serum levels of CRP, TNF-α, IL-6 and IL-8 in colorectal adenoma and CRC patients [88]. Moreover, expression-enhancing polymer-phisms in the genes for IL-6, TNF-α, IL-1β and IL-8 are associated with increased risk for the development of colorectal cancer [89]. The increased release of IL-6 in the sera of CRC patients is associated with CEA-induced production of IL-6 by Kupffer cells, macrophages, lymphocytes and tumour cells [90]. Serum IL-6 > 10 pg/ml values are associated with higher incidence of CRC with distant metastasis and therefore can be an independent, negative prognostic marker for patients' survival [91].

Adipose tissue is considered to be the largest endocrine tissue that secrets various cytokines such as IL-2, IL-6, IL-8, TNF-α, etc. [92]. IL-6 is a poor prognostic factor in obese patients with CRC [93], [94], [95], [96].

Clinical significance of tissue overexpression of IL-6 in CRC cancer tissue

Few studies address the immunohisto-chemical expression of IL-6 in CRC [97], [98], [99]. Some studies show overexpression of IL-6 in tumour tissue in glioblastoma [94], prostate cancer [43], renal cell cancer [57], gastric cancer [61] etc. Additionally, the expression of IL-6R and gp130 was investigated in tumour cells of CRC [97]. The overexpression of IL-6 in cancer tissue correlates to advanced stage, lymph node metastasis, and venous invasion [100], [101]. Therefore, IL-6 cancer cell expression can be a relevant marker of cancer progression.

In conclusion, IL-6 is mainly a pro-tumorigenic cytokine that triggers JAK / STAT3 activation with subsequent promotion of tumour cell growth and suppression of tumour cell apoptosis. IL-6 / STAT3 signalling regulates the balance between Th17 and Tregs in TME with immunosuppressive properties. The anti-tumour activity of IL-6is associated with modulation of T cell polarisation initiated by IL-6 secreting DCs and with the support of T lymphocyte recruitment in lymph nodes. A further investigation is necessary to elucidate the intimate mechanisms of IL-6 regulation.

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