ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1323-1327.
https://doi.org/10.3889/oamjms.2018.315
eISSN: 1857-9655
Basic Science

Serum Midkine Levels in Systemic Lupus Erythematosus

Blondina Marpaung*, Andi Raga Ginting, OK Moehad Sjah

Division of Rheumatology, Faculty of Medicine Universitas of Sumatera Utara, Medan, Indonesia

Citation: Marpaung B, Ginting AR, Sjah OKM. Serum Midkine Levels in Systemic Lupus Erythematosus. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1323-1327. https://doi.org/10.3889/oamjms.2018.315

Keywords: Midkine; Systemic Lupus Erythematosus

***Correspondence:** Blondina Marpaung. Division of Rheumatology, Faculty of Medicine Universitas of Sumatera Utara, Medan, Indonesia. E-mail: blondina@usu.ac.id

Received: 02-Jun-2018; **Revised:** 22-Jul-2018; **Accepted:** 27-Jul-2018; **Online first:** 14-Aug-2018

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Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: Midkine (MK) induces inflammation and could inhibit inducible regulatory T cell differentiation. These reports suggest that MK may play a role in the pathogenesis of autoimmune disease including SLE, but data about MK in SLE patients was still limited, and the role of Midkine in SLE is largely unknown.

AIM: The purpose of this study was to compare serum level MK in SLE patients and control, also analysed the relationship between the serum MK level and disease activity in SLE.

METHODS: This cross-sectional study was conducted in Adam Malik Hospital from January-June 2017. Diagnosis of SLE was established according to the Systemic Lupus International Collaborating Clinics (SLICC) classification criteria, and disease activity was assessed using the Mexican Systemic lupus erythematosus disease activity index (MEX-SLEDAI). Subjects with evidence of malignancy and systemic disease (pulmonary, kidney, liver, metabolic disorder, etc.) were excluded. Data analysis was performed using SPSS 22nd version. $P < 0.05$ was considered statistically significant.

RESULTS: There were 90 subjects and divided into 2 groups: SLE patients group (n=40) and healthy control groups (n = 50). Midkine levels were increased in the serum of SLE patients compared by health control. There was a significant difference in the median serum Midkine levels between SLE patients and healthy control ($P < 0.001$). Elevated Midkine serum levels were a significant difference between active disease and remission ($P = 0.018$).

CONCLUSION: Elevated Midkine serum level could be a marker of SLE disease activity and have a role in the pathogenesis of SLE.

Introduction

Systemic lupus erythematosus (SLE) is a common systemic inflammatory autoimmune disease. The immune response in SLE can cause chronic inflammation leading to irreversible damage to organ systems [1]. SLE is characterised by many immunologic abnormalities, such as polyclonal activation of circulating B cells that generate a large number of autoreactive antibodies. SLE is also characterised by T lymphocyte abnormalities and immune complex (IC) deposition [2]. Cytokines that are derived from monocyte/macrophage play a key role in SLE pathogenesis. Cytokines collectively play key roles in the regulation of systemic inflammation, local tissue damage, and immunomodulation [3].

Midkine (MK) is a heparin-binding growth factor that was originally identified as the retinoic acid–response gene product. MK gene encodes it on chromosome 11 [4]. Midkine has a critical role in cell growth, survival, migration, angiogenesis, and carcinogenesis [5]. A higher midkine level in peripheral blood was associated with a poor outcome in patients with malignancies [6].

MK might modulate inflammatory responses [7]. MK induces inflammation via increasing leukocytes migration, induction of chemokine synthesis and preventing the development of regulatory T cells [8]. Midkine could inhibit inducible regulatory T cell differentiation by suppressing the development of tolerogenic dendritic cells [9]. It has been reported that MK level was elevated in the serum and synovial fluid of RA patients. These reports suggest that MK may play a role in the pathogenesis

of RA [10]. Data about MK in SLE patients was still limited. Role of midkine in SLE is largely unknown. The purpose of the present study was to compare the serum level of MK in SLE patients and control, also analysed the relationship between the serum MK level and disease activity in SLE.

Methods

This study was a cross-sectional study on 40 consecutive SLE patients that were admitted to the Rheumatology Department and outpatient clinic of Adam Malik General Hospital and affiliated hospitals in Medan, Indonesia between January-June 2017. Diagnosis of SLE was established according to the Systemic Lupus International Collaborating Clinics (SLICC) classification criteria [11]. Subjects with evidence of malignancy and systemic disease (pulmonary, kidney, liver, metabolic disorder, etc.) were excluded.

Disease activity was assessed for all the patients using the Mexican Systemic lupus erythematosus disease activity index (MEX-SLEDAI). MEX-SLEDAI score has a score range of 0 to 32, where the higher the score indicates, the more severe the activity of SLE disease. Active lupus is defined as the MEX-SLEDAI score > 5 [12]. Renal disorder in SLE patients according to SLICC classification criteria was proteinuria (> 0.5 g/24hr) or red blood cell casts [11]. Fifty age and sex-matched subjects were considered as a control group and were recruited from the relatives of the patients and healthcare providers in the hospital. The study was approved by the local ethics committee. All patients gave their informed consent before their inclusion in the study.

Serum MK was measured in all enrolled subjects using ELISA kit (Glory Science, USA). The assay is based on a double-antibody sandwich ELISA technique for the quantitative assay of human MK in samples. In this technique, MK binds to the monoclonal antibody-enzyme well which is precoated with human MK monoclonal antibody, making a solid phase antibody. Then MK antibody is added and combines with Streptavidin-Horseradish Peroxidase (HRP) to form an immune complex. Following incubation, MK is removed during a wash step and then substrates A and B are added to the wells and the colour of the liquid changes into blue. The coloured product is formed in proportion to the amount of MK present in the sample. The reaction is terminated by addition of sulphuric acid. The concentration of MK in the samples is then determined by comparing the (optical density) OD of the samples to the standard curve and values were reported as pg/mL.

Numerical data were expressed as mean ± SD, or median (interquartile range, IQR) if they were not in normal distribution. Data analysis was performed through univariate and bivariate analyses using the SPSS 22nd version (SPSS Inc., Chicago) with a 95% confidence interval. Bivariate analysis was performed using an Independent t-test and Mann-Whitney U test with significance $p<0.05$.

Results

This study was followed by 90 subjects and divided into 2 groups: SLE patients group (n = 40) and Healthy control group (n = 50). There were 37 (92.5%) female patients and 3 (7.5%) male patients in group SLE. The mean age of the SLE group and healthy control group were 28.7 (6.1) and 27.3 (5.8) years respectively. The median of disease duration in group SLE was 4.5 (0-9) years. 24 patient (57.5%) in the SLE group had active disease (MEX-SLEDAI score > 5) and 19 patients (47.5%) had renal disorder (Table 1).

Table 1: Basic and clinical characteristics of the subjects

Characteristics	SLE patients (n = 40)	Healthy controls (n = 50)
Gender [a]		
Female	37 (92.5%)	46 (92%)
Male	3 (7.5%)	4 (8%)
Age (years) [b]	28.7 (6.1)	27.3 (5.8)
BMI (kg/m2) [b]	22.7 (3.6)	24.4 (3.7)
Disease duration (years) [c]	4.5 (0 – 9)	NA
Active Disease [a]		NA
Yes	24 (57.5%)	
No	16 (42.5%)	
Renal disorder [a]		NA
Yes	19 (47.5%)	
No	21 (52.5%)	

N = total number of subjects; [a]n (%); [b]mean ± SD; [c]median (min-max).

Midkine levels were increased in the serum of SLE patients compared by health control. There was a significant difference in the median serum Midkine levels between SLE patients and healthy control ($P < 0.001$). Patients SLE with active disease had midkine levels higher than remission group (660.2 ± 84.8) vs (596.9 ± 68.8) pg/ml, there was a significant difference in serum Midkine levels between active disease and remission ($P = 0.018$) but there was no significant difference in serum Midkine levels in renal disorder group ($P = 0.092$) (Table 2).

Table 2: Comparison of serum midkine between SLE patients, active disease and renal disorder groups

Group	Number	Midkine Levels (pg/ml)	p
SLE patients[a]	40	658 (502-795)	<0.001
Healthy controls	50	510 (370-750)	
Active Disease			
Yes	24	660.2 ± 84.8	0.018
No	16	596.9 ± 68.8	
Renal Disorder[s]			
Yes	19	658.4 ± 75.2	0.092
No	21	613.6 ± 87.5	

[a] Mean ± SD; [b]Median (min-max).

SLE patients had serum Midkine levels higher than the control group; it can be seen in Figure 1, that median Midkine levels were 658 (502-795) pg/ml in SLE patient group and 510 (370-750) pg/ml in healthy control group.

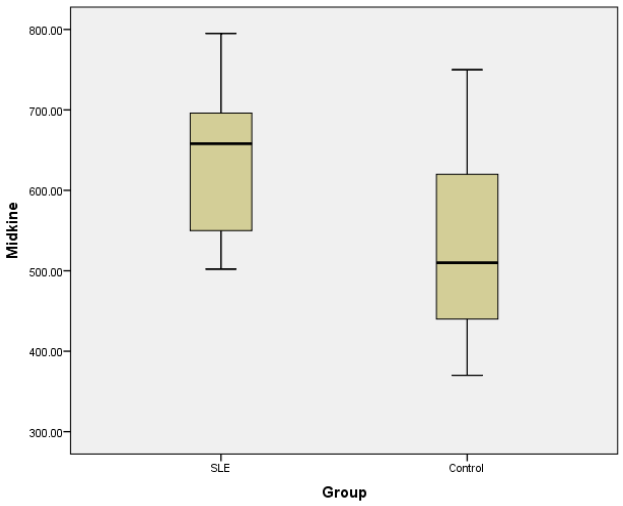

Figure 1: Boxplot diagram of Midkine level between SLE patients and controls

SLE patients, especially with active disease, had serum Midkine levels higher than remission group because of elevated levels of autoimmune inflammatory response (Figure 2).

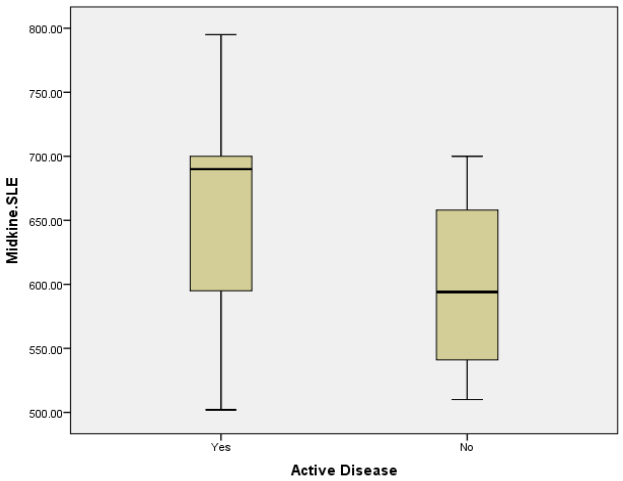

Figure 2: Boxplot diagram of Midkine level between Active disease and remission

Analysis result using the ROC curve obtained that cut off serum Midkine was >569pg/ml. The area under the curve (AUC) was 75.1% (p<0.001) with sensitivity and specificity of Midkine to predict diagnosis SLE were 70% and 60%. Positive Prediction Value (PPV), Negative Prediction Value (NPV), Positive Likelihood ratio (PLR), and Negative Likelihood Ratio (NLR) were 58.3%, 71.4%, 1.75 and 0.5 respectively (Table 3).

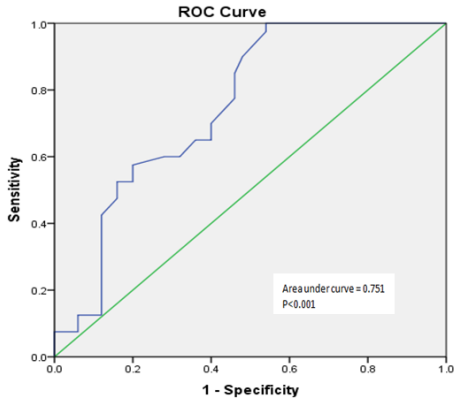

Figure 3: ROC curve of midkine level in the diagnosis of SLE

Discussion

SLE pathogenesis is closely related to the regulation of T lymphocytes. Regulatory T (Treg) cells are a subset of CD4+ T cells that maintain self-tolerance by suppressing autoreactive lymphocytes. Treg cells are required to restore the disturbed immune homeostasis in SLE, and the failure of Treg is related to the persistent inflammatory cytokine. Defects in Treg cells or a lack of Treg cells are contributed to SLE pathogenesis [13] [14].

Table 3: Accuracy of Midkine in the diagnosis of SLE

	Cut off	Sensitivity	Specificity	PPV	NPV	PLR	NLR	Accuracy
Midkine	>569 pg/ml	70%	60%	58.3%	71.4%	1.75	0.5	75.1%

The previous study explained that there was a negative correlation between Tregs and disease activity of SLE [15].

Midkine (MK) derived from CD4+ T cells has a role in promoting cell proliferation, cell survival, migration cells, and antiapoptotic activity in the nervous system, cancer and inflammation area. In inflammation process, MK induces cytokines and modulates migration of neutrophils and macrophages. MK activates T splenocytes, Th1 cell differentiation and has functioned as a negative immune modulator of Tregs in peripheral lymph nodes [16]. Inhibition of MK leads to an increase in Treg expansion and then suppresses the autoreactive Th1 cell population thus causing the reduced severity of the autoimmune disease [8].

This study examined Midkine serum level in SLE patients and healthy control. Median Midkine levels in this study were 658 (502-795) pg/ml and 510 (370-750) pg/ml in SLE patient group and healthy control group. Our result was comparable to a study conducted by Wu GC et al., that reported median Midkine levels were 698.37 (516.09-767.07) pg/ml

and 628.22 (373.66-712.41) pg/ml in SLE patients and healthy control group. Our study result was consistent with Wu GC et al. that Midkine serum levels were significantly increased in SLE patient (P < 0.05) [17]. Elevated Midkine serum level also occurs in other autoimmune diseases such as rheumatoid arthritis and multiple sclerosis [10] [18].

Our result was consistent with Wu et al. that there was no significant difference in serum Midkine levels in SLE patients with and without the renal disorder (P > 0.05). But, there was a significant difference in serum Midkine levels between flare and remission in our result (P = 0.018) contrary to a previous study (P > 0.05) [17]. In Rheumatoid Arthritis (RA), Shindo et al. reported that MK level could be a biomarker of RA disease activity. There was correlation between serum MK and RA disease activity (DAS28-ESR) (r = 0.223, P = 0.019) [10]. Same result in Crohn's Disease and Ulcerative Colitis that serum MK level was positive associated with Disease Activity Index [19] [20]. Midkine plays an important role in the pathogenesis of autoimmune diseases including SLE. Inhibiting Midkine can be useful to reduce the disease activity of autoimmune disease. In this study, cut off serum Midkine (> 569 pg/ml) had a moderately accuracy to predict SLE (AUC > 70%) and a higher accuracy level than previous study [17].

This study has some limitations. MEX-SLEDAI was used to assess the disease activity of SLE. MEX-SLEDAI was more simple but has a lower accuracy compared to the SLEDAI score. This study didn't compare the difference of Midkine levels with blood disorders group or neuropsychiatry disorder group of SLE patients. Further studies are required with larger samples to determine the role of Midkine as a predictive marker to diagnose SLE.

In conclusion, elevated Midkine serum level could be a marker of SLE disease activity and have a role in the pathogenesis of SLE.

Acknowledgements

We would like to express our gratitude to Prodia Education and Research Institute for assistance in Midkine testing, to Rumondang who has assisted the SLE patients in this study.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1328-1334.
https://doi.org/10.3889/oamjms.2018.266
eISSN: 1857-9655
Basic Science

Association between *VEGF-634G>C* Gene Polymorphism with Gastric Premalignant Lesions and Serum VEGF Levels in *Helicobacter pylori* Gastritis Patients

Gontar Alamsyah Siregar[1*], Ida Parwati[2], Tri Hanggono Achmad[3], Yoni Fuadah Syukriani[4]

[1]Division of Gastroentero-Hepatology, Department of Internal Medicine, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia; [2]Department of Clinical Pathology, Faculty of Medicine, Universitas Padjajaran, Bandung, Indonesia; [3]Department of Biochemistry, Faculty of Medicine, Universitas Padjajaran, Bandung, Indonesia; [4]Department of Forensic and Legal Medicine, Faculty of Medicine, Universitas Padjajaran, Bandung, Indonesia

Citation: Siregar GA, Parwati I, Achmad TH, Syukriani YF. Association between *VEGF-634G>C* Gene Polymorphism with Gastric Premalignant Lesions and Serum VEGF Levels in *Helicobacter pylori* Gastritis Patients. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1328-1334. https://doi.org/10.3889/oamjms.2018.266

Keywords: Gastric premalignant lesion; Helicobacter pylori; VEGF; polymorphism

***Correspondence:** Gontar Alamsyah Siregar, Division of Gastroentero-Hepatology, Department of Internal Medicine, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia. E-mail: gontarsir@gmail.com

Received: 26-May-2018; **Revised:** 25-Jun-2018; **Accepted:** 26-May-2018; **Online first:** 02-Aug-2018

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

AIM: To evaluate the association between *VEGF-634G>C* gene polymorphism with premalignant gastric lesions as well as the level of VEGF.

METHODS: This cross-sectional study included patients with *H. pylori* gastritis at Haji Adam Malik General Hospital, Permata Bunda General Hospital, and Universitas Sumatera Utara Hospital, Medan, Indonesia. Detection of *H. pylori* infection was made using positive results of ^{14}C-UBT, rapid urease test, and/or immunohistochemistry. Gastric premalignant lesion diagnosis was made when one or more of the following were present: chronic atrophic gastritis, intestinal metaplasia, or dysplasia. Real-time polymerase chain reaction (RT-PCR) was used to examine VEGF-634G>C gene polymorphism. Additionally, serum samples of patients with *H. pylori* gastritis were obtained to determine the level of circulating VEGF. Data were analysed using SPSS version 22.

RESULTS: A total number of 87 patients with *H. pylori* gastritis were included in this study. Of all participants, 26 patients (29.9%) showed gastric premalignancy. There was a significant association between *GG+GC* genotype of *VEGF-634G>C* and gastric premalignant lesions (P = 0.003; OR (CI 95%) = 6.07 (1.88-41.71)). *VEGF-634 G>C* polymorphism also showed an association with VEGF serum levels (P = 0.005). Patients with the *GG+GC* genotype would be at risk of 3.16 times to have high VEGF levels compared to *CC* genotypes.

CONCLUSION: *VEGF-634G>C* polymorphism, in particular, *GG+GC* genotype was associated with an increased risk of gastric premalignant transformation as well as having high VEGF levels in patients with *H.pylori* gastritis.

Introduction

Gastric cancer remains to be the second leading cause of all cancer mortality, causing approximately 700.000 death worldwide [1]. Gastric carcinogenesis is a continuous process commonly initiated with atrophic gastritis [2]. Chronic atrophic gastritis is considered the first step in a sequence of mucosal changes in the stomach leading to cancer. The current model for stomach carcinogenesis begins with gastritis, proceeding to chronic atrophic gastritis, then to intestinal metaplasia, dysplasia and, finally, carcinoma [3]. Risk factors of gastric cancer are *Helicobacter pylori* (*H. pylori*) infection, salt intake, smoking, alcohol, family history of gastric cancer, and presence of premalignant gastric lesions such as atrophic gastritis, intestinal metaplasia, and dysplasia. Gastric premalignant lesions are well-known risk factors for the development of gastric cancer [4] [5] [6] [7] [8] [9] [10] [11] [12].

H. pylori infection has been well studied as the main aetiology of chronic gastritis. Subsequently, atrophic gastritis, intestinal metaplasia, dysplasia, and gastric cancer may follow. Not all patients with *H. pylori* infection would develop such progression. One

other factor that may contribute to the malignant progression is genetic [13].

Previous evidence suggests the Vascular Endothelial Growth Factor (VEGF) may play a role in gastric carcinogenesis, as VEGF promotes angiogenesis that is required for tumour survival and metastasis [14]. Patients with premalignant lesions such as atrophic gastritis, intestinal metaplasia, and dysplasia also show overexpression of VEGF that may contribute in the early step of carcinogenesis [15].

The VEGF gene, located at 6p21.3 chromosome, composed of 8 exons separated by 7 introns, is a very polymorphic gene (about 140 variants) [16] [17]. Previous studies show *VEGF-634G>C* polymorphism to be associated with an increased risk of gastric cancer as well as elevated VEGF levels. This polymorphism is influenced by ethnicity to previous studies showed different results [18] [19] [20] [21]. There were no similar studies in Southeast Asia after searching on PubMed. To the authors' knowledge, no published studies evaluating the association between *VEGF-634G>C* polymorphism with gastric premalignant lesions and serum VEGF levels in *H. pylori* gastritis patients are available. This study was conducted to determine the association between *VEGF-634G>C* polymorphism with premalignant gastric lesions and serum level of VEGF in *H. pylori* gastritis patients.

Methods

Patients Selection

This study was a cross-sectional study on 87 consecutive *H. pylori* gastritis patients that were admitted to the Endoscopy Unit at Haji Adam Malik General Hospital, Permata Bunda General Hospital, and Universitas Sumatera Utara Hospital, Medan, Indonesia between October and December 2017. Inclusion criteria include gastritis patients diagnosed based on histopathological examination, at least 18 years old, and willing to take part in the study. Patients with one of the following criteria were excluded: a history of *H. pylori* eradication treatment in the last 6 months or currently on antibiotics therapy commonly used in *H. pylori* eradication; history of proton pump inhibitor, H2 receptor antagonist use within the past 1 month; patients with systemic disease, malignancy or currently pregnant. This study was approved by the Institutional Review Board of Universitas Sumatera Utara.

Endoscopy was conducted to evaluate gastric mucosae such as the presence of oedema, erythema (spotted, patchy, linear), exudate, bleeding, erosion; as well as to take a tissue sample for the rapid urease test, immunohistochemistry test for *H. pylori* and histopathology. Tissue biopsy was performed within the greater and lesser curvature of the distal antrum, the lesser curvature at incisura angular, the anterior and posterior wall of the proximal corpus. An additional biopsy was also done in suspicious regions that were not included in the areas mentioned previously.

Diagnosis of Gastric Premalignant Lesion

Microscopic evaluation was done to diagnose premalignant gastric lesions such as chronic atrophic gastritis, intestinal metaplasia, and dysplasia. The presence of one or more findings is positive for a premalignant gastric lesion. Histopathologic examination was done by two pathologists blindly at Universitas Sumatera Utara Medan. If there were differences in the results of the examination of both experts, then a third pathologist was required to perform the histopathological examination.

Helicobacter pylori detection

The diagnosis of *H. pylori* infection was made using positive results of ^{14}C-UBT, rapid urease test, and/ or immunohistochemistry. Before the ^{14}C-UBT examination, the subjects fasted for at least 6 h, usually overnight. Patients swallowed 37 kBq (1 μCi) of encapsulated ^{14}C urea/citric acid composition in 25 ml water. Breath samples of patients were collected into Heliprobe Breath Cards (Noster system) 10 min after administration of ^{14}C urea. Patients exhaled into the breath card until its colour indicator changed from orange to yellow. The breath samples were measured using the Heliprobe analyser (Noster system), and the activity was counted for 250 s. Results were expressed as counts per minute (cpm) and counts < 25 cpm were defined as Heliprobe 0 = not infected, counts between 25 cpm and 50 cpm as Heliprobe 1 = equivocal and counts > 50 cpm as Heliprobe 2 = infected [22].

The rapid urease test (Pronto Dry®, France) was also used to establish the diagnosis of *H. pylori* infection. The positive result is indicated by the colour changing of the indicator from amber to pink-red at room temperature within 24 hours. The yellow indicator colour is considered to be negative [23].

Immunohistochemical (IHC) staining for evaluation of *H. pylori* status carried-out with the procedure as follows [24]. Tissue sections were deparaffinized, rehydrated, and pretreated with Proteinase K for 8 min and incubated with ChemMate peroxidase blocking solution at room temperature for 10 min. The slides were subsequently incubated with the polyclonal rabbit anti- *H. pylori* primary antibody (B0471: Dako Corporation, Glostrup, Denmark) with a dilution of 1:50 was conducted at room temperature for 1 hour. After samples had been washed 3 times with phosphate-buffered saline, the Dako EnVision

Dual Link System–HRP (K4065: Dako Corporation) was applied for 30 minutes. Finally, sections were incubated in diaminobenzidine for 10 minutes, followed by hematoxylin counterstaining and mounting. *H. pylori*-infected gastric mucosa from chronic gastritis patients served as positive controls. Negative controls were obtained by replacing the primary antibody with phosphate-buffered saline. *H. pylori* infection in the tissue sections was confirmed when short, curved or spiral bacilli resting on the epithelial surface, in the mucus layer, or deep in the gastric pits could be observed by light microscopy.

Serum Levels of VEGF

Venous blood samples were drawn into serum separator tubes and allowed to clot for 30-45 minutes at room temperature, before being centrifuged for 15 minutes at approximately 1,000 g. Serum was immediately stored frozen in aliquots at -20^{o}C until assay for VEGF was performed. Circulating VEGF levels were examined in serum using the Quantikine Human VEGF-ELISA (Quantikine, R&D Systems, Inc., Minneapolis).

VEGF-634 G>C Polymorphism

Genomic DNA was extracted and purified from peripheral blood using the High Pure PCR Template Preparation Kit (Roche Applied Science) and stored until processed for genotyping. Analysis of the *VEGF* SNP *-634G>C* was performed using real-time polymerase chain reaction (RT-PCR). The PCR primers used for the *–634G>C* polymorphisms were 5'-CGACGGCTTGGGGAGATTGC-3' (forward) and 5'-GGGCGGTGTCTGTCTGTCTG-3' (reverse). The PCR cycle conditions consisted of an initial denaturation step at 94°C for 5 min, followed by 35 cycles of 30 s at 94°C, 30 s at 62°C, 30 s at 72°C and a final elongation at 72°C for 10 min.

Statistical Methods

Data analysis was performed through univariate and bivariate (Chi-Square test) analyses using SPSS 22nd version (SPSS Inc., Chicago). A value of $P < 0.05$ with a 95% confidence interval was considered statistically significant.

Results

Baseline and clinical characteristics of subjects

A total number of 87 *H. pylori* gastritis patients were included in this study. The diagnosis of *H. pylori* infection was made using positive results of ^{14}C-UBT, rapid urease test, and/ or IHC. Figure 1 shows *H. pylori* in the gaster tissues by IHC.

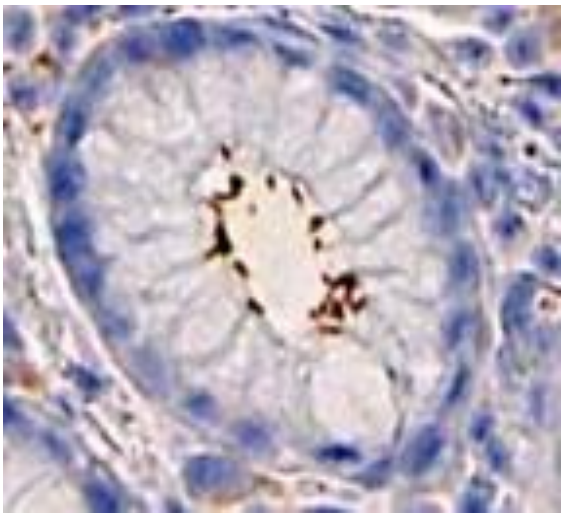

Figure 1: H. pylori in the gaster tissues by immunohistochemistry (IHC)

Patients' characteristics show a majority of males (63.2%) with a mean age of 50.8 years, with mostly Batak ethnicity (52.9%). There were 46 patients (52.9%) having *VEGF-634G>C* polymorphism *GC* genotype, followed by 24 patients (27.6%) *GG* genotype and 17 patients (19.5%) CC genotype. The median value of VEGF levels was 441.7 pg/mL with a minimum level of 80.7 pg/mL and a maximum level of 2185.2 pg/mL. We grouped VEGF levels into 2 categories based on the median value, ≥ 442 and <442. As many as 48.3% of patients had VEGF ≥442 pg/mL, 51.7% of patients had VEGF <442 pg/mL. There were 26 patients (29.9%) with premalignant gastric lesions (Table 1).

Table 1: Baseline and clinical characteristics of subjects

Characteristics	n (%)
Gender	
Male	55 (63.2%)
Female	32 (36.8%)
Age, mean ± SD (years)	50.8 ± 12.2
≥51 years	58 (66.7%)
<51 years	29 (33.3%)
Ethnicity	
Batak	46 (52.9%)
Javanese	20 (23%)
Aceh	12 (13.8%)
Malay	9 (10.3%)
Education	
Elementary school	7 (8%)
Middle school	12 (13.8%)
High school	57 (65.5%)
University	11 (12.6%)
BMI, mean ± SD (kg/m^2)	22.2 ± 3.8
Overweight	39 (44.8%)
Not overweight	48 (55.2%)
Gastric Premalignant Lesion	
Yes	26 (29.9%)
No	61 (70.1%)
VEGF-634 G>C polymorphism	
GG genotype	24 (27.6%)
GC genotype	46 (52.9%)
CC genotype	17 (19.5%)
VEGF level, median (min-max) (pg/ml)	441.7 (80.7 – 2185.2)
High (≥ 442)	42 (48.3%)
Low (< 442)	45 (51.7%)

Association between patient's characteristics and VEGF levels with premalignant gastric lesions

There was a significant association between age and gastric premalignant lesions (P = 0.020), patients ≥ 51 years were at risk of 2.75 times to get premalignant gastric lesions compared to age < 51 years. There was a significant association between ethnic group and gastric premalignant lesions (P = 0.016), where the Batak ethnic group had a 2.97 times risk of having premalignant gastric lesions compared to non-Bataks. There was a significant association between VEGF levels and premalignant gastric lesions (P <0.001), where patients with high VEGF levels had a 12.86 times risk of having gastric premalignant lesions compared to patients with low VEGF levels. There were no associations between gender, education, and overweight with premalignant gastric lesions (P > 0.05) (Table 2).

Table 2: Association between patients' characteristic and VEGF level with premalignant gastric lesions

Variables	Gastric premalignant lesions		Total	p	OR (95% CI)
	Present	Absent			
Gender					
Male	14 (25.5%)	41 (74.5%)	55 (100%)	0.237	0.68 (0.36-1.28)
Female	12 (37.5%)	20 (62.5%)	32 (100%)		
Age					
≥51 years	22 (37.9%)	36 (62.1%)	58 (100%)	0.020*	2.75 (1.05-7.24)
<51 years	4 (13.8%)	25 (86.2%)	29 (100%)		
Education					
Low level	7 (36.8%)	12 (63.2%)	19 (100%)	0.454	1.32 (0.65-2.66)
High level	19 (27.9%)	49 (72.1%)	68 (100%)		
Ethnic group					
Batak	20 (43.5%)	26 (56.5%)	46 (100%)	0.016*	2.97 (1.32-6.67)
Non-Batak	6 (14.6%)	35 (85.4%)	41 (100%)		
Overweight					
Present	10 (2.6%)	29 (74.4%)	39 (100%)	0.436	0.77 (0.4-1.5)
Absent	16 (33.3%)	32 (66.7%)	48 (100%)		
VEGF level					
High	24 (57.1%)	18 (42.9%)	42 (100%)	<0.001*	12.86 (3.24-51.1)
Low	2 (4.4%)	43 (95.6%)	45 (100%)		

*P < 0.05; Low level of education : elementary school + middle school; High level of education: high school + university.

Association between VEGF-634G>C polymorphism and premalignant gastric lesions

There was a significant association between *VEGF-634G>C* polymorphism and premalignant gastric lesions. *GG* genotype increased the risk of 7.08 times for premalignant gastric lesions compared to *CC* genotype (P = 0.014). *GC* genotype increased the risk of 5.54 times for premalignant gastric lesions compared to *CC* genotype (P = 0.048). *GG* + *GC* genotype was 6.07 times more likely to have premalignant gastric lesions than patients with *CC* genotypes (P = 0.003). Patients with the *G* allele were at risk of 1.75 times for premalignant gastric lesions compared to *C* allele (p = 0.022) (Table 3).

Table 3: Association between *VEGF-634G>C* polymorphism and premalignant gastric lesions

VEGF-634G>C Polymorphism	Gastric premalignant lesion		Total	p	OR (95% CI)
	Present	Absent			
GG	10 (41.7%)	14 (58.3%)	24 (100%)	0.014*	7.08 (1.2-50.26)
GC	15 (32.6%)	31 (67.4%)	46 (100%)	0.048*	5.54 (1.79-38.82)
CC	1 (5.9%)	16 (94.1%)	17 (100%)		1 (ref.)
GG+GC	25 (35.7%)	45 (64.3%)	70 (100%)	0.003*	6.07 (1.88-41.71)
CC	1 (5.9%)	16 (94.1%)	17 (100%)		1 (ref.)
Allele *G*	35 (37.2%)	59 (62.8%)	94 (100%)	0.022*	1.75 (1.07 – 2.88)
Allele *C*	17 (21.3%)	63 (78.8%)	80 (100%)		1 (ref.)

*P <0.05.

Association between VEGF-634G>C polymorphism and serum VEGF levels

Further analysis was done to evaluate the association between *VEGF-634G>C* polymorphism and serum levels of VEGF. *GG* genotype increased the risk of 3.54 times to have high VEGF levels compared to *CC* genotype (P = 0.004). GC genotype increased the risk of 2.96 times to have high VEGF levels compared to *CC* genotype (P = 0.014). *GG* + *GC* genotype was 3.16 times more likely to have high VEGF levels than patients with *CC* genotypes (P = 0.005). Patients with *G* allele were at risk 1.53 times to have high VEGF levels compared to *C* allele (P = 0.009) (Table 4).

Table 4: Association between VEGF -634 G>C polymorphism and the level of serum VEGF

VEGF-634G>C Polymorphism	VEGF Level		Total	P	OR (95% CI)
	High	Low			
GG	15 (62.5%)	9 (37.5%)	24 (100%)	0.004*	3.54 (1.21-10.35)
GC	24 (52.2%)	22 (47.8%)	46 (100%)	0.014*	2.96 (1.02-8.56)
CC	3 (17.6%)	14 (82.4%)	17 (100%)		1 (ref.)
GG+GC	39 (55.7%)	31 (44.3%)	70 (100%)	0.005*	3.16 (1.11-9)
CC	3 (17.6%)	14 (82.4%)	17 (100%)		1 (ref.)
Allele G	54 (57.4%)	40 (42.6%)	94 (100%)	0.009*	1.53 (1.1 – 2.13)
Allele C	30 (37.5%)	50 (62.5%)	80 (100%)		1 (ref.)

*P < 0.05.

Discussion

H. pylori is a type 1 carcinogen according to the International Agency for Research on Cancer (IARC). It promotes malignant changes through triggering an inflammatory cascade, including neutrophil and monocyte recruitment and upregulation of proinflammatory cytokines destroying the gastric mucosa. Epidemiological studies have shown an association between chronic inflammation and malignant transformation. *H. pylori* may also play a role in angiogenesis, essential for malignant cells survival. Among angiogenic factors, VEGF is known to be the most potent stimulus for neoangiogenesis [3]. This VEGF has a mitogenic effect on endothelial cells, promotes tumour cell growth, stimulates cell migration, and metastasis from the primary site [25].

The previous meta-analysis by Chen et al. reported higher VEGF expression in Asian patients with gastric cancer compared to controls (OR=

112.41, 95% CI= 64.12 – 197.06). High expression of VEGF also showed poor 5-year survival rates (RR= 2.45, P = 0.000), indicating its potential use as a marker for gastric cancer prognosis [26]. In gastric adenocarcinoma, a higher level of VEGF was also observed along with the increased density of intratumour small vessels. Moreover, higher expression of VEGF was also seen in gastric premalignant lesions such as chronic atrophic gastritis, intestinal metaplasia, and gastric dysplasia; as well as gastric carcinoma [15] [27] [28]. Raica et al also reported there was an association between increased levels of VEGF expression with gastric carcinogenesis [28]. These results are consistent with this study, that there was a significant association between high VEGF levels and premalignant gastric lesions (P <0.001).

Several SNP on the VEGF gene is thought to affect its expression. Certain allele variation may lead to overexpression of the transcription factor that will bind to the promoter site, which serves as the initial RNA polymerase binding site that will initiate transcription [29]. A previous study by Oh et al. evaluated 190 gastric cancer patients in Korea reported that the GG genotype of *VEGF-634G>C* polymorphism was associated with higher VEGF serum levels as well as poor outcome in patients with advanced stage [21]. Similar with that study, this present study also found a significant association between *VEGF-634G>C* polymorphism and premalignant gastric lesions, in particular, those with the *GG+GC* genotype had an increased risk of 3.16 fold to have high VEGF levels compared to those with the CC genotype.

A gene polymorphism was not only related to an increase or decrease in serum levels, but also with susceptibility to certain diseases [16] [30]. A study in Texas by Guan et al. included 171 gastric cancer patients (vs 353 controls) found that *VEGF-634CG+CC* showed an increased risk of gastric cancer compared to *-634GG* genotype [18]. Patients with VEGF heterozygous *-634GC* showed a poorer 1-year survival compared to patients with *VEGF-634GG* genotype [31]. Similar findings were reported by Tzanakis et al. (2006) in Greece that evaluated 100 gastric cancer patients. Tzanakis et al. found the *VEGF-634CC* genotype was associated with increased risk of gastric cancer and lower survival rates [19]. Meanwhile, a study by Chae et al. (2006) in Korea on 413 gastric cancer patients and 413 subjects as controls showed that *VEGF-634CC* was significantly associated with a lower risk of gastric cancer. *VEGF-634 C* allele was associated with a significant reduction in gastric cancer susceptibility (OR 0.686; 95% CI 0.564-0.834) [20]. Another study in Oman by Al-Moundhri (2009) found a significant association between *VEGF-634 CC* genotype with poor tumour differentiation and lymph nodes metastasis [32]. There were still limited studies that evaluate the relationship of *VEGF* polymorphism with premalignant gastric lesions. Tahara et al. reported that *1612G>A* polymorphism of the *VEGF* gene was associated with premalignant gastric lesions in older individuals. The *1612 GA* genotype showed a significantly higher incidence of intestinal metaplasia in *H. pylori*-positive individuals whose age was more than 65 years old [13]. This current study showed that *VEGF-634G>C* polymorphism was associated with premalignant gastric lesions, where *-634GG+GC* genotype increased the risk of 6.07 times for premalignant gastric lesions, presumably due to elevated serum *VEGF* levels in those genotypes.

These study indicated that older age was associated with an increased risk of premalignant gastric lesions. Previous studies reported a similar result [33] [34]. Benberin et al. showed the prevalence of premalignant gastric lesions increased with age. This condition was rarely seen in individuals under the age of 40 years [35]. The majority ethnic group in this study were Bataks (52,9%) because Bataks inhabit most of the North Sumatera region. There was a significant association between ethnicity and premalignant gastric lesions. Batak ethnic group increased the risk of 2.97 times for premalignant gastric lesions (P = 0.016). Further study is required to evaluate the high prevalence of premalignant gastric lesions in Bataks. Bataks have a habit of consuming alcohol both in a traditional ceremony and daily life [36]. Background of genetic factors, nutritional factors or lifestyle, immune responses to *H. pylori* infection may be considered. Ethnic differences may influence the risk of premalignant gastric lesions, which may be due to genetic variation [37].

In conclusion, the polymorphism of VEGF-634 G>C, in particular, GG+GC genotype, was associated with an increased risk of premalignant gastric lesions and high serum levels of VEGF in patients with *H. pylori* gastritis in Medan, Indonesia. There was a significant association between age, ethnic, and VEGF levels with premalignant gastric lesions.

Acknowledgements

We would like to express our gratitude to Lidya Imelda Laksmi who has conducted the histopathological analysis in this study, as well as to Khairani, Sulasmi, Tiolan, Tiambun who have assisted the endoscopy procedure in all patients.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1335-1341.
https://doi.org/10.3889/oamjms.2018.246
eISSN: 1857-9655
Basic Science

Detection of Biofilm Producing Staphylococci among Different Clinical Isolates and Relation to Methicillin Susceptibility

Rania M. Abdel Halim*, Nevine N. Kassem, Basma S. Mahmoud

Clinical Pathology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt

Citation: Abdel Halim RM, Kassem NN, Mahmoud BS. Detection of Biofilm Producing Staphylococci among Different Clinical Isolates and Relation to Methicillin Susceptibility. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1335-1341. https://doi.org/10.3889/oamjms.2018.246

Keywords: Biofilm; Staphylococci; Methicillin; TCP; TM; CRA

***Correspondence:** Rania M Abdel Halim. Clinical Pathology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt. E-mail: rona169@yahoo.com

Received: 31-Dec-2017; **Revised:** 12-Feb-2018; **Accepted:** 25-Jun-2018; **Online first:** 05-Aug-2018

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

AIMS: To evaluate three in vitro phenotypic methods; tissue culture plate, tube method, and Congo red agar for detection of biofilm formation in staphylococci and assess the relation of biofilm formation with methicillin resistance and anti-microbial resistance.

METHODS: The study included 150 staphylococcal isolates. Biofilm detection in staphylococci was performed using tissue culture plate, tube method, and Congo red agar.

RESULTS: Tissue culture plate, tube method, and Congo red agar detected 74%, 42.7%, and 1.3% biofilm producing staphylococci respectively. *S. aureus* isolates were more common biofilm producers (53.2%) than CONS (46.8%). Biofilm production in CONS species was highest in *S. hemolyticus* (57.7%). Tube method was 51.4% sensitive, 82.1% specific. As for Congo red agar, sensitivity was very low (0.9%), but specificity was 97.4%. Biofilm producers were mostly; isolated from blood specimens (82.6%) and detected in methicillin-resistant strains 96/111 (86.5%). They were resistant to most antibiotics except vancomycin and linezolid.

CONCLUSIONS: Tissue culture plate is a more quantitative and reliable method for detection of biofilm producing staphylococci compared to tube method and Congo red agar. Hence, it can still be used as a screening method for biofilm detection. Vancomycin and Linezolid are the most sensitive antibiotics among biofilm producing staphylococci.

Introduction

Staphylococcus aureus is a virulent organism that is resistant to most of the conventionally available antibiotics. This is attributed to the fact that they are capable of biofilms formation [1]. Biofilm consists of multilayered cell clusters embedded in a matrix of extracellular polysaccharide, which facilitate the adherence of microorganism [2]. The interior of the bacterial biofilms presents greater resistance to the opsonisation by antibodies and phagocytosis. This explains the chronic character of these infections such as endocarditis, osteomyelitis and especially those infections associated with implanted medical devices that are difficult to be treated [1].

Coagulase-negative staphylococci especially *S. epidermidis* is the most frequent cause of hospital-acquired infections. Most *S. epidermidis* infections are subacute or chronic and occur mainly in immunocompromised individuals or patients with indwelling medical devices. Biofilm formation on the surface of indwelling devices is often involved in the pathogenesis [3]. Biofilms can resist antibiotic concentration 10-10,000 folds higher than those required to inhibit the growth of free-floating Staphylococci. Biofilm producing staphylococci have also been isolated from various clinical samples like blood, urine, pus, skin surface etc. The differentiation of staphylococci concerning its biofilm phenotype might help in their diagnosis and thereby, prevention of infections [4]. Biofilm is an increasing cause of morbidity and mortality associated with chronic and nosocomial

infections, so a greater understanding of the nature of intracellular bacterial communities in infections, their early detection and management will aid in the development of new and more effective treatments [5]. A number of tests are available to detect slime production by staphylococci; which include quantitative methods such as tissue culture plate (TCP), which is considered as the gold-standard method for biofilm detection [6], and qualitative methods such as tube method (TM) [7], and Congo red agar (CRA) [8].

Materials and Methods

This study was conducted on 150 staphylococcal isolates randomly selected from different clinical specimens submitted to the Microbiology Laboratory of Ain Shams University Hospitals. They were isolated from different specimens; 30 pus, 46 blood, nine (9) wound, 15 urine, 22 sputa, 17 central line, five body fluids and six others (two ear swabs, two throat swabs, one bile drain and one radivac). All the isolates were identified morphologically by Gram stain, colonial morphology on culture, catalase test to differentiate it from Streptococcus species and DNase test to differentiate *S. aureus* from coagulase- negative staphylococci (CONS). Identification of CONS species and antibiotic susceptibility testing for all isolates were made using a n automated identification system (Vitek 2, bioMérieux, France) according to CLSI guidelines 2015 [9].

Biofilm detection was performed using TCP [6], TM [7] and CRA [8]. *S. aureus* (ATCC 25923) was used as negative control.

Tissue culture plate method was performed as described by Christensen et al., 1985 [6] for quantitative measurement of biofilm production in Staphylococcus spp. Using a microtiter assay. A single colony from each subcultured plate on blood agar was inoculated in a glass tube containing two ml TSBglu. The tubes were incubated overnight at 36°C ± 1 under aerobic conditions. Two hundred microlitres from each of the inoculated TSBglu tubes were aseptically transferred in the wells of a flat-bottomed microwell plastic plate. The inoculated microwell plastic plate was incubated overnight at 36°C ± 1 without sealing of the plate for proper oxygenation. Next day, the contents were discarded by inverting the plate and striking it on filter paper. The microwell plastic plate was washed once by adding 200 µl PBS (pH 7.2) into each well and then discarded. Then 200ul of freshly prepared sodium acetate (2%) was added to each well (for biofilm fixation) for 10 minutes and then discarded. This was followed by adding 200 µl crystal violet (0.1%) to each well for biofilm staining. The Plates was kept at room temperature for 30 minutes, and then the stain was discarded. The washing step was repeated once more. Finally, the plate was left to dry at room temperature for one hour, after which, the absorbance was read on a spectrophotometer at 620 nm OD (Figure 1).

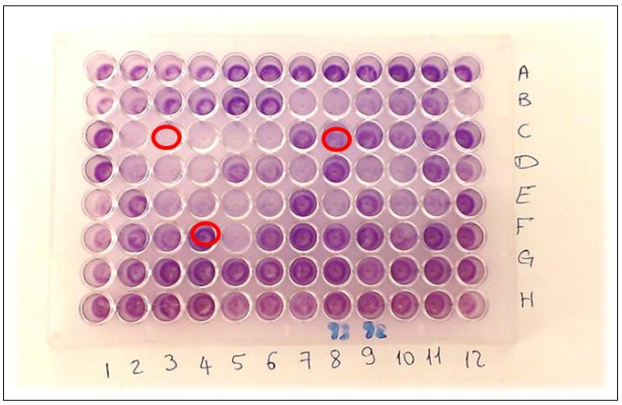

Figure 1: Tissue culture plate showing different biofilm intensities; C3: Non-biofilm producer; C8: moderate biofilm producer; F4: strong biofilm producer

The optical density (OD) value of each isolate was interpreted according to the following table to assess the degree of the biofilm (Table 1).

Table 1: Interpretation of results of Tissue Culture Plate method

OD Value	Biofilm Formation
<0.120	Non-biofilm producer
0.120-0.240	Moderate biofilm producer
>0.240	Strong biofilm producer

Tube method was done as described by Christensen et al., 1982 [7] for qualitative assessment of biofilm production. A loopful inoculum was inoculated on 10 ml TSBglu in plastic tubes. Tubes were incubated aerobically at 36°C ± 1 for 24 hours. Tubes content was discarded, and tubes were washed once with 9 ml phosphate buffer saline pH 7.2 and then discarded. For biofilm fixation, 10 ml of freshly prepared sodium acetate (2%) was added to each tube for 10 minutes and then discarded. For biofilm staining, 10 ml crystal violet (0.1%) was then added to each tube, and tubes were left at room temperature for 30 minutes after which the stain was discarded. The washing step was repeated, and tubes were left to dry in an inverted position at room temperature. Biofilm formation was detected by the presence of visible film on the wall and bottom of the tube. The amount of biofilm formation was interpreted according

to the results of the control strain and graded visually as absent, moderate and strong biofilm formation respectively (Figure 2).

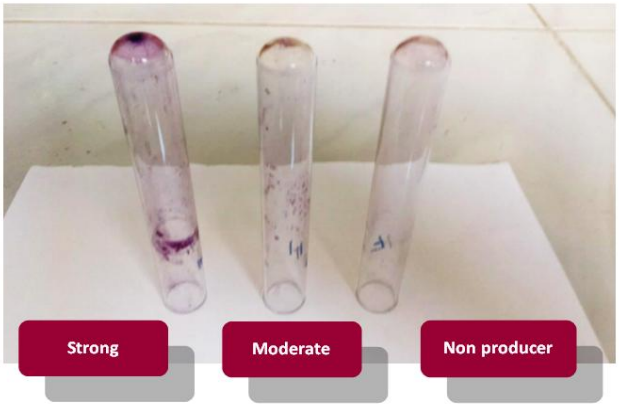

Figure 2: Tube method with different degrees of biofilm formation

The Congo red method was done as described by Freeman et al., 1989 [8] for qualitative assessment of biofilm production. Congo red stain (Research lab fine chem. Industries, India) was prepared as a concentrated aqueous solution of 0.8 g/200 ml distilled water and autoclaved separately from other medium constituents. The dye directly interacts with certain polysaccharides in the biofilm forming coloured complexes. Brain heart infusion agar (37 g) and sucrose (50 g) were dissolved in 800ml distilled water and autoclaved. Congo red stain (200ml) was then added when the agar cooled to 55˚C. Staphylococcal strains were inoculated on the prepared media and incubated aerobically at 37°C for 24 hours. Black colonies with dry crystalline consistency indicate strong biofilm formation. Red colonies with occasional darkening at the centre of the colonies were considered non-biofilm producers (Figure 3).

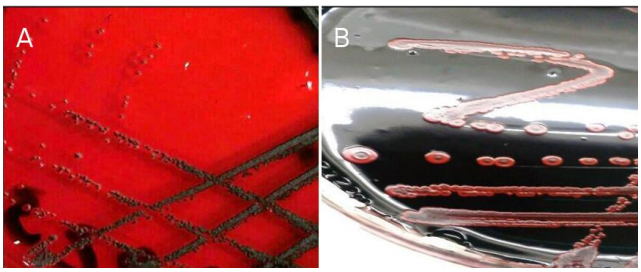

Figure 3: A) CRA showing black colonies with dry crystalline consistency (positive for biofilm formation); B) CRA showing red colonies with darkening at the centre (negative for biofilm formation)

Results

In this study, 150 clinical isolates of staphylococci were isolated; 78 (52%) were *S. aureus,* and 72 (48%) were CONS. Identification of CONS by Vitek 2 system revealed 41 *S. hemolyticus,* 18 *S. epidermidis,* 11 *S. hominis,* one *S. simulans* and one *S. warneri.* Biofilm detection in staphylococci was performed using TCP method, TM and CRA method.

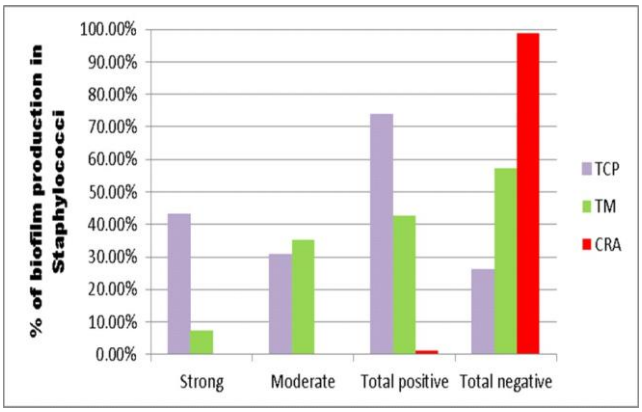

Figure 4: Comparison between TCP, TM and CRA as regards detection of biofilm formation in Staphylococci

The TCP method detected total positive biofilm production in 111 (74%) staphylococcal isolates, t h e strong positive was 65 (43.3%), and moderate positive were 46 (30.7%). As for the TM method, total positive biofilm production was 64 (42.7%), the strong positive was 11 (7.3%), and 53 (35.3%) were moderately positive. CRA method detected biofilm production only in 2 isolates (1.3%) (Figure 4).

Table 2: Comparison between TM and TCP as regards biofilm production

		TCP					
		Non-producers	Moderate producers	Strong producers	Total Staph. isolates	P value	Chi-square
TM	Non-producers	32	29	25	86	<0.001	20.445
		82.1%	63.0%	38.5%	57.3%		
	Moderate producers	6	13	34	53		
		15.4%	28.3%	52.3%	35.3%		
	Strong producers	1	4	6	11		
		2.6%	8.7%	9.2%	7.3%		
Total Staph. isolates		39	46	65	150		

Table 2 shows a comparison between the results of biofilm production by TM and TCP, using the Chi- square test. TM is considered a highly significant test, P value (< 0.001). As for CRA, it detected a very low number of biofilm producers (two) compared to total positive biofilm producers by TCP (111), according to the P value (0.579) CRA method is considered the non-significant test.

In our study, Sixty- eight out of 78 (87.1%) of *S. aureus* were MRSA, and 63 out of 72 (87.5%) of CONS were methicillin resistant. (Table 3) Shows detection of biofilm formation in staphylococci in relation to methicillin susceptibility. By TCP method, biofilm

production was detected in 73.3% of MRS (75% of MRSA, 71.4% of MRCONS) and 78.9% of MSS were biofilm producers. The 65 strong biofilm producers were; 30 (46.1%) MRSA, 6 (9.2%) MSSA, 24 (36.9%) MRCONS and 5 (7.7%) MSCONS. The 46 moderate biofilm producers were; 21 MRSA, 2 MSSA, 21 MRCONS and 2 MSCONS). As for TM, the 11 strong biofilm producers were 6(54.5%) MRSA and 5 (45.5%) MRCONS. By TM, The 53 moderate biofilm producers were 28 S. aureus [21 (39.6%) MRSA, 7(13.2%) MSSA], 25 CONS [22] (41.5%) MRCONS, 3 (5.7%) MSCONS]. Congo red agar method detected only two MRCONS biofilm producers. Biofilm producers were mostly detected in methicillin resistant strains [96/111(86.5%)]

Table 3: Detection of biofilm formation in staphylococci in relation to methicillin susceptibility

	S. aureus (no 78)		CoNS (no 72)				
	MRSA 68	MSSA 10	MRCoNS 63	MSCoNS 9	MRS 131	MSS 19	Total staphylococci 150
Tissue culture plate method (strong)	30	6	24	5	54	11	65
%	46.2%	9.2%	36.9%	7.7%	36%	7.3%	43.3%
Tissue culture plate method (moderate)	21	2	21	2	42	4	46
%	45.7%	4.3%	45.7%	4.3%	28%	2.6%	30.7%
Tissue culture plate method (negative)	17	2	18	2	35	4	39
%	43.6%	5.1%	46.2%	5.1%	23.3 %	2.6%	26%
Total positive	51	8	45	7	96	15	111
%	45.9%	7.2%	40.5%	6.3%	64%	10%	74%
Tube method (strong)	6	0	5	0	11	0	11
%	54.5%	0%	45.5%	0%	7.3%	0%	7.3%
Tube method (moderate)	21	7	22	3	43	10	53
%	39.6%	13.2%	41.5%	5.7%	28.6 %	6.7%	35.3%
Tube method (negative)	41	3	36	6	77	9	86
Total positive	27	7	27	3	54	10	64
%	42.2%	10.9%	42.2%	4.7%	36.6 %	6.7%	42.7%

MRSA: Methicillin-Resistant Staphylococcus aureus; MSSA: Methicillin-Sensitive Staphylococcus aureus; MRCONS: Methicillin-Resistant Coagulase negative Staphylococci; MSCONS: Methicillin Sensitive Coagulase negative Staphylococci; MRS: Methicillin-Resistant Staphylococci; MSS: Methicillin Sensitive Staphylococci.

Table 4 shows biofilm production in different CONS species. By TCP, biofilm production in CONS species was highest in *S. hemolyticus* (57.7%), followed by *S. epidermidis* (21.2%) and then *S. hominis* (19.2%). By TM, biofilm production in CONS species was highest in *S. hemolyticus* (46.7%), followed by *S. epidermidis* (33.3%) and S. hominis (20%). Whereas, Only two *S. epidermidis* isolates were biofilm producers by CRA method.

Biofilm production in staphylococci among various clinical specimens as detected by TCP method showed that the highest percentage were isolated from Blood cultures (82.6%) followed by urine (80%) and body fluids (80%) (Figure 5).

Comparative analytical study of TM and CRA methods about TCP method which is considered as the standard gold test showed that tube method was 51.4% sensitive, 82.1% specific for biofilm detection, PPV and NPV were 89% and 37.2% respectively.

Table 4: Biofilm production in CONS species

	CONS species				
	S. hemolyticus	*S. epidermidis*	*S. hominis*	*S. simulans*	*S. warneri*
Biofilm producers by TCP (111)	30	11	10	0	1
% from total biofilm producers	57.7%	21.2%	19.2%	0%	1.9%
Biofilm producers by TM (64)	14	10	6	0	0
% from total biofilm producers	46.7%	33.3%	20%	0%	0%

As for congo red agar method, sensitivity was very low (0.9%), but specificity was 97.4% for biofilm detection, PPV and NPV were 50% and 25.7% respectively.

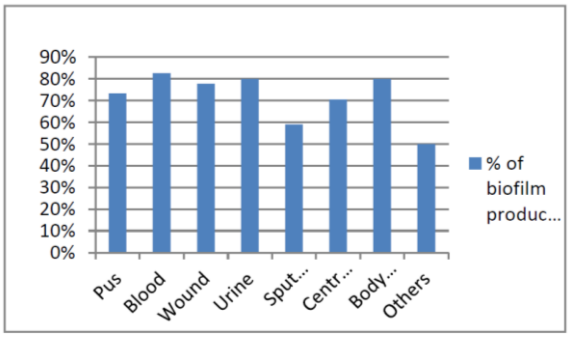

Figure 5: Biofilm production in staphylococci isolated from various clinical specimens (TCP method)

Biofilm producing strains were more resistant to almost all the classes of antibiotics showing resistance to Cefoxitin in 87.4% of staphylococci, Levofloxacin 57.7%, Gentamycin 53.2%, Clindamycin 60.4%, Erythromycin 69.5%, Doxycycline 40.5%, and Linezolid 3.6%. Biofilm non-producers were comparatively less resistant; Cefoxitin resistance was detected in 87.2% of staphylococci, Levofloxacin 43.6%, Gentamycin 23.6%, Clindamycin 53.8%, Erythromycin 61.5%, Doxycycline 18% and Linezolid 1.3%. All isolates were sensitive to vancomycin.

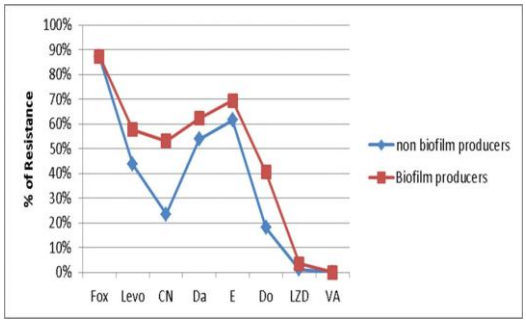

Figure 6: Antibiotic resistance pattern of Biofilm producers and non-producers staphylococci; Fox: Cefoxitin; LZD: Linezolid; levo: Levofloxacin; CN: Gentamycin; Da: Clindamycin; E: Erythromycin; Do: Doxycycline; VA: Vancomycin

Figure 6 shows the antibiotic resistance pattern of Biofilm producers and non- producers as detected by the TCP method.

Discussion

Biofilm formation is considered an important cause of all staphylococcal species associated with the infection of biomedical devices. Biofilm producing staphylococci isolated from other clinical samples are also of clinical significance as biofilm constitutes a reservoir of pathogens and are associated with resistance to antimicrobial agents and chronic infections. So, a reliable and easy method for their diagnosis is necessary [10].

Our study tested 150 clinical isolates of staphylococci by three in-vitro screening procedures for their ability to form a biofilm. TCP method is the standard gold method as reported by Mathur et al., 2006 [11], hence it was considered a standard method for interpretation of our results. Biofilm producing staphylococcal isolates were 74% when TCP was performed (43.3% strong producers and 30.7% moderate producers). This method gave the best discrimination between strong, moderate and non-production of biofilm as it used cut off values. Results of our study were higher than other studies [11] [12] [13] [14] [15] [16] who reported 53.9%, 54.2%, 21.8%, 46%, 43.3%, and 39.7% of staphylococci as biofilm producers respectively.

We detected biofilm production in 53.2% of *S. aureus* and 46.8% of CONS by TCP method. Fatima et al., 2011 [17] also reported a high percentage of *S. aureus* as biofilm producers (64.89%). However, Akinkunmi and Lamikanra 2012 [13] reported 36% of *S. aureus* and 32.9% of CONS as biofilm producers and Ramakrishna et al., 2014 [10] reported 38% of *S. aureus* while 84% of CONS as biofilm producers. This might be attributed to the difference in the sources from which their strains were isolated.

Our results revealed that *S. hemolyticus* was the most frequently isolated species among CONS. However, Oliveira and Cunha, 2010 [18] reported *S. epidermidis* as the most frequently isolated species among CONS. This could be explained by the difference in the type of specimens selected; where half of the specimens collected in their study were catheter tips from which *S. epidermidis* is usually isolated (their study included 100 specimens, 50 catheter tips, 30 blood cultures and 20 nasal swabs).

We detected a high percentage of biofilm production in staphylococci isolated from blood culture specimens (82.6%). On the other hand, Sharvari and Chitra, 2012 [15] found a very high incidence of biofilm production in staphylococcal isolates from patients with artificial devices (89.5%), whereas, biofilm production in staphylococcal isolates from blood culture specimens were 45.9%. Also, Oliveira and Cunha, 2010 [18] detected 54.3% and 28.4% biofilm producing staphylococcal from catheter tips whereas and blood culture specimens respectively.

However, our sputum samples gave the least percentage of biofilm production (59.1%). Sharvari and Chitra, 2012 [15] also detected the least biofilm producing specimens among their sputum samples (26.3%).

Biofilm producers were mostly detected in methicillin resistant strains 96/111 (86.5%). This is discordant with O'neill et al. 2007 [19] who reported biofilm production in 74% among MRSA and 84% among MSSA isolate.

They stated that the significant association between methicillin susceptibility in *S. aureus* and ica-dependent biofilm formation was first reported when PIA production was found to be essential for biofilm formation by MSSA but not MRSA. Furthermore, MSSA biofilms are significantly induced in growth media supplemented with NaCl, which is known to activate ica operon expression.

However, this was not the case in Sharvari and Chitra, 2012 [15] who reported biofilm production in 72.3% of methicillin- resistant and 30.3% methicillin sensitive staphylococci (80.8% of MRSA, 31.6 of MSSA, 60% of MRCONS and 28% of MSCONS). Also, Rewatkar and Wadher 2013 [20] reported biofilm production in 85% among MRSA and 15% among MSSA isolates. Eiichi et al., 2004 [21] found a very high percentage (95.4%) of biofilm production in MRSA and Fatima et al., 2011 [17] reported 87.6% of MRSA as biofilm producers.

In our work, Tube method detected less number of biofilm producers, 42.7% which was lower compared to the TCP method. This difference may be due to the inter-observer variability in the reading of results, also may be due to performing the test using plastic tubes instead of glass, hindering visual interpretation. This was concordant with Saha et al., 2014 [22] where the TCP method detected 69% of biofilm producers, whereas, TM detected only 36%. They further stated that this method could discriminate between strong and moderate biofilm producers. However, the interpretation is observer dependent and there are chances of subjective errors. Our results were nearly similar to Mathur et al., 2006 [11] and Umadevi and Sailaja 2014 [23] who reported 41.4% and 42.5% as biofilm producers by TM respectively. On the other

hand, Oliveira and Cunha, 2010 [18] and Reddy 2017 [24] reported a higher percentage; 82% and 63% biofilm producers by TM respectively.

Congo red agar method was found to be easier and faster to perform than other phenotypic methods, but it only detected 1.3% of biofilm producing staphylococci in our study. Knobloch et al., 2002 [25], Mathur et al. 2006 [11], and Taj et al., 2012 [26] also reported very low percentage of positive biofilm producers by CRA method, 3.8%, 5.3%, and 3.4% respectively. The low percentage of positive results by CRA in our work might be attributed to the technique of preparation of the CRA, where congo red stain was autoclaved before being added to the agar. On the contrary, the study performed by Sharvari and Chitra, 2012 [15] gave higher results (25.3%). This could be attributed to congo red stain being prepared separately without autoclaving in sterile distilled water and then added to sterile molten autoclaved agar. Also, modified CRA method as described by Kaiser et al., 2013 [27] can be used instead to increase capacity of biofilm detection (the formula included BHIA with sucrose (5%), Congo red (0.08%), NaCl (1.5%), glucose (2%), and vancomycin (0.5 mg/mL). According to their study, this formula showed a high percentage of correlation among biofilm production in *S. epidermidis* and the presence of the *icaAB* gene (82.9%). The addition of vancomycin at a sub-MIC concentration (0.5 µg/mL) to modified CRA led to phenotype change in 64.8% of their strains, all of which were classified as a non-biofilm producer by the original CRA method and presenting the *icaAB* genes. The presence of a minimum concentration of vancomycin probably acts as a stress factor against the bacterial cells, which may lead to some alterations such as cell wall thickening [28] and may induce an increased expression of genes related to biofilm formation [29] [30].

Mathur et al., 2006 [11] recommended performing CRA method from *S. epidermidis* strains freshly isolated from clinical specimens of patients when the strains still retain their virulence characteristics expressed in “in vivo” conditions.

Also, some studies revealed higher results than ours as [31] [18] [20] who reported 83%, 73% and 90% of positive biofilm producers respectively. Cafiso et al., 2004 [31] explained the detection of a high percentage of biofilm producers (83%) by CRA by the addition of glucose 1%w/v in the congo red medium enhancing the production of biofilm in almost all isolates, where two is-positive non-biofilm producers by TCP became producers in CRA.

In the present study, the sensitivity of tube method was 51.4%, and specificity was 82.1% for biofilm detection which was higher than Saha et al., 2014 [22] who reported 34.21% sensitivity and 58.82% specificity for biofilm detection. However, Mathur et al., 2006 [11] and Bose et al., 2009 [12] reported higher sensitivity and specificity 73.6%, 76.3%, 92.6% and 97.6% respectively.

On the other hand, we report very low sensitivity for congo red agar method (0.9%) but higher specificity (97.4%) for biofilm detection. Also, (Mathur et al., 2006 [11], Bose et al., 2009 [12], and Saha et al., 2014 [22] reported 6.8%, 8.3% and and 21.1% sensitivity, 90.2%, 96.3% and 58.8% specificity respectively. Our results were discordant with the findings of Oliveira and Cunha, 2010 [18] who reported higher sensitivity 89% and specificity 100% for biofilm detection.

Biofilm producing strains in our work were resistant to almost all groups of antibiotics. Among our isolates, 87.4% were resistant to Cefoxitin, Levofloxacin (57.7%), Gentamicin (53.2%), Clindamycin (60.4%), Erythromycin (69.5%) and Doxycycline (40.5%) which was lower compared to biofilm non-producing strains where resistance to Cefoxitin was 87.2%, Levofloxacin 53.6%, Gentamycin 23.6%, Clindamycin %, Erythromycin 61.5%, and Doxycycline 18%. This is concordant with Sharvari and Chitra, 2012 [15], Ramakrishna et al., 2014 [10] and Singh et al., 2017 [32] who found that staphylococci biofilm producers were more resistant to commonly used antibiotics.

In our study, all the strains were sensitive to vancomycin (100%) while only (3.6%) were resistant to linezolid. This is concordant with Sharvari and Chitra, 2012 [15] who reported 100% of their isolates sensitive to vancomycin and (4.1%) resistant to linezolid. However, in Ramakrishna et al., 2014 [10] and Hashem et al., 2017 [33] studies all the strains were sensitive to both linezolid and vancomycin.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1342-1345.
https://doi.org/10.3889/oamjms.2018.311
eISSN: 1857-9655
Basic Science

Male Infertility and Sperm DNA Fragmentation

Afrim Zeqiraj[1*], Sheqibe Beadini[2], Nexhbedin Beadini[2], Hesat Aliu[2], Zafer Gashi[1], Shkelzen Elezaj[3], Sadi Bexheti[4], Agim Shabani[5]

[1]Department of Biochemistry, FAMA Colleague, Prishtina, Kosovo; [2]Department of Biology, University of Tetovo, Republic of Macedonia; [3]Clinic of Urology, Regional Hospital of Peja, Kosovo; [4]Faculty of Medicine, University of Tetovo, Republic of Macedonia; [5]Department of Chemistry, University of Tetovo, Republic of Macedonia

Citation: Zeqiraj A, Beadini S, Beadini N, Aliu H, Gashi Z, Elezaj S, Bexheti S, Shabani A. Male Infertility and Sperm DNA Fragmentation. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1342-1345. https://doi.org/10.3889/oamjms.2018.311

Keywords: Sperm DNA fragmentation; (SCD); IVF/ICSI

***Correspondence:** Afrim Zeqiraj. Department of Biochemistry, FAMA Colleague, Prishtina, Kosovo. E-mail: afi_istog@hotmail.com

Received: 27-May-2018; **Revised:** 23-Jul-2018; **Accepted:** 26-Jul-2018; **Online first:** 14-Aug-2018

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: One of the main factors affecting male infertility is DNA fragmentation in sperm. Male infertility is a heterogeneous group of disorders, known causes account for only 30-50%, and unknown cause (idiopathic) constitute the rest. Infertility involves nearly 15% of couples in the reproductive age, and only the male problem involves about 40% of the problems.

AIM: We have studied our DNA damage to sperm cells of a group of infertile males (113 patients) with abnormal sperm parameters (oligoasthenospermia and oligospermia) and a group of male patients (80 patients) with normal semen parameters (normospermia) to document whether the Sperm Chromatin Dispersion (SCD) analysis could increase the information obtained from the sperm routine analysis to explain the causes of infertility.

MATERIALS: A group of 193 patients were analysed, 113 patients in the working group and 80 patients in the control group were screened. The ejaculate samples were taken by the patient to whom the reason for the analysis was explained. All patients were from the Republic of Kosovo. Samples are collected from 2014/2018. Sperm Chromatin Dispersion (SCD) analyses in the ejaculate were analysed by the Biolab Zafi laboratory in Peja.

RESULTS: Clinical data were compared between the two groups by one-way ANOVA, mean ± SD, student's t-test. A p-value of less than $P < 0.05\%$ was considered statistically significant. Outcomes: In our study, we have gained significant ($P < 0.05$) results in the workgroup and the control group across all hormonal parameters, sperm parameters, and fragmented DNA in the sperm.

CONCLUSION: Based on our obtained results we can conclude that DNA fragmentation in spermatozoa is useful in the selection of unsuitable DNA sperm for use in ART methods. We conclude that our DNA fragmentation analysis results are encouraging and can be used for diagnostic purposes in determining male infertility.

Introduction

Male-factor infertility reportedly accounts for 30–40% of cases of couple infertility. Infertility is defined as the inability to achieve a successful pregnancy after 12 months of unprotected intercourse or therapeutic donor insemination (Practice Committee of the American Society for Reproductive Medicine, 2013) [1]. The natural desire of human beings is to propagate their lineage and is part and parcel of human evolution. The inability to conceive and produce a pregnancy results in the depression in couples. This infertility or subfertility forces couples to seek a solution as the problem results in social stigma. One in eight couples encounters problems when attempting to conceive a first child and one in six when attempting to conceive a subsequent child. Three per cent of women remains involuntarily childless, while 6% of parous women are not able to have as many children as they would wish [2]. Male fertility can be reduced as a result of congenital or acquired urogenital abnormalities, malignancies, urogenital tract infections, increased scrotal temperature (e.g. as a consequence of varicocele), endocrine disturbances, genetic abnormalities and immunological factors [2]. In about 15% of infertile male subjects, genetic abnormalities may be present, including chromosome aberrations and single gene mutations [3]. The cause of infertility can be attributed to male, female or due to both the partners and in a significant number the cause of infertility is unexplained. Hence simultaneous evaluation of both male and female partners of the infertile couple should

be done. Appropriate investigative modalities and the proper assisted reproductive procedure adopted will bring a definitive change in partners affected by infertility. Affected male partner of infertile couples should undergo semen analysis as a primary procedure. When the male partner alone is involved in infertility, it can save time and procedures can be accelerated which is also less expensive. One of the main factors affecting male infertility is DNA fragmentation in the sperm.

Male infertility is a heterogeneous group of disorders, the known causes account for only 30-50%, and the unknown cause (idiopathic) is the rest of infertility. It is of great importance to consider the effects of DNA damage on the sperm because half of the DNA in the progeny comes from the father's unit [4]. As a consequence of the complex anatomical and functional integration of the reproductive system, spermatogenesis in the germinal epithelium and the regulatory role of the hypothalamohypophysial-testicular axis are very susceptible; their changes become apparent even in the deterioration of fertility [5]. The hormone responsible for spermatogenesis is LH [6]. This glycoprotein regulates the testosterone synthesis of the extra tubular Leydig cells. FSH controls spermatogenesis by affecting both the germinal epithelium and Sertoli cells [7].

However, LH secretion is regulated by the negative feedback of the testosterone in the vascular system. The serum LH concentration reflects the function of Leydig cells; it is an important factor in the differential diagnosis between primary sociopathy and hypothalamo-hypophyseal hormone deficient [8]. Despite some deficiencies, spermogram analysis is generally acceptable and is considered reliable in evaluating male fertility [9]. Spermogram analysis does not always indicate the quality and sperm health [10]. For the accurate disclosure of genetic information, the integrity of the DNA molecule is essential [11].

The main causes of early pregnancy loss are genetic abnormalities in the sperm genome (EPL) [12]. Independent fertility indicators in couples subject to ART can be used for DFI [13]. The diagnostic test used today (SCD) is a lightweight and fast test based on the sperm chromatin dispersion [14]. Normal sperm creates DNA halo zones [15]. It has been established that if sperm DNA fragmentation exceeds 30%, sperm quality is significantly reduced [16]. Based on the percentage of DNA fragmentation, it is possible to choose the right technique in assisted medical care clinics. Patient selection based on sperm parameters to perform DNA fragmentation analysis is advisable.

We have studied our DNA damage to sperm cells of a group of infertile males (113 patients) with abnormal sperm parameters (oligoasthenospermia and oligospermia) and a group of male patients (80 patients) with normal semen parameters (normospermia) to document whether the Sperm Chromatin Dispersion (SCD) analysis could increase the information obtained from the sperm routine analysis to explain the causes of infertility.

Materials and Methods

There were analysed 193 patients, 113 patients in the working group and 80 patients in the control group. Patients received for analysis were all from the Republic of Kosovo. The sampling period was 2014/2018. All analyses were performed at Biolab Zafi, Laboratory in Peja.

Sperm analysis was done according to World Health Organization guidelines (WHO, 2010) [17].

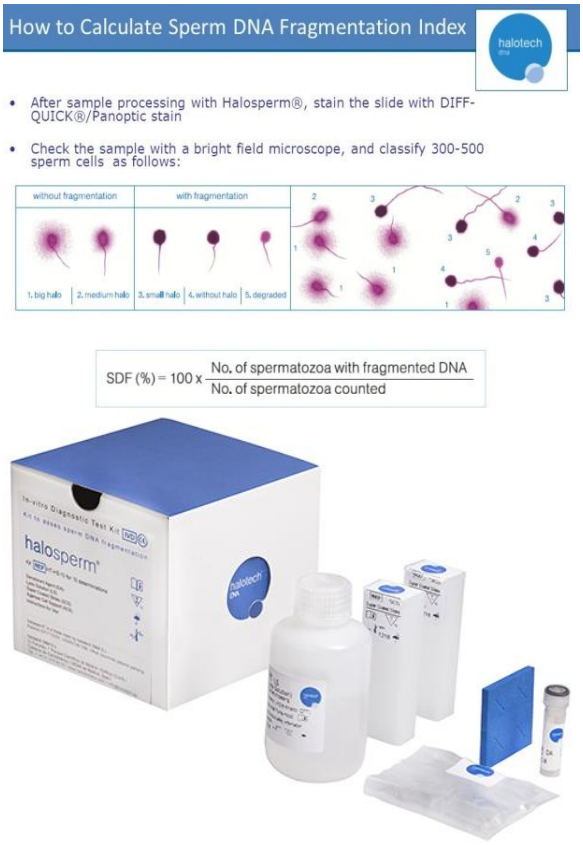

Figure 1: Sperm DNA fragmentation kit

Analysis of hormone levels of FSH, LH, prolactin, testosterone, will be made by ECIA (Eng. electrochemiluminescence immunoassay) in a mini VIDAS ® (bioMérieux, Marcy l'Etoile, France), in the laboratory clinic "BIOLAB-Zafi" Peja, R. of Kosovo.

Clinical data were compared between the two groups by one-way ANOVA, mean ± SD, student's t-test. A p-value of less than $p < 0.05\%$ was considered statistically significant.

Results

From the results obtained after analysing and comparing hormonal parameters in both groups of patients to take analysis (working group and group control) received the following results.

Table 1: Table presentation of the two groups of patients taken for analysis

	Infertility group (113 patients) Average/Std	Fertile group (80 patients) Average/Std	t-test	Significant P < 0.05
FSH	10.35 ± 8.17	6.42 ± 4.12	4.748	p<0.00001
LH	8.14 ± 4.96	4.98 ± 3.15	2.759	p<0.01
Prolactin	16.56 ± 4.61	10.29 ± 8.97	3.543	p<0.00001
Testosterone	2.85 ± 1.78	5.46 ± 3.76	2.754	p<0.001
Number in 1 million sperm	19.67 ± 19.79	61.43 ± 34.47	-8.247	p<0.00001
The general moving	28.29 ± 18.37	57.15 ± 10.83	-10.151	p<0.00001
Movement A	14.09 ± 10.86	25.21 ± 7.46	-7.359	p<0.00001
Movement B+C	14.03 ± 10.88	31.73 ± 10.09	-9.928	p<0.00001
Without moving	71.59 ± 18.34	42.7 ± 10.9	11.554	p<0.00001
Normal morphology	16.90 ± 14.47	42.6 ± 15.2	-9.696	p<0.00001
Abnormal morphology	83.09 ± 14.47	57.2 ± 16.2	8.991	p<0.00001
DNA- fragmentation (SDF%)	34.53 ± 4.68	14.91 ± 4.02	11.476	p<0.00001

The Table 1, shows that all hormones (FSH, LH, prolactin, and testosterone) defined in patients with infertility are at the significantly higher ($p < 0.05$) degree compared with hormones designated the control group.

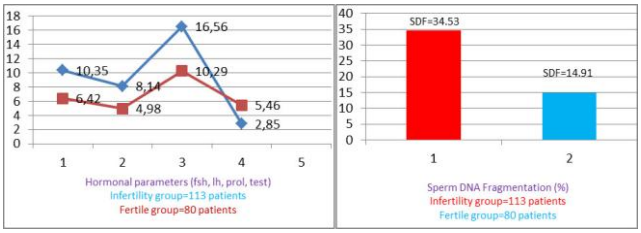

Figure 2: Graphical presentation of Sperm DNA Fragmentation (%)

In our work, we have gained significant results between the working group and control group on all spermogram parameters ($p < 0.001001$), and in DNA fragmentation in the sperm ($p < 0.001001$).

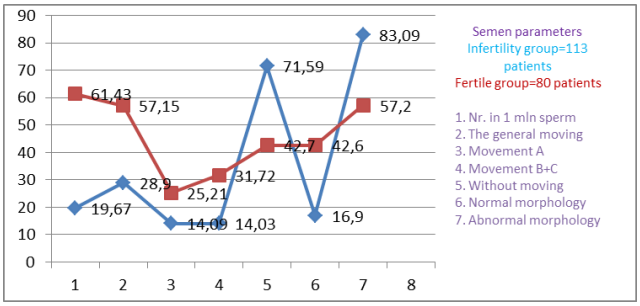

Figure 3: Graphical presentation of sperm parameters between two groups of patients taken for analysis

Discussion

The FSH, LH and testosterone evaluation is useful in the management of male infertility. FSH is necessary for initiation of spermatogenesis and maturation of spermatozoa [18]. In infertile men, a higher concentration of FSH is considered to be a reliable indicator of germinal epithelial damage, and was shown to be associated with severe oligozoospermia [19] [20] and reported that elevated levels of serum FSH with increasing severity of seminiferous epithelial destruction.

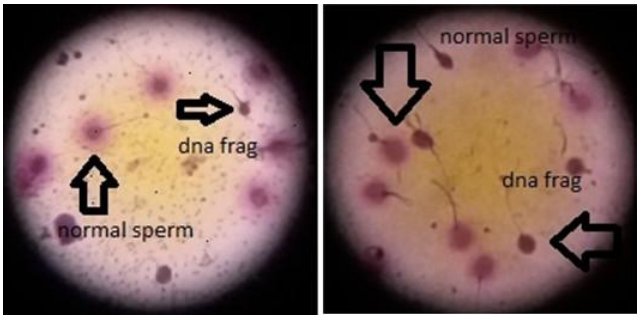

Figure 4: Photo by microscope during Sperm DNA Fragmentation analysis

However, FSH acts directly on the seminiferous tubules whereas LH stimulates spermatogenesis indirectly via testosterone. FSH plays a key role in stimulating mitotic and meiotic DNA synthesis in spermatogonia [21]. The increase in serum levels of gonadotropins might have disrupted the spermatogenic process leading to the decline in the sperm count and infertility [22]. In the present study, elevated serum levels of FSH and LH were observed in oligozoospermia and asthenozoospermia males when compared with normozoospermic men [23]. Also found that the high values of FSH, LH, Testosterone affect the reduction of sperm parameters [24].

From this paper, we have gained higher percentages of DNA than fragmented into infertile male sperm that had abnormal morphology and decreased mobility. Our results are consistent with the studies (Sergerie et al., 2005) [25] that found a DFI in the infertility group was significantly higher than in the fertile group (40.9 ± 14.3% compared to 13.1 ± 7.3%) and the mean sperm concentration in the infertile group was also significantly lower compared to the fertile group ($62.9 \pm 33.2 \times 10^6$/ml compared to $102.4 \pm 66.4 \times 10^6$/ml). DFI can be used to distinguish infertile men from fertile men [26]. A man with DFI sperm ≥ 26.1% has 2.84 times higher risk for infertility than the male with DFI sperm of < 26.1%.

In conclusion, determining Hormones (FSH, LH, Prolactin, Testosterone) of infertile men is a major step towards predicting infertility. Results of hormonal analysis and sperm parameters serve as an indicator

for medical personnel to determine male infertility. From our original work, we can conclude that with the increase of hormone parameters there is a reduction of sperm parameters (total number, total movement, movement A, movement B, normal form, abnormal form) and reduction of male reproductive capacity. We have concluded in our paper that there is a negative correlation between DNA fragmentation, mobility and morphology of sperm in male infertile. Based on our obtained results we can conclude that DNA fragmentation in spermatozoa is useful in the selection of unsuitable DNA sperm for use in ART methods. We conclude that our DNA fragmentation analysis results are encouraging and can be used for diagnostic purposes in determining male infertility.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1346-1348.
https://doi.org/10.3889/oamjms.2018.277
eISSN: 1857-9655
Basic Science

IgM Anti PGL-1 Antibody Level in Patients with Leprosy: A Comparative Study between Ear Lobes Capillary and Median Cubital Vein Blood Samples

Khairina Nasution*, Kristina Nadeak, Syahril Rahmat Lubis

Department of Dermatology and Venereology, Faculty of Medicine, Universitas Sumatera Utara, Adam Malik Medan, Medan, North Sumatera, Indonesia

Citation: K Nasution, K Nadeak, SR Lubis. IgM Anti PGL-1 Antibody Level in Patients with Leprosy: A Comparative Study between Ear lobes Capillary and Median Cubital Vein Blood Samples. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1346-1348. https://doi.org/10.3889/oamjms.2018.277

Keywords: Leprosy; PGL-1; ELISA; Antibody levels; Diagnosis

***Correspondence:** Khairina Nasution. Department of Dermatology and Venereology Faculty of Medicine, Universitas Sumatera Utara/RSUP. H. Adam Malik Medan, Medan, Indonesia. E-mail: khairina_nst@yahoo.com

Received: 15-May-2018; **Revised:** 07-Jun-2018; **Accepted:** 08-Jun-2018; **Online first:** 16-Aug-2018

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: To establish the diagnosis of leprosy accurately, additional examination such as serologic examination with ELISA is required. There are considerations about taking a blood sample from the earlobe region.

AIM: To determine the differences in IgM anti-PGL-1 antibody levels from earlobe capillary and median cubital vein blood sample in leprosy patients.

METHODS: An observational analytic study using a cross-sectional study involving 30 patients with leprosy. ELISA examination of earlobe blood samples with filter paper, and the median cubital vein blood samples with filter paper and conventional methods were performed to determine IgM anti-PGL-1 antibody levels.

RESULTS: The mean value of IgM anti PGL-1 antibody levels from earlobe blood samples with filter paper (1476.62 µ/ml) was relatively similar with median cubital vein blood samples with conventional method (1476.77 µ/ml), but the mean value of IgM anti PGL-1 antibody levels from median cubital vein blood samples with filter paper (1210.37 µ/ml) was lower from other methods. However, there was no statistically significant difference between them.

CONCLUSION: There are no significant differences between the mean levels of IgM anti-PGL-1 antibody from earlobe and the median cubital vein blood samples.

Introduction

Leprosy is a disease caused by *Mycobacterium leprae (M. leprae)* that mostly affect peripheral nerve, then skin, mouth mucosal, upper part of the airway, reticuloendothelial system, eyes, muscles, bones and testicle, except central nerve system [1] [2] [3] [4] [5].

According to data from the World Health Organization (WHO) in 2011, there were 210.074 new leprosy cases in the world with a prevalence of 4.06 per 10.000 people. While in the same year, data from the Ministry of Health Republic of Indonesia showed that there were 19.371 new leprosy cases with a prevalence of 8.03 per 100.000 people. In North Sumatera, there were 170 new leprosy cases with a prevalence of 1.3 per 100.000 people [2] [6] [7].

Leprosy usually diagnosed based on clinical examination, supported by slit skin smear, but in certain cases, additional tests are needed histopathology examination, inoculation on the animal, serologic test and *polymerase chain reaction (PCR)* [8] [9] [10]. Numerous studies have been conducted for these past few years to measure the antibody anti *phenolic glycolipid-1* (PGL-1) using ELISA [8] [9] [10] [11]. There are few problems using blood samples from a median cubital vein for the serologic test because it needs centrifugation and special way of storing and delivering. This process can be difficult to do, especially the peripheral area. Therefore, some studies were done to find solutions for this matter.

A study has been done in detecting IgM antibody against PGL-1 by using filter paper with a *finger prick* method using ELISA test [12]. While in Indonesia, a study was conducted to compare examination of antibody anti-PGL-1 level in blood samples from leprosy patient with and without using filter paper. However, this study showed no significant differences between examination using filter paper and conventional method [13].

Although the explanation on how *M. leprae* could get into the human body is still unknown, some studies showed that it commonly enter through the blistered skin, body parts with low temperature and nasal mucosa [13] [14] [15]. Earlobes have a relatively low temperature, and skin slit smear from earlobes usually shows a positive result for fast acid bacilli, even though it shows negative results in another area [16]. Therefore, we conducted research to compare antibody anti-PGL-1 level in leprosy patient using ELISA test from capillary blood in earlobe using filter paper with blood samples from a median cubital vein with filter paper and the conventional method.

Methods

This research is an observational analytic study with a *cross-sectional* design. It was conducted at Pulau Sicanang Leprosy Hospital, Belawan. The inclusion criteria for this research are a leprosy patient diagnosed based on one of the cardinal symptoms, leprosy patient above 14 years old and willing to participate in this research by signing *informed consent*. The exclusion criterion for this research is if we are unable to take blood smear sample.

The characteristics of research subjects were then compiled and presented according to gender distribution, age group, and type of leprosy. Subjects proceeded to ELISA test using a blood sample from earlobe with filter paper, and blood samples from median cubital vein were also tested using filter paper and conventional method to find out the antibody IgM anti-PGL-1 level. Then it was tested and analysed statistically using ANOVA test to see if there are differences between these methods. If the data is not normally distributed, the statistical analysis will be done using the Kruskal Wallis test. The study was conducted after it was approved by the Research Ethics Commission of the Faculty of Medicine, Universitas Sumatera Utara.

Results

The subject characteristics in this research were showed based on gender distribution, age group and type of leprosy. From a total of 30 subjects, 23 of them (76.7%) were male, and 7 of them were female (23.3%). This showed that the number of male leprosy patients was more than female. Most patients based on age group is > 45 years old of age group with the total of 12 patients (40%) followed by 15-25 years old of age group with 11 patients (36.7%), and the lowest proportion is 26-35 years old of age group with 2 patients (6.7%). From 30 subjects of research, there were more patients with multibasiler type of leprosy, 28 patients (93.3%) than pausibasiler type of leprosy, 2 patients (6.7%).

Table 1: Characteristics of research subjects

Characteristics	Research subjects	
	n	%
Sex		
Male	23	76.7
Female	7	23.3
Total	30	100.0
Age (year)		
15-25	11	36.7
26-35	2	6.7
36-45	5	16.7
>45	12	40
Total	30	100.0
Leprosy Type		
Paucibacillary	2	6.7
Multibacillary	28	93.3
Total	30	100.0

Different amount of anti-PGL-1 IgM antibody from earlobe capillary blood using filter paper and median cubital vein blood using filter paper and the conventional method.

To compare the amount of anti-PGL-1 IgM antibody from earlobe capillary blood using filter paper with median cubital vein blood using filter paper or conventional method, the scale conversion was made based on regression linear analysis result. The results between this different examination were then analysed using Kruskal-Wallis test due to an abnormality of data distribution.

Table 2: The difference amount of anti-PGL-1 IgM antibody from earlobe blood using filter paper, median cubital vein blood using filter paper and median cubital vein blood using the conventional method on Kruskal Wallis test

		n	*p*
The amount of anti-PGL-1 IgM antibody	Earlobe blood (filter paper)	30	0.164
	Median cubital vein (filter paper)	30	
	Median cubital vein (conventional method)	30	

Kruskal-Wallis test.

The average amount of anti-PGL-1 IgM antibody from earlobe capillary blood using filter paper (1,476.62 µ/ml) is relatively similar with the average amount of anti-PGL-1 IgM antibody from a median cubital vein using conventional method (1,476.77 µ/ml). However, the average amount of anti-PGL-1 IgM antibody from a median cubital vein using filter paper (1210,37 µ/ml) is lower than both data. After, statistical analysis using the Kruskal-Wallis test, we found that there is no any significant difference between each examination (P = 0.164).

Discussion

From the total number of 30 research subjects, there were more male than a female with most of them aged more than 45 years old and suffered from Multibacillary Leprosy. By data from the Ministry of Health Republic of Indonesia in 2007, most countries in the world, except in some countries in Africa, leprosy is more commonly found in male than female. This probably influenced by environmental or biological factors. Although leprosy can be found at all ages, this disease particularly affected young and productive age people [2]. Furthermore, data from Indonesia in 2011 showed that Multibacillary leprosy cases are higher (15,634 people) than Paucibacillary Leprosy (3,737 people) [7].

Filter paper has been considered to be used for diagnosis of new cases in leprosy. A study showed that there is a straight correlation between antibody anti-PGL-1 test using a blood sample from filter paper and classical method [12]. However, another study in Indonesia found that there are no significant differences between the examination of antibody anti-PGL-1 level using filter paper and conventional method [13]. From our study, we did not find a significant difference between each methods (P = 0.164). There are other similar study and it showed that blood samples from skin smear site had a higher level of antibody compared with sera and may be more sensitive for antibodies measurement [16].

Statistic analysis results from the examination of anti-PGL-1 IgM antibody amount from earlobe blood using filter paper, median cubital vein blood using both filter paper and conventional method indicate a similar relative interpretation. However, there are some advantages in taking blood samples from the earlobe, as it can also be taken while obtaining slit skin smear samples for bacteriological examination. Furthermore, special storage and delivery process are not needed. Therefore it is more convenient in the peripheral area.

In conclusion, the study showed similar results between mean antibody IgM anti-PGL-1 level from earlobe capillary blood samples using filter paper and median cubital vein blood samples using the conventional method. However, the mean antibody IgM anti-PGL-1 level from median cubital vein blood samples using filter paper is lower than both data, although the difference is not significant.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1349-1353.
https://doi.org/10.3889/oamjms.2018.323
eISSN: 1857-9655
Basic Science

Role of Procalcitonin As an Inflammatory Marker in a Sample of Egyptian Children with Simple Obesity

Ghada M. El Kassas[1], Manal A. Shehata[1*], Maged A. El Wakeel[1], Ahmed F. Amer[1], Fatma A. Elzaree[1], Marwa K. Darwish[2], Marwa F. Amer[3]

[1]Department of Child Health, National Research Centre, Cairo, Egypt; [2]Biochemistry Department, Faculty of Science, Suez University, Egypt; [3]Department of Medical Biochemistry, Cairo University, Cairo, Egypt

Citation: El Kassas GM, Shehata MA, El Wakeel MA, Amer AF, Elzaree FA, Darwish MK, Amer MF. Role of Procalcitonin As an Inflammatory Marker in a Sample of Egyptian Children with Simple Obesity. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1349-1353. https://doi.org/10.3889/oamjms.2018.323

Keywords: Procalcitonin; Inflammatory Marker; Egyptian Children; Obesity

***Correspondence:** Manal A Shehata. Department of Child Health, National Research Centre, Cairo, Egypt. E-mail: dr_manalabdelkader@hotmail.com

Received: 27-Jul-2018; **Revised:** 27-Jul-2018; **Accepted:** 31-Jul-2018; **Online first:** 17-Aug-2018

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Funding: This work was supported and funded by the National Research Centre, Egypt

Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Obesity is a multifactorial disease, associated with metabolic disorders and chronic low-grade inflammation. Procalcitonin (PCT) is well known as a biomarker of infection, and systemic inflammation. Recently, it has potential as a marker for chronic low-grade inflammation.

AIM: This study aims to evaluate the role of serum PCT as an inflammatory biomarker in the diagnosis of obesity-related low-grade inflammation.

METHOD: In this case-control study, 50 obese and 35 normal weight children and adolescents aged 5–15 years were enrolled. Anthropometric parameters were measured in all subjects. Blood samples were collected for measurement of lipid profile, blood glucose, insulin, high sensitivity-CRP (Hs-CRP) and serum procalcitonin. Serum (PCT) levels were assessed using enzyme-linked immunosorbent assay.

RESULTS: Obese participants had higher concentrations of serum PCT, total cholesterol, triglycerides, LDL-c, glucose and Hs-CRP than control group. On correlation analysis, procalcitonin had significant positive correlation with (BMI) z-score (P = 0.02), insulin (P = 0.00), insulin resistance (HOMA-IR) (P = 0.006), Hs-CRP (P = 0.02), total cholesterol (P = 0.04) and triglycerides (P = 0.00) in obese group.

CONCLUSION: The increased serum procalcitonin concentrations were closely related to measures of adiposity, Hs-CRP and insulin resistance, suggesting that PCT may be an excellent biomarker for obesity-related chronic low-grade inflammation in children and adolescents.

Introduction

Childhood obesity is one of the most important public health problems with increasing prevalence worldwide in this century. Childhood obesity is more prevalent in low and middle-income countries, especially in urban areas. In 2016, the number of overweight children below 5 years old, worldwide, is approximately 41 million. About fifty per cent of overweight children below five years are from Asia, and twenty-five per cent are from Africa [1]. The aetiology of obesity is complicated, a range of factors are suggested to play a role, including factors related to the lifestyle preferences, genetic, neuroendocrine, metabolic, immunologic, environmental, social and cultural factors [2] Obesity is associated with the chronic low-grade inflammatory reaction. This type of inflammation can be differentiated from normal inflammation by the absence of ordinary signs of inflammatory reactions. However, it shares the same diseases caused by typical inflammatory mediators and signalling pathway [2] [3].

Procalcitonin is the precursor to the hormone calcitonin, which is produced by all tissues throughout the body [4]. Production of procalcitonin occurs mainly in response to bacterial toxins and some inflammatory mediators. On the contrary, the downregulation of procalcitonin occurs in the course of viral infection. The definite physiological role of procalcitonin is not yet completely recognised [5]. Procalcitonin level can be detected in serum after about 3-6 hours after the onset of inflammation and stay raised for 12-36 hours after recovery [6].

Procalcitonin has been identified as a

marker of infections and significant systemic inflammatory states [7]. Previously, research proved the ability of adipose tissue to express and produce procalcitonin [8]. This provides evidence for the relation between inflammation and obesity, considering procalcitonin a potential marker for it [9].

The objective of this study is to investigate the role of procalcitonin as a marker of inflammation in childhood obesity and its relationship with markers of obesity and other metabolic indices.

Subjects and Method

The present study included fifty children with simple obesity. Their age ranged from 5 to 15 years, with mean age 10.1 ± 2.5 years and 35 non-obese healthy children were enrolled as a control group with a mean age of 9.3 ± 2.1 years. Children with a diagnosis of obesity were recruited from the child health clinic in Medical and Scientific Centre of Excellence, National Research Centre. Obesity is defined as BMI greater than the 95th percentile on the growth charts from the National Center of Health and Statistics (NCHS). Exclusion criteria included genetic and endocrinal causes of obesity, children with chronic debilitating diseases, mental retardation, and use of drugs that affect blood pressure, lipid profile, or glucose level. Informed consents were obtained from the parents of the children studied, and the study was approved by the medical ethical committee of the National Research Centre, Cairo, Egypt.

A full history was taken from all participants. Also, thorough clinical examination and anthropometric measurements were done. A calibrated Seca scale was used to weigh children to the nearest 0.1 kg (Seca, Hamburg, Germany), whereas a Seca 225 stadiometer was used to measure height to the nearest 0.1 cm, with the children dressed in minimal clothes and without shoes [10]. Each measurement was taken as the mean of three consecutive readings following the recommendations of the International Biological Program [11]. BMI for age was recorded according to WHO standards using AnthroPlus software for personal computers [12]. Weight for age, height for age and BMI Z-score were determined using the new WHO reference [13]. Measurements of waist circumference, hip circumference, W/H ratio and blood pressure were done.

Morning venous blood sample (3 ml) was withdrawn after 12 hours overnight fasting into a plain tube and left to clot. The serum was separated by centrifugation for 10 minutes at 5000 rpm and stored at-20 until assays done. Fasting serum glucose, fasting serum insulin, cholesterol, triglycerides (TG), high-density lipoprotein cholesterol (HDL-c) were measured by calorimetric method.

Serum LDL-C levels were calculated using the Friedewald formula [LDL-C=Total cholesterol-HDL-C- (Triglyceride/5)] [14].

C-reactive protein was determined using a latex agglutination technique [15]. Procalcitonin (Human) ELISA Kit was used for the quantitative measurement of human Procalcitonin in serum (Bioassay Technology Laboratory). The detection range of this kit was 5 pg/ml - 20000 pg/ml [16].

Data entry was carried out in excel sheet, and statistical analysis was done using SPSS software program version 20.0, the measurement data presented as a mean ± standard deviation. A t-test was done for comparison between two means. Simple linear correlation (Pearson correlation) for quantitative data was also done. P value was considered statistically significant when P was <0.05 and considered statistically highly significant when its value was < 0.001.

Results

Comparisons between mean ± SD values of studied parameters in obese and non-obese groups are shown in (Table 1 & 2). The study comprised fifty obese children (34 females and 16 males) with mean age 10.1 ± 2.5 years and 35 non-obese healthy children (18 females and 17 males) with mean age 9.3 ± 2.1 years, considered as control group, there were highly significant statistical differences between them as regard weight z-score, body mass index z-score, waist circumference, hip circumference, mid-arm circumference, fasting blood glucose, cholesterol, triglycerides, LDL, hs-CRP, and PCT. Also significant statistical differences between them as regard waist/hip ratio, insulin and HOMA-IR. The comparison between males and females as regards the mean PCT level revealed a non-significant difference.

Table 1: Characteristics of the study group

Characteristics	Obese N = 50 Mean ± SD	Non Obese N = 35 Mean±SD	t	p- value
Age (years)	10.1 ± 2.5	9.3 ± 2.1	1.34	0.185
Weight z-score	2.54 ± 1.07	0.96 ± 0.6	7.02	0.000**
Height z-score	-0.91 ± 0.79	-0.70 ± 0.9	0.79	0.4
Z score-BMI	2.77 ± 0.6	1.7 ± 0.46	7.72	0.00**
Waist circumference	100.7 ± 18.6	69.9 ± 9.8	7.23	0.000**
Hip circumference	109.6 ± 17.8	79.4 ± 14.8	7.84	0.000**
Waist/hip ratio (WHR)	0.96 ± 0.34	0.79 ± 0.41	2.74	0.04*
Mid arm circumference (MAC)	33.4 ± 7.5	18.4 ± 5.4	9.75	0.000**

SD: standard deviation, BMI: body mass index. * if p≤0.05, then the relation is statistically significant. ** if p≤0.001, then the relation is statistically highly significant.

Table 2: Laboratory characteristics of the study group

Characteristics	Obese N = 50 Mean ± SD	Non Obese N = 35 Mean ± SD	t	P- value
Insulin (mg/dl)	14.80 ± 4.5	9.50 ± 3.4	2.208	0.03*
HOMA IR	3.87 ± 1.12	1.75 ± 0.93	3.243	0.00**
FBG (mg/dl)	97.43 ± 15.5	72.64 ± 11.78	7.323	0.00**
Cholesterol (mg/dl)	201.46 ± 39.84	89.81 ± 17.5	13.244	0.00**
LDL (mg/dl)	119.8 ± 40.63	40.74 ± 9.53	12.069	0.00**
HDL (mg/dl)	47.39 ± 10.3	61.47 ± 9.4	-3.864	0.00**
TG (mg/dl)	121.74 ± 39.76	79.89 ± 19.74	3.644	0.00**
Hs-CRP (mg/dl)	4.89 ± 2.2	1.02 ± 0.66	9.462	0.00**
Procalcitonin (pg/ml)	388.68 ± 114.19	122.38 ± 21.48	12.815	0.000**

Hs-CRP: high sensitive C-reactive protein, FBG: fasting blood glucose; HOMA-IR: homeostasis model assessment of insulin resistance, LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TG: triglycerides. * if P ≤ 0.05, then the relation is statistically significant. ** if P ≤ 0.001, then the relation is statistically highly significant.

Results of the correlations of the various parameters with procalcitonin in obese children showed that there was strong significant positive association between procalcitonin and Weight z-score (r = 0.34; P = 0.01), BMI z-score (r = 0.31; P = 0.02) as shown in Fig. 1, insulin (r = 0.4; P = 0.00), HOMA-IR (r = 0.37; P = 0.006) as shown in Fig. 2, Hs-CRP (P = 0.02), cholesterol (r = 0.3; P = 0.04), and triglycerides (r = 0.41; P = 0.00) as shown in Fig. 3, while there were no significant correlations with age, height z-score, waist circumference, fasting blood glucose, LDL-cholesterol and HDL-cholesterol.

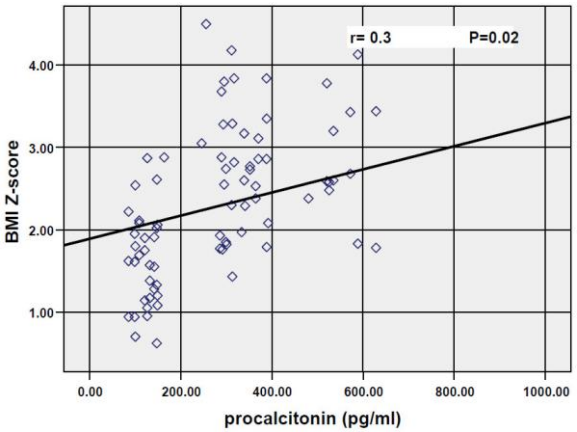

Figure 1: Correlation between procalcitonin and BMI z-score

Discussion

The research for the pathogenesis of obesity during the last decades had shown a strong link between excessive nutrient intake and activating the innate immune response in many organs relevant to energy homeostasis [17] [18].

Strong correlations were reported between plasma concentrations of procalcitonin and the degree of inflammatory responses [19]. Procalcitonin is indicated mainly for diagnosing bacterial infections that precipitate systemic inflammatory responses. It shows high degrees of stability, with prolonged half-life and easy method of determination, making it perfect for clinical application [20].

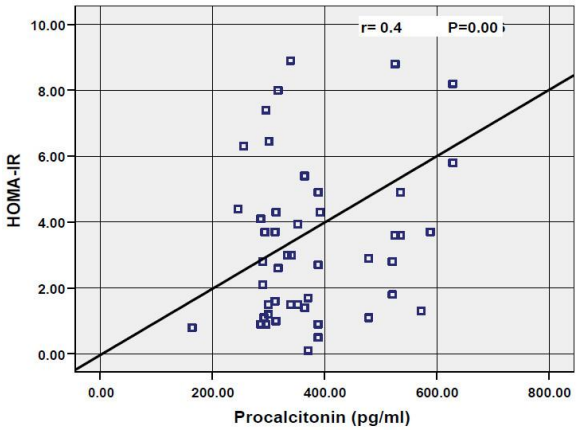

Figure 2: Correlation between procalcitonin and HOMA-IR

Moreover, it was suggested that plasma PCT could be a marker of inflammation without the manifestations of systemic infection or sepsis [21] [22]. Adipose tissues have been considered as an endocrine organ, expressing calcitonin mRNA [23]. Also, it was found that adipocyte excretion of procalcitonin in vitro was triggered by activated macrophages [24], and the existence of those macrophages in adipose tissue has been reported to be associated proportionately to the extent of obesity [25].

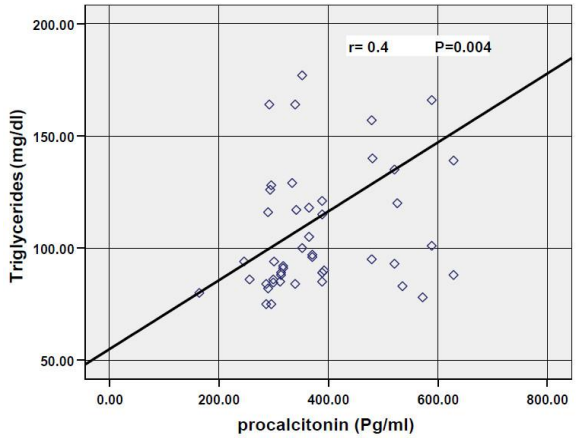

Figure 3: Correlation between procalcitonin and triglycerides

The objective of this study is to investigate procalcitonin serum concentrations in a group of obese children in comparison with non-obese children, and studying the correlation of procalcitonin with some metabolic indices and anthropometric measurements.

The study findings showed a significant difference in procalcitonin serum concentration between obese and non-obese children and a significant positive association between procalcitonin and BMI z-score. In accordance, it was reported that, in obesity-related inflammation, there is a

proportional association between the amount of adipose tissue and the increased generation of inflammatory mediators [26]. Also, our results showed a positive correlation between procalcitonin and weight z-score, insulin and HOMA-IR.

In agreement with our results, Abbasi and colleagues who conducted a cross-sectional study on a general population, reported a higher procalcitonin level in more obese subjects, our results were also matching regarding the association of plasma procalcitonin with insulin resistance [9]. A recent study in Egypt, investigated procalcitonin level in type 2 diabetic patients and assessed its relation with obesity, the authors reported, significantly higher concentrations of procalcitonin, hs-CRP and HOMA-IR in obese compared to non-obese patients [27]. Moreover, Boursier et al., [28] found high plasma procalcitonin levels of their subjects associated with the degree of obesity, but in contrast to our results, they found no association between procalcitonin and insulin resistance. However, it is well known that increased adiposity is one of the major predisposing factors in developing insulin resistance [29]. Moreover, several studies suggest that inflammatory reactions that occur as a result of obesity may be implicated in the generation of insulin resistance, deficient insulin production, and disrupted energy homeostasis [30]. In accordance, Chen and his colleagues found a significant positive correlation between inflammatory markers and insulin resistance [31]. Also, Indulekha and his colleagues suggested that the relation between inflammatory reactions and insulin resistance indicates a continued cytokine- generated acute phase reactions [32].

On the other hand, the group of obese children in this study presented a state of disturbed lipid profile, and there was a significant correlation between procalcitonin, total cholesterol and triglycerides levels. These findings are supported by the accumulating evidence that reveals the association of systemic-obesity-related inflammation with the risk of developing cardiovascular disease (CVD). Hence, several obesity-associated factors including dyslipidemia are involved in CVD risk. In this regard, pro-inflammatory cytokines, are suggested to affect the liver, leading to alterations in the release of lipoproteins and inflammatory mediators [33] [34]. Particularly, they cause an elevation of very low-density lipoprotein, apolipoprotein B, and triglyceride levels [35]. C-reactive protein is a highly sensitive inflammatory marker, it is produced from the liver, and its production is controlled mostly by IL-6 [36]. Previous research has evidenced that concentrations of C-reactive protein have a positive relationship with BMI in healthy subjects [37].

Moreover, several studies have shown that CRP is associated with most obesity markers [38]. Our results showed significantly higher hs-CRP levels in obese children compared to non- obese. In accordance, Ahmed et al., [39], evaluated the role of some inflammatory mediators and adipokines in obese Egyptian children; they reported that the mean level of CRP was significantly elevated in obese children compared with controls.

In conclusion, the findings of this study revealed the significance of serum procalcitonin as a marker of obesity-related low-grade inflammation in obese children.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1354-1358.
https://doi.org/10.3889/oamjms.2018.312
eISSN: 1857-9655
Basic Science

Serum Apelin and Obesity-Related Complications in Egyptian Children

Maged A. El Wakeel[1*], Ghada M. El-Kassas[1], Alyaa H. Kamhawy[1], Essam M. Galal[1], Maysa S. Nassar[1], Elsayed Mahmoud Hammad[2], Salwa Refat El-Zayat[3]

[1]Child Health Department, Medical Division, National Research Centre, Cairo, Egypt; [2]Clinical Nutrition Department, National Nutrition Institute, Cairo, Egypt; [3]Department of Medical Physiology, Medical Division, National Research Centre, Cairo, Egypt

Citation: El Wakeel MA, El-Kassas GM, Kamhawy AH, Galal EM, Nassar MS, Hammad EM, El-Zayat SR. Serum Apelin and Obesity-Related Complications in Egyptian Children. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1354-1358. https://doi.org/10.3889/oamjms.2018.312

Keywords: Apelin; Obesity; Children; Metabolic syndrome

***Correspondence:** Maged A. El Wakeel. Child Health Department, Medical Division, National Research Centre, Cairo, Egypt. E-mail: maged_elwakeel@yahoo.com

Received: 27-May-2018; **Revised:** 23-Jul-2018; **Accepted:** 27-Jul-2018; **Online first:** 17-Aug-2018

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Funding: This research was supported by National Research Centre, Cairo, Egypt

Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: The rapidly increasing prevalence of childhood obesity became a major burden on health worldwide, giving an alarm to clinicians and researchers. Adipocytes act as an active endocrine organ by releasing plenty of bioactive mediators (adipokines) that play a major role in regulating metabolic processes. Apelin is a recently identified adipokine that is expressed in adipocytes.

AIM: The current work aimed to uncover the relation between serum apelin and childhood obesity and its related complications as hypertension and hyperglycemia

METHOD: A group of 50 obese and 31 non-obese; sex- and age-matched children were enrolled in our study with a mean age of (9.5 ± 2.1) and (8.7 ± 1.3) respectively. Anthropometric measurements, blood pressure, were assessed in all studied participants, we also determined the lipid profile, serum insulin, fasting blood glucose (FBG) level, HOMA-IR and serum apelin.

RESULTS: Obese children had higher levels of HbA1c, FBG, serum insulin, HOMA-IR, total cholesterol, triglycerides, low-density lipoprotein (LDL) and diastolic blood pressure (DBP Z-score); compared to controls (all $P < 0.05$). Apelin was significantly higher in obese children versus controls and correlated positively with BMI Z-Score ($P = 0.008$), DBP Z-Score ($P = 0.02$), cholesterol, TG (both $P = 0.02$), serum insulin ($P = 0.003$), FBG and HOMA-IR (both $P = 0.001$). Linear regression analysis showed that FBG was the most effective factor in predicting the level of serum apelin ($P = 0.04$).

CONCLUSION: This work supports the hypothesis that apelin may have a crucial role in the pathogenesis of health hazards related to obesity in children including insulin resistance, hypertension and a higher risk of occurrence of metabolic syndrome.

Introduction

Obesity in childhood is related to a large number of metabolic disorders,

including insulin resistance [1], dyslipidemia. [2], hyperglycemia [3], type 2 diabetes mellitus [4], and the risk of cardiovascular complications [5].

Moreover, obese children tend to become obese adults [6]. However, studying childhood obesity is important to reduce its incidence and the possibility of developing obesity-related complications and other metabolic diseases.

Adipose tissue depots are the most important target to mediate significant immune cells infiltration contributing to systemic inflammation and insulin resistance in obese humans [7]. They represent powerful acting endocrine organs by releasing a huge amount of bioactive adipokines which play an effective role in regulating glucose homeostasis and inflammatory process [8]. This pattern of secretion reflects adipose tissue function and accounts essential for detecting the human susceptibility to develop cardiovascular and metabolic complications of obesity [9].

On initiating adipose tissue inflammation, adipokine release is markedly produced by a

diabetogenic, pro-inflammatory and atherogenic mode [10].

The recently known adipokine, Apelin which is a peptide of a 12-amino acid, encoded by the APLN gene and expressed in human adipocytes [11]. Apelin synthesis in adipocytes is stimulated by insulin, and its serum levels are found to be higher about diabetes mellitus, hyperinsulinemia and insulin resistance [12] [13].

Apelin expression helps in regulation of blood pressure [14], cardiac contractility [15], fluid balance [16] and activation of ACTH release by the pituitary [17]. Most of the previously mentioned studies demonstrate that contention presents around the levels and the associations of apelin in metabolic and cardiovascular disorders. Although relations have been reported between apelin and atopic dermatitis [18] and insulin resistance in adolescents with polycystic ovary syndrome [19].

Till now, there is no acceptance on if apelin levels have a direct relation with childhood obesity or not. So, this work aimed to study the relations between apelin levels and obesity in children and to find out the associations between those levels and obesity-related complications including hyperlipidemia, insulin resistance and hypertension.

Patients and Methods

This case-control study included 81children classified as 50 obese children compared to 31 healthy control who were recruited from child health Clinic in Medical and Scientific Centre of Excellence, National Research Center.

This includes full personal, history of systemic diseases, drug administration (as corticosteroids), and symptoms covering various systems, and family history of chronic non- communicable diseases (obesity, diabetes, cardiovascular diseases and hypertension).

All anthropometric measurements have been obtained using standardised equipment and following the recommendations of the International Biological program [20].

Assessment of body mass index (BMI) was done using categories reported by the World Health Organization (WHO) Child Growth Charts Standards for age and sex defined as the weight in kilograms divided by the square of the height (kg/m^2) [21]. Weight for age, height for age and BMI Z-score were determined using the new WHO reference [22].

Waist Circumference was measured using inelastic insertion tape to the nearest 0.1 cm, with the subject in a standing position; the tape was applied horizontally midway between the lowest rib margin and the iliac crest. Assessment of waist circumference was done using categories reported by Fernandez et al., 2004 [23]. Thorough medical general examination (head & neck, chest, heart, abdomen, upper &lower limbs) including measurement of systolic blood pressure (SBP) and diastolic blood pressure (DBP) then blood pressure Z-score was determined using the German references [24].

Blood samples were withdrawn from patients and controls after overnight fasting (> 12 hours). Fasting venous blood samples were collected in heparinised centrifuge tubes. Serum was separated by centrifugation (3000 rpm, 15 min). Separated serum aliquots were removed and stored frozen at -20^0C until further analyses were carried out, following tests were performed: Fasting serum glucose, fasting serum insulin, Cholesterol, Triglycerides (TG), high-density lipoprotein (HDL)-cholesterol, lowh-density lipoprotein (LDL)-cholesterol were measured by calorimetric method. While serum apelin levels measured by quantitative commercial enzyme-linked immunosorbent assay ELISA kit supplied from Elabscience Biotechnology Co., Ltd, WuHan, China-Catalog No: E-EL-H0456 (www.elabscience.com), detection range was between 62.5-400 pg/ml [25].

Insulin resistance was estimated by using the Homeostasis Model Assessment for insulin resistance (HOMA-IR), which was calculated according to the known formula, Insulin resistance being defined as a HOMA-IR index > 3.16). The greater the HOMA-IR value, the greater the level of insulin resistance [26].

Data management and analysis were performed using the Statistical Package for Social Sciences (SPSS) v. 21.

Numerical data were summarised using means±standard deviations. Comparisons between groups for normally distributed numeric variables were made using the Student's t-test while for non-normally distributed numeric variables were done by Mann-Whitney test. To measure the strength of association between numeric variables, Pearson's correlation coefficients were computed. All p-values are two-sided. Linear regression analysis was performed to predict risk factors significantly associated with an increased level of Apelin. P value was considered statistically significant when it was less than 0.05.

Results

Fifty obese and thirty-one non-obese Egyptian children were included in this study; all were age-and sex-matched. Mean age was 9.5 ± 2.1 and 8.7 ± 1.3 years in obese children and control groups, respectively.

Table 1: Anthropometric data of obese children versus controls

variable	Obese (n = 50) Mean ± SD	Control (n = 31) Mean ± SD	P-value
Age	9.5 ± 2.1	8.7 ± 1.3	0.185
Wt. z-score	2.4 ± 1.1	0.97 ± 0.42	0.000*
Ht. z-score	-0.7 ± 0.98	-0.9 ± 1.2	0.456
BMI z-score	2.8 ± 0.7	1.6 ± 0.6	0.000*
(DBP) Z-Score	0.7 ± 0.8	0.2 ± 0.4	0.013*
(SBP) Z-score	0.4 ± 0.8	0.1 ± 0.7	0.178
Waist circumference	99.4 ± 17.5	64.7 ± 10.9	0.000*
Hip circumference	110.5 ± 16.8	86.7 ± 12.2	0.000*
Waist/hip ratio (WHR)	0.9 ± 0.2	0.8 ± 0.3	0.04*
Mid arm circumference (MAC)	32.1 ± 6.3	17.3 ± 4.2	0.000*

*(P ≤ 0.05) is significant.

Table 1 shows that the obese group had significantly higher weight Z-score, BMI Z-score, DBP Z-score, waist circumference, hip circumference, WHR, MAC (all P < 0.05) than the control group.

Table 2: Biochemical features of the studied groups

Variable	Obese (n = 50) Mean ± SD	Control (n = 31) Mean ± SD	P-value
Apelin	2531 ± 547.8	1107.1 ± 436.7	0.000**
Cholesterol	196.7 ± 41.9	94.5 ± 11.1	0.000**
TG	114.6 ± 34.4	83.7 ± 20.6	0.000**
HDL	45.3 ± 9.5	53.3 ± 8.2	0.000**
LDL	121.4 ± 37.4	38.8 ± 8.4	0.000**
Fasting blood Glucose (FBG)	98.8 ± 16.4	73.3 ± 12.9	0.000**
Insulin	15.1 ± 3.9	9.4 ± 2.3	0.038*
HOMA-IR	4.7 ± 1.3	1.8 ± 0.9	0.001*

* Significant difference (P ≤ 0.05); ** Highly significant difference (P ≤ 0.01).

Obese children had significantly higher Cholesterol, TG, LDL, FBG, Insulin, HOMA-IR, and significantly low HDL compared to the control group. Also apelin levels were found to be higher in obesity group (Table 2).

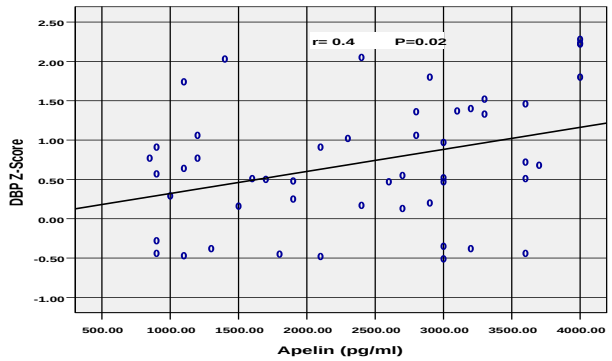

Figure 1: Correlation between apelin level and diastolic blood pressure in obese cases

Correlation analysis of levels of serum apelin with anthropometric parameters and biochemical findings showed significant positive correlations between apelin and weight z score, BMI z score, waist, SBP, DBP z score (Figure 1), cholesterol, TG, Glucose, insulin and HOMA-IR (Figure 2) in the obese group.

No correlations were found between apelin levels and age, height, skinfold thickness, WHR, MAC, HDL, LDL.

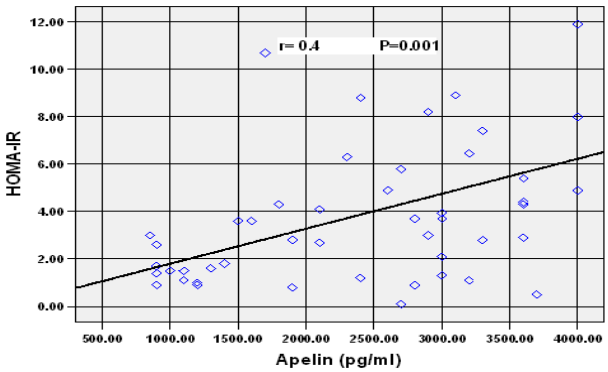

Figure 2: Correlation between apelin level and HOMA-IR in obese cases

Discussion

Obesity is considered a widely spread chronic illness and a major risk factor for the development of metabolic syndrome, type 2 diabetes mellitus and cardiovascular diseases [27]. Many studies have agreed that visceral obesity is strongly related to hypertension, dyslipidemia, hyperglycemia, and IR [28]. Severe adiposity may be the cause of some metabolic diseases such as type 2 diabetes (T2D), hypertension, insulin resistance, polycystic ovarian diseases and some types of cancer [29].

Table 3: Predictive factors for an increased level of Apelin in obese children as estimated by linear regression

Variable	Apelin	
	B	Sig.
BMI Z-score	0.176	0.605
SBP Z-score	0.266	0.132
DBP Z-Score	0.037	0.842
Cholesterol	0.160	0.344
TG	0.117	0.402
FBG	0.455	0.038*
Insulin	0.488	0.371
HOMA-IR	-0.622	0.308

*P < 0.05, the relationship is statistically significant; Factors entered: BMI.SBP Z-score, DBP Z-score, TG, Cholesterol, glucose, Insulin, HOMA-IR; Dependent variable: Apelin; Linear regression analysis showed that FBG was the main predictor serum apelin levels (P = 0.038).

Adipose tissue hormones called adipokines such as adiponectin, leptin, and apelin have a major role in obesity-related comorbidities and complications [12] [30]. High plasma levels of apelin have been detected by many authors in severe obesity and related to adiposity [31] [32].

The current work investigated the data of obese and non-obese groups of Egyptian children to find out correlations if present between apelin and obesity-related hazards, especially insulin resistance, hyperlipidemia, hypertension, and insulin sensitivity.

Serum insulin, TG, TC, LDL, and HOMA-IR were found to be significantly higher in all obese subjects when compared to controls in our study, these results were in agreement with Ba et al., 2014

[33], also we observed a significantly higher Apelin level in obese cases when compared to non-obese and this agreed with Boucher et al., 2005 [12] who found similar results and supposed that adipose tissue is a major source of apelin release, and that expression of apelin and apelin receptors (APJ) both increase in fat cells of obese subjects.

In the current study, we found that serum apelin levels were positively correlated with BMI. This came in agreement with a study done by Sheibani et al., 2012 supposing an important role of apelin in the pathogenesis of obesity and obesity-related complications [34]. Also, many studies revealed that apelin levels were higher in obese individuals in comparison to none-obese and correlated positively with BMI [31] [32]. In contrast, Reinehr et al., 2011 found that changes in apelin levels were not linked to weight reduction in obese children in a study assessed risk factors before and after one year of regular lifestyle modulations [35].

Moreover, in our study we found that there was a significantly positive correlation between Apelin and insulin, similar finding had been detected by other authors and explained by that the increased levels of both serum apelin and insulin could reflect impairment apelin homeostasis and also supposed that higher serum insulin concentrations could increase serum apelin levels [12].

In the current work, there was a significant increase in HOMA-IR in obese children in comparison to control group, also we found a significant positive correlation between Apelin and HOMA-IR and this in concordance with Li et al., 2006 who described a positive correlation with HOMA-IR in patients with type 2 diabetes and impaired glucose tolerance [36].

There is a potent correlation between obesity and parameters of insulin sensitivity caused by apelin released by adipose tissue [12]. It was mentioned that apelin suppresses the secretion of insulin plasma systems [37] [38]. Many authors also suggested that apelin may act as powerful insulin sensitising factor and could be a potent target for diabetes elimination and management due to its ability to increase insulin sensitivity [11].

In the present study, we observed a significant difference in diastolic blood pressure between obese and control groups, and there was a significant positive correlation between Apelin and SBP, DBP. These findings don't agree with Samir et al., 2015, who found a similar increase in serum apelin in obese hypertensive and nonhypertensive obese individuals but they found no correlation between systolic blood pressure, diastolic blood pressure, and serum apelin [38]. Also, Rittig et al., 2011, who evaluated the relation between apelin serum levels, body fat distribution and insulin sensitivity/resistance as dependent cardiovascular risk factors; blood pressure was reported to be unaffected by serum apelin levels [39].

In conclusion, apelin levels are significantly higher in obese children when compared to control group and correlate significantly with insulin, HOMA-IR, lipid profile and hypertension in these children suggesting that this adipokine may act as potential biomarkers for evaluation of metabolic risk factors in obesity.

Further studies with large sample size are in need to explain the role and mechanisms of action of apelin in association with obesity-related markers and metabolic diseases

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1359-1364.
https://doi.org/10.3889/oamjms.2018.313
eISSN: 1857-9655
Basic Science

Investigating of Moringa Oleifera Role on Gut Microbiota Composition and Inflammation Associated with Obesity Following High Fat Diet Feeding

Elham M. Youssef Elabd[1*], Safaa M. Morsy[2], Heba A. Elmalt[2]

[1]Biochemistry Department, National Research Centre, Cairo, Egypt; [2]Medical Biochemistry Department, National Research Centre, Cairo, Egypt

Citation: Elabd EMY, Morsy SM, Elmalt HA. Investigating of Moringa Oleifera Role on Gut Microbiota Composition and Inflammation Associated with Obesity Following High Fat Diet Feeding. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1359-1364. https://doi.org/10.3889/oamjms.2018.313

Keywords: Moringa oleifera; Obesity; Gut bacteria; Bifidobacterium; Lactobacillus

***Correspondence:** Elham M Youssef Elabd. Biochemistry Department, National Research Centre, Cairo, Egypt. E-mail: elhamelabd@yahoo.com

Received: 28-May-2018; **Revised:** 09-Jul-2018; **Accepted:** 27-Jul-2018; **Online first:** 19-Aug-2018

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

AIM: The alteration in the gut microbial community has been regarded as one of the main factors related to obesity and metabolic disorders. To date, little is known about Moringa oleifera as a nutritional intervention to modulate the microbiota imbalance associated with obesity. Therefore we aim to explore the role of aqueous Moringa oleiferous leaf extract on Lactobacilli and Bifidobacteria in high-fat diet-induced obesity and to investigate whether any restoration in the number of caecal Lactobacilli and Bifidobacteria could modulate obesity-induced inflammation.

METHODS: Young Swiss albino mice were divided into three groups according to their diet. Two of them were fed on either high fat diet or high fat diet+aqueous extract of Moringa oleifera leaf, while the third group was fed on the control diet. Bacterial DNAs were isolated from the mice digesta samples for bacteria level estimation using Quantitative real-time polymerase chain reaction along with serum interleukin-6 and lipid profile

RESULTS: Compared to the normal control mice, high-fat diet feeding mice showed significantly reduced intestinal levels of Bifidobacteria, and increased body weight, interleukin 6, and levels of Lactobacilli. Upon treatment with Moringa oleifera, body weight, interleukin 6, and both bacteria levels were significantly restored

CONCLUSIONS: Our findings suggest that Moringa oliefera aqueous leaf extract may contribute towards the pathophysiological regulation of weight gain, inflammation associated with high-fat-induced-obesity through gut bacteria modulation.

Introduction

Obesity is well known to be a consequence of contributing factors, including hereditary, metabolic, behavioural and environmental factors [1] [2] [3]. Gut microbiota has been viewed as one of the main environmental factors identified with obesity and metabolic disorders [4] [5] [6]. Indeed the major cause of the development of obesity is the imbalance between energy intake and energy expenditure. The microorganisms residing in the gut influence the entire body metabolism by affecting such energy balance, in addition to other mechanisms including inflammation, gut barrier function, integrate peripheral and central nutritionally relevant information, therefore, increase adiposity and body weight [4] [7] [8] [9].

Snaz et al., and other researchers have supported this view and gained great insight into the relationship between gut bacteria and metabolic disorders [10]. However, certain phyla and classes of bacteria are associated with the improved transfer of calories from the diet to the host, and with alterations in the host metabolism of absorbed calories [6] [11] [12] [13] [14]. They ferment dietary polysaccharides and convert them into monosaccharides and short-chain fatty acids (SCFAs). These metabolites are subsequently absorbed and react as a source of energy by the host. Moreover, SCFAs, through free fatty acid receptors 2 (FFAR2) and 3 (FFAR3), are thought to participate in the regulating of different gut hormones. In addition to their vital role in nutrient absorption and metabolism regulation they control the proliferation of some pathogenic bacteria existed in the intestinal tract [15], induce the immune system, contribute in the production of vitamins and enzymes

such as vitamin K and biotin, and in the synthesis of useful compounds for the mucosa and cell renewal [16] [17].

Lactobacillus (Lb) and Bifidobacteria are the major component of gut microbiota that are recognised for their basic roles in normal physiological processes. Moreover, they include such probiotics as Lactic acid bacteria (LAB), which have been proved benefit effects to human health. Interestingly, they show an alteration in diet-induced obesity via different mechanisms. Those beneficial intestinal Lactobacilli and Bifidobacteria can synthesise bioactive isomers of conjugated linoleic acid that have antidiabetic, anti-atherosclerotic, immunomodulatory, and anti-obesity properties [18]. For the close connection between gut microbiota composition and metabolic disorders, more studies are needed to focus on how the composition of gut bacteria community be modified to reduce the risk of obesity and the associated metabolic changes.

Currently, considerable efforts have been made using plants as traditional natural medicines for reducing body weight and healing many diseases. Among them is M. oleifera that belongs to the Moringaceae family. The medicinal values of M. oleifera plant parts such as roots, bark, leaves, flowers, fruits, and seeds have been documented to have antimicrobial activity [19] [20] [21] [22], antidiabetic [23] [24], hepatoprotective [25]. Recently, hypocholesterolemic and antiobesity activity of crude extract of M. oleifera leaf was explored [26] [27], but whether the anti-obesity property is related to modulating the gut microbiome has not been yet investigated; hence our study was conducted to investigate the effect of aqueous extract of M. oleifera leaves on caecal Lactobacillus and Bifidobacteria in experimentally induced obesity.

Material and Methods

The experimental procedures were carried out in biochemistry and molecular biology labs in the national research centre and Clinilab, Cairo, Egypt, following the actual law of animal protection which was approved by the Ethical Committee of National Research Center.

Moringa oleifera leaf extract was used in this study after being collected from Pilbis, Sharqia Governorate, Egypt, and processed in the labs of Moringa Unit, National Research Centre. As powdered air dried Leaves (1 Kg) were extracted with (100 mL) of distilled water by boiling at a temperature from 80 to 100°C in reflux for 3 h to achieve an initial extract. The extract was then filtered with filter paper after cooling to room temperature. The aqueous extract stock solution (100 mg/mL) was stored at 4°C until further use according to the method used by Isitua et al., in 2013 [28].

Two types of diets were used in feeding a total of 45 young Swiss albino mice of either sex, (18–20 g). A normal diet has fat content 3% of energy, and a high-fat diet (HFD) has fat content 20% of energy [29]. The fat content of the HFD was beef tallow 15% and corn oil 5%. The diets have a formula covering the nutrient requirements of the mice which were divided into three groups of 15 in a completely randomized design: Group 1, Normal control group in which the animals were fed on a standard chow diet and water ad libitum; Group 2, H.F.D (obese) group in which the animals were fed on H.F.D (standard chow diet+20% beef tallow) and water ad libitum; Group 3, H.F.D+M. oleifera (treated) group, in which the animals were fed on H.F.D and received M. oleifera leaf extract orally along the experiment duration (200 mg/Kg).

After 3 months of treatment, all mice were anaesthetised with ether and sacrificed. The abdominal cavity was opened to expose the gastrointestinal tract. The digest of the cecum was removed immediately and were stored at -80°C until further analyses. Blood samples were collected by carotid bleeding separately into sterilised dry centrifugation tubes and allowed to stand for 30 minutes at 20–25°C. The clear serum was separated at 2500 rpm for 10 min using a centrifuge. Serum total cholesterol (Tc), serum high-density lipoprotein cholesterol (HDLc), serum triglycerides (TG), all were performed using Biodiagnostic Chemical Company kit (Egypt) according to the instructions of the suppliers.

Serum low-density cholesterol LDL-C was calculated according to the equation, as follows:

LDL-C = Total cholesterol-HDL-C-(Triglyceride/5) [30].

Serum Interleukin 6 as an inflammatory marker was performed using Enzyme Immunoassay kit (ELSA) according to the instructions of the suppliers (BIOS Company) Bacterial DNA was isolated from the digested samples using QIAamp DNA Stool Mini Kit according to the manufacturer's protocol. The pellet was suspended in 180 µl TE buffer [10 mmol/L Tris-HCl (pH 8.0), 2 mmol/L EDTA], and the mixture was briefly mixed on a vortex mixer. The suspension was incubated for 30 min at 37°C, 20 µl proteinase K and 200 µl Buffer were added. The pellets were mixed by vortexing and incubated at 56°C for 30 min and then for further 15 min at 95°C. The supernatant was transferred to a sterile tube and was stored at -20°C until PCR testing (Qi gene protocol). Quantitative real-time PCR was carried out with QuantiTect SYBR Green real-time PCR detection system (Qi gene). Two different genus-specific primer sets were used in this study, (g-Bifid-F/g-Bifid-R) for Bifidobacterium and (Lacto-16S-F/Lacto-16S-R) for Lactobacillus [31].

The 20-µl reaction mixture contained 10 µl of QuantiTectTM SYBR® green master mix, 1 µl of 25 mmol l−1 $MgCl_2$, 1 µl (0·2%) of BSA, 2 µl of each primer (5 mmol l−1) and 4 µl of template DNA extraction. Each PCR run included a 15-min activation time at 95°C as required by the instrument. The three-step cycle included denaturing (94°C, 15 s), annealing (60°C, 30 s) and extension (72°C, 30 s). At the end of each PCR run, melting curve analysis was performed from 72 to 95°C for detecting nonspecific PCR product and primer-dimers.

The agarose gel electrophoresis was performed to confirm further the specific PCR products. The PCR products were separated by an electrophoresis system at a constant voltage of 80V for 50 min. Then, the gel was stained with ethidium bromide (Sigma, USA) staining (0.5 μgml^{-1}) for 5 min and followed by washing with distilled water for about 30 min. Finally, the gel was visualised under UV transilluminator (VilberLourmat, Cedex, France) and the photos were taken using a gel documentation system (Bio-Rad Gel Doc 2000 Model Imaging System).

Standards were set-up before starting quantitative sample DNA extraction by real-time PCR for quantifying the sample DNA.

Results

Our results showed the high efficiency of dietary fat in the significant (P < 0.05) increase of the blood lipid, IL-6 levels along with the significant (P < 0.05) increase in total body weigh in mice fed on HFD compared to control (Table 1 & 2).

Table 1: Statistical significant differences of lipid profile and IL-6 in high-fat diet group after 3 months of feeding P < 0.05

Parameters	Groups	N	Min.	Max.	Mean±S.D.	Per cent change	P value
Cholesterol (mg/dl)	Control group	15	106	177	36.64 ± 23.59	100%	0.001
	H.F.D. (Obese) group	15	200	247	221.79 ± 14.38	162.31%	
Triglycerides (mg/dl)	Control group	15	65	94	82.14 ± 9.36	100%	0.001
	H.F.D. (Obese) group	15	130	228	167.29 ± 30.30	203.65%	
HDL (mg/dl)	Control group	15	39	80	54.29 ± 13.47	100%	0.001
	H.F.D. (Obese) group	15	30	45	36.79 ± 5.09	67.76%	
LDL (mg/dl)	Control group	15	46	101	70.93 ± 18.41	100%	0.001
	H.F.D. (Obese) group	15	82	132	102.93 ± 14.95	145.12%	
IL-6 (mg/dl)	Control group	15	2.16	7.40	3.95 ± 1.54	100%	0.001
	H.F.D. (Obese) group	15	2.29	14.22	7.67 ± 2.72	194.23%	

Upon high fat-diet feeding, the amount of Lactobacilli in caecal digesta was significantly (P < 0.05) higher than those in control group while the amount of Bifidobacteria was significantly (P < 0.05) lower (Table 3).

Table 2: Statistical significant differences in total body weight in the obese group after 3 months of feeding P < 0.05

Parameters	Groups	N	Min.	Max.	Mean ± S.D.	P value
Total body weight (g)	Control group	15	18	21	19.5 ± 1.20	0.001
	Obese group	15	27	35	31.0 ± 1.11	

Table 3: Statistical significant differences of Bifidobacteria and Lactobacillus levels in the obese group after 3 months of feeding P < 0.05

Parameters	Groups	N	Min.	Max.	Mean ± S.D.	Per cent Change	P value
Bifidobacteria	Control group	15	7.50	11.00	9.25 ± 1.29	100%	0.001
	Obese group	15	3.00	6.50	4.65 ± 1.11	50.27%	
Lactobacilli	Control group	15	7.00	10.90	8.72 ± 1.06	100%	0.001
	Obese group	15	17.5	24.00	21.08 ± 2.04	241.74%	

After three months of treatment with the aqueous extract of Moringa oleifera leaf, these mice showed a significant (P < 0.05) decrease in total body weight, Lactobacillus, blood cholesterol, triglycerides, LDLc and a significant (P < 0.05) increase in Bifidobacteria, HDLc compared to H.F.D (obese group). However, the decrease in IL-6 was not significant (Table 4, 5, 6).

Table 4: Statistical significant differences in biochemical parameters in obese and Moringa treated groups after 3 months of treatment with Moringa oleifera aqueous leaf extract P < 0.05

Parameters	Groups	N	Min.	Max.	Mean ± S.D.	Per cent change	P value
Cholesterol (mg/dl)	H.F.D. (Obese) group	15	200	247	221.79 ± 14.38	100%	0.001
	Moringa oleifera treated group	15	150	200	170.50 ± 16.98	76.88%	
Triglycerides (mg/dl)	H.F.D. (Obese) group	15	130	228	167.29 ± 30.30	100%	0.001
	Moringa oleifera treated group	15	100	150	119.50 ± 15.79	71.43%	
HDL (mg/dl)	H.F.D. (Obese) group	15	30	45	36.79 ± 5.09	100%	0.001
	Moringa oleifera treated group	15	45	85	63.71 ± 11.98	173.20%	
LDL (mg/dl)	H.F.D. (Obese) group	15	82	132	102.93 ± 14.95	100%	0.001
	Moringa oleifera treated group	15	54	120	75.86 ± 23.33	73.70%	
IL-6 (mg/dl)	H.F.D. (Obese) group	15	2.29	14	7.67 ± 2.72	100%	0.478
	Moringa oleifera treated group	15	3.02	11.21	6.98 ± 2.36	90.98%	

Discussion

The significant metabolic disturbance in sort of total body weight gain, and blood lipid increase, in our high-fat diet group mice, is obviously due to the positive fat balance and consequently to adipose mass accumulation [32] [33].

Table 5: Statistical significant differences of total body weight in obese and Moringa treated groups after 3 months of treatment with Moringa oleifera aqueous leaf extract P = 0.05

Parameters	Groups	N	Min.	Max.	Mean ± S.D.	P value
Total body weight (g)	Obese group	15	27	35	31 ± 1.11	0.05
	Moringa oleifera treated group	15	26	29	28 ± 2.3	

Energy balance not only depends on the diet but also on the microbiome as the amount of energy harvested is hypothesised to be influenced by the composition of the gut microbiota [34] [35] [36].

The HF diet group mice in this study had lower numbers of Bifidobacteria and higher numbers of Lactobacilli compared to control. Hildebrandt et al. approved in 2009 such alteration which associated with switching to the HF diet, a decrease in the Bacteroidetes level and an increase in both Firmicutes and Proteobacteria levels, in particular [37].

Table 6: Statistical significant differences of Bifidobacteria and Lactobacillus levels in obese and Moringa treated groups after 3 months of treatment with Moringa oleifera aqueous leaf extract P < 0.05

Parameters	Groups	N	Min.	Max.	Mean ± S.D.	Per cent Change	P value
Bifidobacteria	Obese group	15	3.0	6.50	4.65 ± 1.11	100%	0.001
	Moringa oleifera treated group	15	6.00	9.10	7.92 ± 0.96	170.35%	
Lactobacilli	Obese group	15	17.50	24.00	21.08 ± 2.04	100%	0.001
	Moringa oleifera treated group	15	12.50	18.00	14.73 ± 1.65	69.86%	

Recent work that was conducted by the Turnbaugh team showed that specific enzymatic activities of obese individuals were found in Gram-positive bacteria of the Firmicutes phylum (of which Lactobacillus belong to) rather than in Bacteroidetes [38] [39] [40]. They also described a decrease in Proteobacteria and Bifidobacterium spp. Upon high fat-diet feeding [41].Other work by Million supports that obesity is linked to enrichment in the concentrations of gut Lactobacilli, especially Lactobacilli reuteri [40] [42].

There are several possible suggested mechanisms as a causal link between gut microbiota alterations and obesity. Among them are:

1. Increasing energy recovery from the diet by gut microbiota which influences host energy storage.

2. Modulating liver lipogenesis.

3. Regulating appetite through gut satiety hormones.

4. Activating innate immunity through LPS-Toll-like receptors [43] [44].

In the framework of microbiota-related nutrition, the clear energy imbalance could affect the gut barrier through changing the intestinal wall leading to a state of inflammation; a potential condition caused an increase in blood IL-6 level shown in our high-fat feeding mice. In 2016, Agus A et al., confirmed a modification of intestinal mucosa by dysbiosis becoming thinner and more permeable to pathogens and antigens with a consequent establishment of chronic low-grade, inflammation [45].

Although attempts to use natural remedies for weight loss has increased. Very little is known about the impact of Moringa on the gut microbiome which is a significant factor in the pathogenesis of obesity. Available evidence approved that leaves of M. oleifera possess hypolipidemic and antiobesity effects [19] [20] [21] [22] [23] [24] [25] [26] [27]. Whether its contribution is part of improving dysbiosis associated with diet-induced obesity is poorly understood. Therefore our study was conducted to explore the link between the gut microbiota alteration and the anti-obesity effect of the Moringa leaf extract.

Our results confirmed that treatment with Moringa oleifera leaf extract was effective in reducing weight gain and the consequent metabolic disturbance in obese group mice following H.F.D feeding. Apart from a reduction in body weight, treatment with aqueous extract of Moringa oleifera leaf was observed to attenuate the levels of total cholesterol and LDL significantly and increased the level of HDL level in mice fed on HFD. According to Farooq F et al., the hypolipidaemic effect of different medicinal plants has been related to their bioactive components [46]. A mechanism by which these compounds may decrease plasma cholesterol in our obese mice is that flavonoids that are contained in Moringa leaf extract may augment the activity of lecithin acyltransferase (LCAT), which regulates blood lipids. LCAT plays a key role in the incorporation of free cholesterol into HDL (this may increase HDL) and transferring it back to VLDL and LDL which are taken back later in liver cells [46] [47].

Moreover, mice in Moringa leaf extract treated group showed an improvement in dysbiosis by increasing Bifidobacteria level compared to those fed on HF diet. It is likely that changes in such gut resident microbe by Moringa treatment have a significant impact on another metabolic disturbance progression such as lipid levels and weight gain because Bifidobacteria can help Bacteroides degrade polysaccharides and inhibit exogenous cholesterol absorption from the small intestine [48].

In the last few decades, some researches linked the weight lowering effect of Moringa oleifera to the polyphenols present in their leaf extract. However, a more recent work added that polyphenols could modify the gut microbial composition, and had been converted by them to bioactive compounds in a two-way relationship “polyphenols↔microbiota” which greatly influence host health. On the one hand, the first direction Polyphenol, microbiota relationship has been investigated by Pacheco-Ordaz R and his team in very recent work in 2018. They reported that phenolic compounds inhibit the growth of some pathogenic bacteria such as *Escherichia coli*, while they promote the growth of some probiotics such as Lactobacillus. On the other hand, the reverse direction of microbiota, polyphenolic relationship, evidence showed that Bifidobacterium sp. and Lactobacillus sp. are among the few bacterial species catalyzing the metabolism of phenolics through some catabolic pathways most likely are responsible for the extensive breakdown of the polyphenolic structures into a series of low-molecular-weight phenolic metabolites that, being absorbable. The mechanism could be either through the glycan-degrading capability of Bacteroides, which is higher than Firmicutes or through the end products of colonic metabolism of polyphenols [50].

In this respect, Moringa oleifera leaf extract in our study could serve as a gut modulator in obesity-induced dysbiosis, however further studies are still needed.

Looking at the significant increase of Bifidobacteria level along with the decreased level of

IL-6 in Moringa oleifera treated group, predicts an improvement effect on the inflammatory status after treatment. This is in parallel with Cani et al., findings who used a rat obesity model to study the mechanism of inflammation associated with dysbiosis in obese mice. A significant correlation between increased levels of Bifidobacteria and reduced leakiness in the gut was observed, therefore allowing fewer LPS to translocate to the serum [51] [52].

Overall, these results support the dysbiosis state in response to a high-fat diet feeding and suggest that the response to Moringa oleifera leaf extract may be one potential approach to manage body weight and inflammation related to obesity through gut microbiota.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1365-1369.
https://doi.org/10.3889/oamjms.2018.297
eISSN: 1857-9655
Clinical Science

Thr83ala Gene Polymorphism Association with Arterial Calcification, Acute Coronary Syndrome and Ischemic Strokes in Older Adults

Yurii O. Ataman[1], Albina V. Zharkova[1*], Andrii V. Grek[2], Tetiana M. Rudenko[1], Maryna A. Gordina[1]

[1]Family Medicine Department, Sumy State University, Sumy, Ukraine; [2]Internal Medicine Department, Sumy State University, Sumy, Ukraine

Citation: Ataman YO, Zharkova AV, Grek AV, Rudenko TM, Gordina MA. *Thr83ala* Gene Polymorphism Association with Arterial Calcification, Acute Coronary Syndrome and Ischemic Strokes in Older Adults. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1365-1369. https://doi.org/10.3889/oamjms.2018.297

Keywords: Lower extremity arterial calcification; Matrix Gla protein; Gene polymorphism

***Correspondence:** Albina V Zharkova. Family Medicine Department, Sumy State University, Sumy, Ukraine. E-mail: a.v.zharkova@gmail.com

Received: 25-Apr-2018; **Revised:** 24-Jun-2018; **Accepted:** 03-Jul-2018; **Online first:** 15-Aug-2018

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Calcification of the arteries of the lower extremities is a very common pathological process, which has an independent significance in the development of cardiovascular diseases. There is evidence that development of calcification of the arteries correlates with a mutation of the MGP protein gene representing by the *Thr83Ala* polymorphism.

AIM: The purpose of the study was to analyse the connection of *Thr83Ala* polymorphism of the MGP gene with the development of calcification of the arteries of the lower extremities.

METHODS: The study involved 80 patients. Half of them had signs of calcification of the arteries of the lower extremities. The allelic *Thr83Ala* polymorphism of the MGP protein gene was determined by polymerase chain reaction, establishing the presence of calcification of the arteries by radiological and dopplerographic methods.

RESULTS: The study aimed to analyse the association of the *Thr83Ala* polymorphism of the matrix Gla protein gene with the development of calcification of the arteries of the lower extremities. The data obtained suggest that the replacement of threonine by alanine in the 83rd position of the MGP molecule can affect the functional properties of the protein and in particular its anticarcinogenic properties. Although there was no difference in the distribution of different variants of the genotype by *Thr83Ala* to the MGP gene polymorphism in patients with CA and healthy patients, but in the distribution of genotypes in the comparison groups separated by sex, it was found that in women, carriage of the Ala allele in a homozygous state is a factor, which protects the development of arterial calcination in the elderly and senile.

CONCLUSION: Differences in the distribution of different variants of the genotype according to *Thr83Ala* to the polymorphism of the MGP gene between patients with CAD and healthy patients do not exceed the limits of statistical significance. In the distribution of genotypes in the comparison groups divided by sex, it was found that in women the carrier of the Ala-allele in the homozygous state is a factor that prevents the development of Menkeberg arteriosclerosis in the elderly and old age.

Introduction

Calcification of the arteries of the lower extremities (CA) is a risk factor for associated acute cardiovascular events, as well as gangrene of the lower extremities [1] [2] [3]. According to modern studies, calcification of peripheral arteries in older age groups is very common and ranges from 33.3% [1] to 50.2% [4].

It is known that the matrix Gla protein (MGP) is a strong inhibitor of vascular calcification. It refers to representatives of the group of vitamin K-dependent proteins containing the residues of γ-carboxyglutamic acid (Gla). The very same group also comprises proteins, participating in blood coagulation: prothrombin, factors VII, IX and X, proteins C, S and Z. The human MGP molecule (molecular weight 10kDa) includes 84 amino acid residues, five of which are represented by γ-carboxyglutamic acid (Gla) [5]. The newly synthesized MGP molecule consists of 103 amino acid residues (84 is a mature protein, 19 is a transmembrane signal peptide) and contains, starting from at the N-terminus, three functional fragments: a transmembrane signal peptide, a probable site that

recognizes γ-carboxylase (putative recognition site for γ-carboxylase), a domain, containing Gla (Gla-containing domain) residues [6].

The Formed in the cells MGP experiences undergo post-translational modification, which consists of the carboxylation of five glutamic acid residues (Glu) to form a γ-carboxyglutamic acid (Gla). This reaction is catalysed by the enzyme γ-glutamyl carboxylase (GGCX) and is conjugated with the oxidation of the reduced form of vitamin K (hydroquinone) to the 2,3-epoxide of vitamin K. Thus, without the oxidation of vitamin K, the carboxylation of the Glu residues of the MGP molecule cannot occur. In turn, an enough amount of vitamin K for the carboxylation reaction of MGP depends on the state of the reverse reaction of its reduction, which is carried out with the help of a vitamin K-epoxy-reductase complex (VKRC). In addition to γ-carboxylation, MGP can undergo other post-translational modifications, specific proteolytic splitting in the C-terminal fragment of the molecule [7] [8] and phosphorylation of three serine residues at the N-terminal tail [9]. The MGP gene in humans is represented by a single copy, which is containing in the short upper arm of the 12th chromosome (12p12.3-13.1) [6]. It encodes 84 amino acid residues of the mature protein and 19 residues of the transmembrane signal peptide. The length of the gene is 3900 nucleotides; it consists of 4 exons, separated by three large intermediate sequences (introns), containing for more than 80% of the total length of the gene.

The single nucleotide polymorphism of Thr83Ala (rs4236) is such rearrangement, when in the 4th exon of the MGP gene, and at 3748 positions, thymine is replaced by adenine. This leads to the fact that the 83rd amino acid of the MGP molecule of threonine is replaced by alanine. The change in the primary structure of protein molecules can request in a variety of functional disorders; in case of MGP, one should expect changes in these familiar protein effects that are associated with its anticarcinogenic properties. These include, for example, binding to calcium ions and hydroxyapatite crystals; binding to extracellular matrix components; interaction with the BMP-2 and elimination of the latter's effects; participation in the regulation of apoptosis [10] [11].

Thus, the purpose of the study was to analyse the connection of Thr83Ala polymorphism of the MGP gene with the development of calcification of the arteries of the lower extremities.

Methods

Eighty patients, attending Sumy Region Veterans Affairs, were involved in this study. Half of them (40 persons) had signs of calcification of the arteries of the lower extremities (main group), and the other 40 persons-had no signs (control group). Patients with diagnosed diabetes mellitus were not added to any of the study groups that allowed it possible to exclude the effect of this factor on the studied connections. The control group and the main group of patients did not differ according to persons of different sexes (P = 0.369), as per middle age (74.4 ± 0.76 vs. 74.4 ± 0.97, P = 0.984) as well as according to body mass index (26 02 ± 0.22 versus 26.01 ± 0.35, P = 0.999). The presence of SC was determined to base on X-ray and Doppler data [12].

The studies were carried out in compliance with the main provisions of the Council of Europe Convention on Human Rights and Biomedicine, as well as the Helsinki Declaration of the World Medical Association on the Ethical Principles of Scientific Medical Research with Human Participation (1964, with subsequent additions, including the 2000 version). All patients signed informed consent to participate in the studies with a subsequent collection of venous blood for genetic analysis.

Determination of gene polymorphism was performed using the polymerase chain reaction (PCR) method with subsequent by analysis of the length of the restriction fragments upon their detection using restriction fragment length polymorphism (RFLP).

For genotyping, venous blood was collected in sterile conditions in a mono volume of 2.7 ml with potassium salt of ethylenediaminetetraacetic acid ("Sarstedt", Germany), which served as an anticoagulant. The blood was frozen and stored at the temperature of -20°C. DNA was extracted from it using the "Izogen" kits (Russia).

Amplification of the fragment of the gene containing the Thr83Ala site of polymorphism in the 4th exon of the MGP gene was performed using a pair of specific primers: sense-5'-TCAATAGGGAAGCCTGTGATG-3` and antisense-5`-AGGGGGATACAAAATCAGGTG-3. The amplification program was as follows: denaturation-94°C (50 c), hybridisation of primers, 64.5°C (45 c), elongation-72°C (1 min), totally 33 cycles.

Later, 6 μl of the amplification product was incubated at the temperature of 37°C during 18 hours with 3 units of Eco477 restriction enzyme in R buffer of the following composition: 10mM Tris-HCl (pH 8.5), 10 mM magnesium chloride 100 mM potassium chloride and 0.1 mg/ml albumin. The presence of adenine in the 3748 position of the MGP gene prevents restriction, and upon substitution of adenine by thymine restriction, it splits the amplified area of the 4th exon (length-173 pairs of nitrogen bases) into two fragments: 127 and 46 base pairs (Figure 1).

Amplifications of the achieved fragment of the MGP gene after restriction were separated in a 2.5% agarose gel, containing ethidium bromide. Horizontal

electrophoresis (0.1 A; 140 V) was performing for 20 minutes. DNA visualisation after electrophoresis was performed using a transilluminator ("Biocom", Russia).

Statistical analysis was carried out by using the SPSS-17 program. In this case, the reliability of the differences was determined by the Pearson χ2-criterion and the Students t-criterion. A value of $p < 0.05$ was considered reliable. The odds ratio (OR) and the 95% confidence interval were calculated using the logistic regression method.

Results

There are three possible variants of the genotype for the *Thr83Ala*: *Thr/Thr* (homozygous for the main allele), *Thr/Ala* (heterozygotes) and *Ala/Ala* (homozygous for the minor allele) polymorphism (Figure 1).

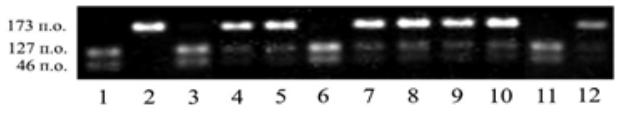

Figure 1: Results of the electrophoresis of fragments of the MGP gene after restriction for the detection of Thr83Ala polymorphism. Tracks 1, 3, 6, 11 correspond to the Thr/Thr genotype; 4, 5, 7, 8, 9, 10, 12-Thr/Ala-genotype; 2-Ala/Ala genotype

Genotyping patients with CA and patients of the control group for this SNP allowed establishing the frequency with which one can come across the certain variants of this gene meet and allow to compare them among the groups and according to gender. When checking the correspondence of the distribution of alleles to the Hardy-Weinberg law, it was established that the ratio of Thr and Ala alleles in both groups does not differ significantly from those expected (Table 1).

Table 1: The frequency of allelic variants and alleles according to *Thr83Ala* polymorphism of the MGP gene in the control group and patients with CA

	Control group	Patients with CA
Homozygotes *Thr/Thr*, n (%)	13 (32.5)	16 (40.0)
Heterozygotes *Thr/Ala*, n (%)	17 (42.5)	19 (47.5)
Homozygotes *Ala/Ala*, n (%)	10 (25.0)	5 (12.5)
Thr-allele	0.54	0.64
Ala-allele	0.46	0.36
χ^2	0.84	0.03
P	> 0.05	> 0.05

Note: n-the number of patients, χ2 and P reflect the deviation in each group from the Hardy-Weinberg equilibrium

Table 2 shows the results of the analysis of the frequencies of individual phenotypes according to the *Thr83Ala* polymorphism in the control group and patients with CA. They show that the ratio of homozygotes for the main allele (*Thr/Thr*), heterozygotes (*Thr/Ala*) and homozygotes for the minor allele (*Ala/Ala*) in patients with arteriocalcinosis comprises 40%, 47.5% and 12.5%, while in the control group the corresponding indices were 32.5%, 42.5% and 25%. Differences in the distribution of different variants of the genotype between patients with CA and healthy patients did not exceed the statistical significance.

Table 2: The Association of the *Thr83Ala* polymorphism of the MGP gene with the CA

		Control	CA	Totally
Thr/Thr	N	13	16	29
		32.5%	40.0%	
Thr/Ala	N	17	19	36
		42.5%	47.5%	
Ala/Ala	n	10	5	15
		25.0%	12.5%	
Totally	n	40	40	80
		100%	100%	
		$X^2 = 2.088$; P = 0.352		

Note. N is the number of patients.

However, the analysis of the distribution of genotypes in sex-disaggregated comparison groups allowed to identify certain significant differences. In general, the ratio of the two variants of homozygotes and heterozygotes (*Thr/Thr: Thr/Ala: Ala/Ala*) in women and men did not differ in individuals with signs and without signs of CA (Table 3). In the first group, it was 40%: 55%: 5% for women and 40%: 40%: 20% for men, and for control group 18.8%: 50%: 31.2% for women (P = 0.081) and 41.7%: 37.5%: 20.8% for men (P = 0.986). However, it is easy to see that for this indicator, female representatives were significantly closer to the level of statistical significance than men.

Table 3: The association of the *Thr83Ala* polymorphism of the MGP gene with CA in females and males

Gender	Genotype		Control	CA	Totally
Female	*Thr/Thr*	n	3	8	11
			18.8%	40.0%	
	Thr/Ala	n	8	11	19
			50.0%	55.0%	
	Ala/Ala	n	5	1	6
			31.3%	5.0%	
	Totally	n	16	20	36
			100%	100%	
			$\chi^2 = 5.031$; P = 0.081		
Male	*Thr/Thr*	n	10	8	18
			41.7%	40.0%	
	Thr/Ala	n	9	8	17
			37.5%	40.0%	
	Ala/Ala	n	5	4	9
			20.8%	20.0%	
	Totally	n	24	20	44
			100%	100%	
			$\chi^2 = 0.029$; P = 0.986		

Note: n - the number of patients.

In women, the difference between patients with CA and patients without signs of arterio-calcinosis was found when comparing individual variants of the genotype with each other. Therefore, it was found that the frequency of homozygotes in the minor allele (*Ala/Ala*) was significantly higher in control than in patients with CA (31.3% vs 5%, P = 0.036).

Using the method of logistic regression, it is shown (Table 4), that in women with the Ala/Ala genotype the risk of CA is 0.075 when comparing with homozygotes over the main allele (P = 0.044). This

means that elderly and senile patients homozygous for the Ala allele have a CA risk more than 10 times lower than *Thr/Thr* homozygotes.

Table 4: Results of the analysis of the *Thr83Ala* association of the MGP gene polymorphism with calcification of the lower extremities arteries in women and men (logistic regression method)

							95% CI for OR	
	Genotype	CR	SE	WS	P	OR	Lower	Higher
Totally	*Thr/Ala*	-0.096	0.501	0.037	0.847	0.908	0.340	2.424
	Ala/Ala	-0.901	0.663	1.847	0.174	0.406	0.111	1.490
Women	*Thr/Ala*	-0.662	0.821	0.651	0.420	0.516	0.103	2.578
	Ala/Ala	-2.590	1.288	4.046	0.044	0.075	0.006	0.936
Men	*Thr/Ala*	0.105	0.679	0.024	0.877	1.111	0.294	4.205
	Ala/Ala	0	0.822	0	1	1	0.200	5.004

Note data on homozygous for the main allele (*Thr/Thr*). CR-coefficient of regression, SE-standard error, WS-Wald statistics, P-indicator of statistical significance, OR-odds ratio, CI-confidence interval.

Reduced risk of CA in women with the Ala/Ala genotype is also evident when comparing them with carriers of the Thr allele (*Thr/Thr+Thr/Ala*) and separately with heterozygotes (*Thr/Ala*). In the first case, the OR ratio is 0.116, and in the second case, 0.145. However, due to an inadequate number of observations, the statistical significance of P did not reach the generally accepted level of confidence and amounted to 0.063 and 0.105, respectively.

Therefore, there is a reason to state that in women the carriage of the Ala-allele in the homozygous state is a factor that prevents the development of arteriocalcinosis in the elderly and old age.

The results of molecular genetic studies, obtained by us in patients with calcification of arteries of the lower extremities, can be compared with the data, obtained in the study of the connection of the Thr83Ala polymorphism of the MGP gene with the most common complications of the atherosclerotic process-acute coronary syndrome (ACS) and ischemic atherothrombotic stroke (IATS). Data for this comparison are taken from the work of the laboratory of molecular genetic studies of Sumy State University [2] [13] and are presented in Table 5.

Table 5: Comparative characteristics of the distribution of genotypes according to *Thr83Ala* polymorphism of the MGP gene in patients with arterial calcification, ACS [2] and IATS [5], %

	Genotype	CA		ACS		IATS	
		Disease (n = 40)	Control (n = 40)	Disease (n = 115)	Control (110)	Disease (170)	Control (124)
Thr83Ala	*Thr/Thr*	40.0	32.5	4..6	43.9	39.4	34.7
	Thr/Ala	47.5	42.5	43.5	45.9	48.8	53.2
	Ala/Ala	12.5	25.0	13.9	10.2	11.8	12.1

Note: n - the number of patients.

The data indicate that the percentage of homozygotes in the minor allele (*Ala/Ala*) in the control group for the CA was more than twice as high (25%) when comparing with the control groups of the other two studies (10.2% and 12.1%). At the same time, the percentage of such homozygotes in patients with CA (12.5%) corresponded to the above data for control in studies with ACS and IATS.

Since the homozygous condition of the minor allele in women interferes with the development of CA, the assumption of the age (physiological) origin of arteriocalcinosis is quite attractive. Since in our work patients with diabetes mellitus, which is one of the main causes of CA, were excluded both from the main and control groups, the presence or absence of lesions of the arteries should occur due to some other factors. One of such factors may be a hereditary predisposition, including the *Thr83Ala* polymorphism of the MGP gene. According to our data, it can be assumed that women homozygous for the Ala-allele are "abnormal" or "sick on the contrary," because their genetic factor interferes with the physiological ageing of the arteries inherent in most people in the elderly and senile ages.

The substitution of threonine for alanine at the 83rd position of the MGP molecule can affect the functional characteristics of the protein and in particular its anticarcinogenic properties. The latter, as is known today, is extremely important for preventing calcification of the arteries, as evidenced by experiments with genetically knocked out MGP (-/-) mice [14] and warfarin-mediated aortic medication in rats [15].

In conclusion: (1) differences in the distribution of different variants of the genotype according to Thr83Ala to the polymorphism of the MGP gene between patients with CA and healthy patients do not exceed the limits of statistical significance; (2) in the distribution of genotypes in the comparison groups divided by gender, it was found that in case of women the carrier of the Ala-allele in the homozygous state is a factor that prevents the development of Menkeberg arteriosclerosis in the elderly and old ages.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1370-1375.
https://doi.org/10.3889/oamjms.2018.274
eISSN: 1857-9655
Clinical Science

Analysis of Cardiovascular Disease Risk Factors in Women

Maria Stanislavovna Tairova[1*], Lucas Odacir Graciolli[1], Olga Sergueevna Tairova[1], Thiago De Marchi[2]

[1]Cardiovascular Rehabilitation Institute of Sports Medicine of Caxias do Sul, Caxias do Sul, Brazil; [2]Faculdade Cenecista of Bento Gonçalves, Rio Grande do Sul, Brazil

Citation: Tairova MS, Graciolli LO, Tairova OS, De Marchi T. Analysis of Cardiovascular Disease Risk Factors in Women. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1370-1375. https://doi.org/10.3889/oamjms.2018.274

Keywords: Cardiovascular risk; Women; Coronary heart disease

***Correspondence:** Maria Stanislavovna Tairova. Cardiovascular Rehabilitation Institute of Sports Medicine of Caxias do Sul, Caxias do Sul, Brazil. E-mail: mariatairova@hotmail.com

Received: 04-Apr-2018; **Revised:** 25-May-2018; **Accepted:** 28-May-2018; **Online first:** 14-Aug-2018

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

AIM: Study the cardiovascular risk factors in a feminine population vulnerable to cardiovascular events particularly to evaluate the principal factors or possible confounding variables.

METHODS: This is a cross-sectional descriptive study. Were analysed all the female patients from the Cardiovascular Rehabilitation Institute of Sports Medicine of Caxias do Sul who had the complete information on cardiovascular disease history, comorbidities and habits and who knew the complete gynaecological history by a phone interview.

RESULTS: A group of 91 patients were analysed. About the comorbidities and habits, 45.2% of these patients presented some tobacco load, 82.4% are hypertensive, 61.5% are dyslipidemic, 25.3% are diabetic and the BMI average was 29.27 (overweight). Between the patients who undergone a hysterectomy and had an episode of the acute coronary syndrome (10 patients), 70% had the event after the procedure. Between the post-menopause women with at least one episode of the acute coronary syndrome, 80.5% (33 patients) had the first event after the menopause.

CONCLUSION: We found multiple lifetime risk factors that predisposed the women of the sample to have cardiovascular disease. Between the women with specific to women risk factors and without, the prevalence of cardiovascular disease was very similar. This information supports the idea that these are just confounding factors of CVD and the principals involved are the genetic factors and habits. For this reason, the focus of CVD prevention and treatment should be directed towards these aspects.

Introduction

Coronary Heart Disease (CHD) is an important Cardiovascular Disease category (CVD) and is considered the most common cause of death in the United States (USA) as well as in Brazil.

Regarding epidemiology, diagnosis, treatment and prognosis of CVD, there are significant differences between men and women. These differences are extremely important for the care of women with a known or suspected disease and should be taken into consideration during medical care. In the past, CVD was considered a “male disease” since most studies excluded female patients or included them as a minority. However, this theory has been gradually disproved in light of new studies.

According to the American Heart Association´s statistics for Heart Disease and Cerebrovascular Accident updated in 2010, 17.6 million people have CVD in the USA, mainly men and women of more advanced age. The Framingham study showed that 40-year-old men have a lifetime risk of 49% to develop CVD while women, on the other hand, have a 32% risk. 70-year-old men have a 35% risk, while women of the same age have 24%. Furthermore, the occurrence of CVD at the age of 65-94 doubles in men and triples in women when compared to the incidence in the age range of 35-64 [1].

CVD generally manifests itself ten years later in women than in men and brings along an increased number of risk factors. Besides this, women tend not to identify its initial symptoms are delaying the moment of diagnosis and resulting in higher risk medical care. In pre-menopausal women, severe manifestations of CVD, such as acute myocardial infarction (MI) and sudden death, are relatively rare. On the other hand, in post-menopausal women, the

occurrence and severity of CVD increase sharply (three times more comparing to pre-menopausal women). Clinical manifestations of CVD have similarities and differences between the sexes. Chest pain is similar in both sexes both in prevalence and in pain level. Acute MI prevalence is higher in men, however, prevalence tends to decrease in men and an increase in women as the years go by. Regarding Heart Failure (HF), women tend to develop asymptomatic HF secondary to CVD more frequently than men. Lastly, men seem to have a more increased rate of sudden cardiac death in all ages and all levels of risk factors.

Apart from the classic CVD risk factors that are the same in men and women, existing literature describes that women also present unique risk factors. The first is menopause [2] during which the risk mechanism is not fully comprehended in the same way that it is not known if there is a direct causal relationship between menopause and CVD. Hysterectomy, oophorectomy, pre-menstrual syndrome, oral contraception and pregnancy complications such as systemic arterial hypertension (SAH), diabetes mellitus (DM), spontaneous abortion and preterm birth are also risk factors exclusive to women [3] [4] [5] [6].

Age, family history, DM, chronic kidney disease and metabolic syndromes are related to a significant increase in the risk of cardiovascular events in both sexes [7]. Nonetheless, risk factors related to lipoproteins present some peculiarities in women: low HDL is more predictive for cardiovascular events in women than a high LDL; Lipoprotein A is a risk determinant for CVD in pre-menopausal and post-menopausal women under the age of 66; the concentration of total cholesterol seems to be associated with CVD only in pre-menopausal women and triglycerides only in older women [8] [9]. A cross-sectional study conducted in Rio Grande de Sul found that obesity is a more prevalent risk factor for women, while SAH is for men [10].

Generally, women are a minority in CVR programs, and there are few studies on the subject, a fact that generates a lot of doubt about the handling and real evolution of women in these programs. Nevertheless, they seem to receive greater or similar benefits comparing to men [11].

The prevalence and mortality of cardiovascular disease are growing, especially when it comes to women, who are increasingly younger. Due to the lack of studies that focus on understanding the natural history, handling and prevention of CVD in women better, we aim to study the cardiovascular risk factors in a feminine population vulnerable to cardiovascular events particularly to evaluate the principal factors or possible confounding variables.

Methods

This is a cross-sectional descriptive study. The population surveyed included patients from the Cardiovascular Rehabilitation Institute of Sports Medicine of Caxias do Sul (SMI).

The data collection process for the study was conducted in three stages. Firstly, initial medical evaluation data were collected from all patients who entered the Cardiac Rehabilitation Service of SMI from 2007 to 2016 with several variables (identification, comorbidities, a medication used and exam results). Secondly, we selected all patients who fulfilled the following criteria: 1-female; 2-complete phone number data. In the end, we collected the gynaecological history by phone after making up to two attempts to contact patients during normal business hours. All data related to the gynaecological history of the patients were collected through an interview. The rest of the data was gathered from the initial evaluation made by a doctor of the service when the patient entered the CVR. After these proceedings, we used the following criteria for including patients in the study: 1-female; 2-Initial medical evaluation with complete information on cardiovascular disease history, comorbidities and habits; 3-Patient knows her complete gynaecological history. Exclusion criteria were as follows: 1-patients that did not recollect their complete gynaecological history; 2-incomplete initial medical evaluation; 3-unable to contact by phone after 2 attempts.

The initial medical evaluation variables taken into consideration were as follows: age of integration in the program, SAH (yes or no), DM (yes or no), dyslipidemia (yes or no), tobacco use (yes or no), body mass index (BMI), maximal oxygen uptake (VO_2 max) in ergometric tests or ergospirometry, acute coronary syndrome episode (ACS) (yes or no) and at what age the episode took place (pre or post-menopause).

The variables analyzed in the phone interview were the following: hysterectomy (yes or no), cause for hysterectomy (benign or malignant), oophorectomy (yes or no, and if yes how many ovaries were removed), use of oral contraception (if the patient has ever taken or not), use of hormonal replacement therapy (HRT), menarche (precocious; before the age of 11 [12], at the expected age, or late: after the age of 15 [13], menopause (didn't take place, precocious: before the age of 40 [14], at the expected age, or late: after the age of 55 [14].

The data was stored in an Excel® 2016 table and analysed with SPSS® software 22.0. We used descriptive statistics and a chi-square test for categorical variables to make the calculations.

This research was approved by the Ethics and Research Committee of Faculdade Cenecista of

Bento Gonçalves and all participants in the study signed a consent statement accepting to participate.

In the first phase, we chose 886 patients, 551 of which were female. Among all women, only 352 had informed their phone number. A first attempt was made to contact during business hours in which we find out that 142 (40.3%) patients had informed the wrong number or the information was out of date. Regarding the rest of the patients, we successfully contacted 112 cases (31.8%) and collected the gynaecological history of the patient through an interview conducted by the researchers. In the remaining 98 cases (27.9%) the phone number was correct. However, the patient did not have sufficient information regarding their gynaecological history or was unavailable to interview at the moment the call was made, even though we made up to two attempts to contact during business hours.

After collecting the gynaecological history from 112 patients, 21 were excluded because of incomplete information in their medical chart. In total, we analysed 91 patients, which represented 16.7% of all female patients in the rehabilitation service.

Results

The evaluation of general clinical characteristics showed that practically one-third of the patients (33%) were ex-smokers and 13.2% were active smokers. In other words, 45.2% of patients presented some tobacco load, and the other 53.8% had never smoked. BMI average was 29.27 kg/m2 with a standard deviation of 5.45. About the VO_2max, 23 patients (25.3%) did an ergometric test with the average VO_2max being 19.87 ml/kg/min and a standard deviation of 7.64. The remaining 68 patients (74.7%) did the ergospirometric test obtaining an average VO_2max of 14.81 ml/kg/min and a standard deviation of 6.23.

Table 1: The prevalence of additional risk factors

Comorbidity	Yes	No
Diabetes	23 (25.3%)	68 (74.7%)
SAH	75 (82.4%)	26 (17.6%)
Dyslipidemia	56 (61.5%)	35 (38.5%)

The analysis of variables related to gynaecological history showed that 66 patients (72.5%) did not have a hysterectomy. In the rest of the patients that informed having a hysterectomy, the majority (68%) did not know the reason for the surgery; one patient reported malignant cause and another 7 (28%) stated that it was a benign cause that motivated them to have the procedure. Regarding oophorectomy, 14 patients (15.4%) had the procedure, 5 of which (35.7%) removed just one ovary and 9 (64.3%) removed both ovaries. The median menarche age was 13 years (average 12.87), taking into consideration that 5 patients (5.5%) had menarche classified as precocious, 82 (90.1%) within the expected period and 4 (4.4%) late. About menopause, 8 patients were still pre-menopausal. From the remaining 83 women, 9 (10.8%) had early menopause, 70 (84.3%) within the expected period and 4 (4.8%) late. When it came to oral contraception, 70 patients (76.9%) informed to have used contraception at some point in their life while 21 (23.1%) denied use. About HRT, 24 patients (26.4%) reported having gone through HRT and 67 (73.6%) reported that they had never been through HRT.

Lastly, around half of the patients (47.3%) had already had an ACS with the first episode taking place approximately at the age of 55 with a standard deviation of 11.42. Furthermore, the lowest age of the first ACS episode was 32 years and the highest 81 years. Regarding the number of events of ACS, 79% of patients only had one episode, 16.3% had two episodes, and 4.7% had 5 episodes.

Using the chi-square test, we analysed the women that had a hysterectomy and presented an ACS episode. This analysis resulted in a total of 10 (11%) patients, 7 of which (70%) experienced the first ACS episode after a hysterectomy and the other 3 (3%) experienced the first ACS episode before having a hysterectomy. The same observation was made about menopause, in that 33 women (80.5%) experienced the first ACS episode after menopause and 8 (19.5%) before menopause. In other words, 41 patients (45%) reported having been through menopause and having had at least one ACS event.

Discussion

Our study analysed 91 female patients of a CVR service. While selecting the patients, we initially noticed a higher number of women than men. This data would contradict most existing studies on this subject [11]. We believe that either this data found in our program is incidental to the period analysed or that women presented more events or they sought out the rehabilitation program more than men.

The metabolic profile of the participating women shows that most of them are hypertensive (82.4%), 61.5% are dyslipidemic, 25.3% are diabetic, and the BMI average was 29.27 (overweight). It is known that women have a greater prevalence of hypertension after the age of 50 and this prevalence tends to increase with age reaching up to 80-90% of women older than 70 years in the population [15] [16]. Regarding dyslipidemia, as it has already been explained, there are particularities in women. Prevalence also matches the population data, as it was shown in a study made in Sao Paulo according to

which the average hovers at around 60% [17]. Women also tend to have a greater prevalence of diabetes in the population; however, the DM prevalence analysed in our sample was significantly superior to the national average of prevalence in women (8.8%), possibly because of being part of a specific group with more CVD risk [7]. In general, despite the reduced number, the resulting metabolic profile matched the results of the population studies with a tendency for greater risk factor prevalence because of being a select group of patients.

Practically half of the patients analysed (47.3%) experienced at least one episode of the acute coronary syndrome (ACS). The majority of the remaining patients have some kind of heart disease, mainly atherosclerotic, which makes us conclude that the group of patients under analysis is at high risk to develop a more severe event or even die. It is known that in Brazil, ischemic heart disease is one of the main causes of death and it is responsible for 31% of deaths caused by cardiovascular diseases while the Ischemic stroke is responsible for 30% of deaths caused by cardiovascular diseases. The age average for the first Acute Myocardial Infarction (AMI) matched the expected age based on other studies in which the age range for this event to be more likely is for women above the age of 50 [18]. Several studies show that the incidence of acute myocardial strokes grows drastically after menopause [19] [20] [21] and in some studies, a greater number of incidents was found among women with early menopause [2] [22]. There are various physiological explanations for such an occurrence. However, there is no concrete proof that menopause is a CVD causal factor. Some hypotheses are based on the fact that estrogen, just like progesterone, suppresses some endothelium and vascular smooth muscle proliferation factors mainly through intracellular receptors. There are studies which suggest that estrogen can stimulate the release of nitric oxide the same way progesterone stimulates COX-2 and consequently PGI2. Furthermore, estrogen aids in the lipolysis and lipogenesis responses, anti-inflammatory effects and antioxidants [21].

Nevertheless, men also present an increase in cardiovascular events as the years go by, which weakens the theory that menopause is an independent causal factor of CVD [1]. Another factor that would contradict the estrogenic protection theory is the great change of habits in women and the increase of cardiovascular events in younger women, mainly related to the smoking habit and the stress at work and routine [23]. Men were not evaluated in our sample, and therefore we shall not hypothesise on this subject. In our sample, approximately 80.5% of the female patients that presented AMI during their lifetime suffered the event after menopause. Even if from a known physiological point of view this makes sense, we still cannot confirm a definite causal relationship based on this information.

An important point to be discussed is about the genetic risk and healthy lifestyle. The evidence on this subject is well established in the literature, and the strong influence of these factors on increased cardiovascular risk must be taken into account. A study based on three prospective cohorts and one cross-sectional study showed that high genetic risk is independent of a healthy lifestyle and is associated with an increased risk (hazard ratio 1.91). There are up to 50 single-nucleotide polymorphisms related to CHD, and it is possible to calculate the polygenic risk score for this disease. Also, adherence into the healthy lifestyle (no current smoking, no obesity, regular physical activity, and a healthy diet) was associated with a significantly decreased risk, within any genetic risk category [24].

Due to the discoveries made about the protective cardiovascular role played by estrogen, many studies were conducted about HRT and cardiovascular prevention. However, many well-conducted studies found exactly the contrary, an increased risk of several incidents with this type of therapy [25]. Given this, most global guidelines do not recommend the prescription of medication with a cardiovascular prevention purpose. Among the patients analysed in our study, 26.4% went through hormonal rehabilitation. There is a lack of data and sample size for the realisation of a persistent comparison of this type of therapy and cardiovascular events in our study.

Apart from menopause, there are also assumptions related to the age of menarche and future risk for CVD. It is believed that early menarche is associated with a greater CVD risk and mortality caused by CVD [25]. In our sample, most patients had menarche at the expected age; therefore, this variable is difficult to analyse to link it to cardiovascular events.

As far as oral contraception is concerned, it is known that the women who used this medication in the past do not have an increased risk for cardiovascular events [26]. There are only a few studies related to oral contraception use and events taking place at the same time and since the use is made at a younger age and cardiovascular events are extremely rare at this age group it is difficult to establish a relationship. Therefore, even if most of our patients had used this type of medication, we can be quite sure that there is little correlation between the number of cardiovascular events.

Other analysed variables in our study were: going under hysterectomy and oophorectomy since there have been hypotheses of connection to increased CVD risk through undefined mechanisms. Very few patients of our study went through this procedure so it would be inappropriate to conclude the existence of any causal relationship. What´s more, according to bigger studies about this subject, the correlation between hysterectomy with or without ovary removal and cardiovascular events was not very

consistent, both in the pre-menopause and post-menopause phase and for this reason, this hypothesis is hardly considered nowadays [3] [27].

Our study was conducted with high-risk cardiovascular patients. The treatment choice for these patients needs to be very well planned with the interaction of drug and non-drug therapies. Taking this into consideration, CVR is a method that presents strong evidence of being a very important tool in the primary and secondary prevention for this kind of patient [28] [29]. However, there are very few studies specific to women and their evolution in this type of program.

Bearing in mind that our study was conducted in a CVR program we can infer that, based on our data, the patients of the program have a very low functional capacity in their initial evaluation regardless their age range [30]. Most patients were evaluated with an ergospirometric test which is quite accurate for measuring such a variable. This is a positive point for our study since most research in CVR programs uses ergometric tests which overestimate results, as it was clearly shown in our findings.

We can conclude that the patients analysed present various accumulated risk factors that predisposed them to CVD. Low functional capacity proves that they are critically ill patients who require meticulous care. Furthermore, there are specific risk factors for women that are still very controversial literature-wise. Although our sample presents risk and a high incidence of ACS, the theoretical risk factor rates specific to women (such as early menopause) were similar to the female population in general. Besides this, among the women in the analysed sample with and without risk factors specific to women, the ACS prevalence was similar.

This information supports the idea that these are just confounding factors of CVD and the principals involved are the genetic factors and habits. For this reason, the focus of CVD prevention and treatment should be directed towards these aspects.

Limitations

The sample of the study ended up being limited due to lack of information in the charts and difficulty to contact patients, apart from the fact that the CVR public is of low numbers. What´s more, the average age of the initial evaluation was not taken into consideration in the study, as it was deemed more important to evaluate only the timeline of the exposure factors and outcome.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1376-1381.
https://doi.org/10.3889/oamjms.2018.292
eISSN: 1857-9655
Clinical Science

Obesity Related Metabolic Disorders and Risk of Renal Disease: Impact of Hypocaloric Diet and Avena Sativa Supplement

Salwa M. El Shebini, Maha I. A. Moaty, Suzanne Fouad*, Nihad H. Ahmed, Salwa T. Tapozada

Nutrition and Food Science Department, National Research Centre, Dokki, Giza, Egypt

Citation: El Shebini SM, Moaty MIA, Fouad S, Ahmed NH, Tapozada ST. Obesity Related Metabolic Disorders and Risk of Renal Disease: Impact of Hypocaloric Diet and Avena Sativa Supplement. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1376-1381. https://doi.org/10.3889/oamjms.2018.292

Keywords: Obesity; Metabolic disorders; Avena sativa oat; Dietary supplement; Hypocaloric diet

***Correspondence:** Suzanne Fouad. Nutrition and Food Science Department, National Research Centre, Dokki, Giza, Egypt. E-mail: suzannefouad6161@yahoo.com

Received: 30-Apr-2018; **Revised:** 28-Jun-2018; **Accepted:** 03-Jul-2018; **Online first:** 29-Jul-2018

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Funding: This work was financially supported by the National Research Centre, Egypt

Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: The recognition of the complications of obesity in various organs and systems should make clinicians and dietitians aware of the importance of early strategies to fight obesity in all age groups.

AIM: The objective of this study was to evaluate the early effect of using *Avena sativa* (oat) flour supplement compared to a hypocaloric diet in the management of obesity-related metabolic disorders.

MATERIAL AND METHODS: Snack was prepared from wholemeal wheat flour (100% extraction) and oat flour. Chemical analysis of the raw materials and the formulae was carried out. 106 obese women with their mean body mass index were 37.73 ± 0.56 kg/m² volunteered for 8 weeks period. They were divided into two groups; group (A), consumed hypocaloric diet supplemented by the prepared snack, while group (B) subjects followed the low caloric balanced diet. All patients were monitored clinically, anthropometrically, dietary 24 h recall and biochemically.

RESULT: Data demonstrated significantly decreased in the mean levels of the anthropometric parameters. Group (A) showed a higher decrease in the waist circumference, WHR, body fat% and SBP; while in group (B) weight, BMI, chest circumference and DBP were the most affected parameters. The reduction in the biochemical parameters was higher in the group (A). At the baseline, high values of cystatin-C were found in both groups which may indicate early renal injury. At the end of the study, a significant reduction of the cystatin concentration was observed among both groups (-24.54 & -12.23%).

CONCLUSION: The healthy effect of the dietary oat supplement on the reduction of central obesity, percentage body fat and different metabolic disorders criteria was confirmed than with hypocaloric diet.

Introduction

James, (2008) reported that obesity is a standout amongst the most genuine general medical issues in the 21st century [1]. Eighty-five per cent of Egyptian women are overweight with 48 per cent of that rate enduring obesity, as indicated by Egypt Demographic and Health Survey (EDHS 2014) reported by Egypt's health minister, the per cent of obese women was twice that of men [2].

Fat accumulation, especially in the abdominal region, is associated with metabolic disturbances such as dyslipidemia, hypertension, insulin resistance, cardiovascular complications and non-alcoholic fatty liver disease. Recent studies demonstrated that obesity independently of diabetes might have a role in the development of kidney disease (obesity-related nephropathy) and may lead to end-stage renal disease. The prevalence of obesity runs in parallel with the prevalence of renal failure. The intrarenal changes associated with the cardiometabolic syndrome result in elevated glomerular filtration rate, impaired pressure natriuresis, endothelial dysfunction related to changes in nitric oxide and, hence, impaired renal autoregulation and enhanced chronic inflammation [3] [4].

Cystatin C is a protein of 120 amino acids delivered in every nucleated cell of the human body and is found in practically all tissues and body liquids. It has a low atomic weight, so it is unreservedly filtered by the glomerulus and reabsorbed and debased by the proximal tubules. It is an endogenous filtration marker that is being considered as a potential substitution of serum creatinine in the evaluation of renal capacity, predominantly for the identification of

little changes in the glomerular filtration rate, since it is asserted to be free of age, sex, skeletal bulk and protein consumption [5]. Sledzinsk and others demonstrated that human obesity is associated with an abnormality in cystatin-C value, and that fat tissue elevated serum cystatin-C in obese subjects [6].

Oat (*Avena sativa*) has a nutritional profile and multifunctional attributes. It is considered a richer source of dietary fibre particularly beta-glucan, minerals and different elements. Wholegrain oat-based breakfast grains considered as prebiotics and have low glycemic index (GI) [7].

The present study aimed to evaluate the early effect of using *Avena sativa* (oat) flour supplement compared to a hypocaloric diet in the management of obesity-related metabolic disorders and the risk of renal disease.

Materials and Methods

The snack was prepared from wholemeal wheat flour (WMWF) (100% extraction) and oat flour (OF) added in equal amounts (at the level of 50%). The flour was mixed with the other ingredients (Skimmed milk, corn oil, baking powder and vanilla) and a suitable amount of water was added. These formulae were baked at 180°C for about 15 minutes [8] (Figure 1). WMWF 100% extractions had more beneficial health effect than Wheat flour (WF) 72% extraction [9].

Table 1: Composition of the supplement (g/100g dry weight)

Items	Snack
Oat Flour	38.7
Whole Meal Wheat Flour 100% extraction	38.7
Vanilla	0.5
Skimmed milk	10.8
Baking powder	0.5
Corn oil	10.8

One hundred and six obese females with BMI more than 30 kg/m² were included in this study with their age ranged from 39 to 57 years old, and their mean body mass index (BMI) was 37.73 ± 0.56 kg/m². They were all enrolled in a program for losing weight in the Nutrition Department, National Research Centre. The participants were informed about the purpose of the study and their permission in the form of written consent was obtained. The protocol was approved by the "Ethical Committee" of the "National Research Centre". All participants have followed a low caloric balanced regimen (1000-1200 KCal/day) for eight weeks. They were divided into two groups, sixty-six patients (group A) consumed the snack, two with breakfast and one with dinner (each weighed 20 g), and the rest of the sample (40) group (B) followed a low caloric balanced regimen without supplement for eight weeks. All the subjects were examined at baseline, and the end of the study with weekly follow up.

Figure 1: Prepared snack

Exclusion criteria: Obese patients on pharmacological treatment, known to have renal failure or thyroid dysfunction.

Body weight and height (subjects were standing with minimum clothing and no shoes to the nearest 0.01 centimetre). Minimal waist circumference (MWC) was measured in centimetre using non-extendable tape during minimal normal respiration and hip circumference. The body mass index (BMI) was Calculated, where BMI = weight in kg/square height in meters [10] (Jelliffe, 1966). Body fat (BF) as a per cent of body weight, body muscle mass and the basal metabolic rate was measured using Geratherm Body Fitness (B-5010), German.

Blood pressure was measured by cuff sphygmomanometer while the subjects sat quietly on a chair, and the mean of three readings was taken.

Data on dietary intake before the intervention were reported using the 24 hours dietary intake recall. All food items and portions were recorded in details. Total nutrients intake was calculated using Nutrisurvey 2007.

Blood samples were obtained on the day of clinical examination after an overnight fast. Fasting blood glucose (FBG) was determined in fresh samples using the glucose oxidase method [11]. Serum total cholesterol (TC), High-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) were done using; cholesterol proceed No 1010, StanBio Liquicolor [12]. HDL-C proceed No 0599 StanBio Liquicolor [13], and triglycerides proceed No 2100, (Enzymatic method) [14] respectively. Low density lipoprotein- cholesterol (LDL-C) was calculated according to the Friedewald equation [15]. Fasting C-peptide level was measured by ELISA method [16] (Monobind Inc. Lake Forest, CA 92630, USA). According to Li et al., [17] (2004), insulin resistance was expressed by modified homeostasis model assessment-insulin resistance (modified HOMA-IR = 1.5 + FBG (mg/dl) × fasting C-peptide (nanograms per milliliter)/2,800), in which insulin was replaced by C-peptide so as to be applied

on diabetic patients using exogenous insulin. Aspartate aminotransferase (AST/GOT) and alanine aminotransferase (ALT/GPT) were measured by colourimetric method [18]. DeRitis ratio was calculated as AST/ALT. Serum Cystatin-C was determined using Human Cystatin-C ELISA, Lot E12-076, BioVendor Research and Diagnostic Product CZECH REPUBLIC [19].

All values were expressed as mean value ± SE. Two-tailed student t-test was used to compare between data in the same group and between groups. P values < 0.05 were considered statistically significant. SPSS window software version 17.0 (SPSS Inc. Chicago, IL, USA, 2008) was used. Changes in different data were expressed as % change from baseline.

Results

Table 2 summarised the average of protein, fat and crude fibre of the raw materials and the oat prepared snack, in addition to some mineral contents of the product.

Table 2: Chemical composition of raw materials and snack (mean ± SE)

Samples	Protein (%)	Fat (%)	Fibre (%)
WMWF	13.5 ± 0.10	2.5 ± 0.01	1.75 ± 0.001
Oat flour	16.8 ± 0.17	5.0 ± 0.05	4.82 ± 0.002
Snack	16.32 ± 0.12	12.15 ± 0.06	3.50 ± 0.007
Minerals	K %	Zn%	Na %
Snack	320.96	5.34	302.34

WMWF: Whole Meal Wheat Flour (100% extraction); Snack prepared by 50% WMWF+50% Oat Flour.

Table 3 showed a comparison between the different macronutrients and micronutrients and the per cent caloric distribution of the diet of the whole sample. The data showed the balanced and healthy distribution of the macronutrients in the two regimens compared to the habitual diet of the patients.

Table 3: Daily intake of calories, macronutrient (as a % of calories), Mean values and % of the recommended dietary allowance (RDAs) of some micronutrient intake of three types of diet

Macronutrient Intake	Habitual Diet	Low Caloric Regimen	Diet with Supplement
Energy (Kcal)	2802.61	1239.91	1229.87
Protein (% cal. supply)	13.99%	21.48%	25.44%
Fat (% cal. supply)	40.92%	29.94%	28.96%
Carbohydrate (% cal. supply)	45.09%	48.58%	45.60%
Micronutrient intake (RDAs)		Mean value %RDAs	
Vit. A (µg)	567.24	765.24	774.21
(800)	70.91	95.66	96.78
Vit. D (µg)	1.97	3.21	3.23
(5)	39.40	64.20	64.60
Potassium (mg)	929.93	1633.51	1669.95
(2000)	46.49	81.68	83.49
Calcium (mg)	731.63	891.46	920.32
(1000)	73.16	89.15	92.03
Iron (mg)	6.35	11.12	11.83
(15)	42.33	74.13	78.87
Zinc (mg)	6.27	10.45	10.98
(12)	52.25	87.08	91.50

Table 4 showed the mean ± SE of age, anthropometric, blood pressure measurements of the two groups at the start and the end of the study. All the anthropometric measurements except body muscle mass of the two groups decreased significantly at p < 0.05-0.01 by the end of the study. Comparing the % changes between the two groups; group (A) showed more % reduction of waist circumference, WHR, body fat % and SBP, while in the group (B) the higher per cent of reduction was observed in weight, BMI, chest circumference and the DBP.

Table 4: Mean ± SE of anthropometric parameters and blood pressure of obese women at the baseline and the end of the study

	Groups A (n = 66)			Groups B (n = 40)		
Parameters	Baseline	Last	% changes	Baseline	last	% changes
Age (year)	50.18±0.64			50.11±0.80		
Height (cm)	155.5±0.78			155.00±0.76		
Weight (Kg)	91.39±1.33	87.63±1.40^{**a}	-4.11	90.10±1.04	86.09±1.02^{**b}	-4.45^{**c}
BMI (Kg/m^2)	37.88±0.56	37.04±0.58^{**a}	-2.21	37.59±0.46	36.27±0.47^{**b}	-3.51^{**c}
Body fat (%)	47.83±0.65	46.37±0.73^{**a}	-3.05	48.83±0.84	47.94±0.84^{**b}	-1.82^{**c}
Body muscle (kg)	42.45±0.46	42.43±0.40	-0.04	42.15±0.36	41.98±0.37	-0.40
Chest (cm)	96.05±0.67	94.46±0.57^{**a}	-1.66	98.18±0.55	95.14±0.53^{**b}	-3.06^{**c}
Waist (cm)	95.32±0.76	92.33±0.99^{**a}	-3.14	95.00±1.19	93.62±1.03^{**b}	-1.45^{**c}
WHR	0.79±0.01	0.77±0.00^{**a}	-2.53	0.81±0.01	0.80±0.11^{**b}	-1.23^{**c}
SBP (mmHg)	132.67±1.37	116.67±1.77^{**a}	-12.06	126.88±2.80	117.64±1.79^{**b}	-7.85^{**c}
DBP (mmHg)	79.33±0.82	76.67±1.18^{*a}	-3.35	82.22±1.55	78.33±1.43^{**b}	-4.97^{*c}

*BMI: body mass index, WHR: waist-hip ratio, SBP: systolic blood pressure, DBP: diastolic blood pressure *P<0.05 **P<0.01, a: Baseline vs. last visit of group A; b: Baseline vs. last visit of group B; c: % changes group A vs. group B.*

Table 5 showed the mean± SE of the biochemical parameters of the two groups at the two visits. The level of HDL-C increased significantly, while the FBG concentration, other lipid parameters, liver enzymes and cystatin-C levels showed a significant reduction in the mean concentrations of both groups with more effect on group A except for AST serum level. The comparison between the two groups regarding the mean per cent changes approached significance on all parameters.

Table 5: Mean ± SE of the Obesity-related metabolic disorders of obese women at the baseline and the end of the study

	Groups A (n = 66)			Groups B (n = 40)		
Parameters	Baseline	last	% changes	Baseline	last	% changes
FBG (mg/dl)	95.76±2.45	79.67±1.50^{**a}	-16.80	84.83±1.32	83.35±1'12	-1.74^{**c}
TC (mg/dl)	232.94±6.29	184.02±4.41^{**a}	-21.00	218.76±6.66	205.68±7.24^{**b}	-5.99^{**c}
LDL-C (mg/dl)	151.29±6.41	97.09±4.77^{**a}	-35.89	151.19±4.82	126.89±4.80^{**b}	-16.07^{**c}
HDL-C (mg/dl)	49.71±1.20	62.19±1.44^{**a}	+25.11	49.23±1.61	53.39±2.61^{**b}	+8.45^{**c}
Non HDL-C (mg/dl)	183.23±6.82	121.83±4.99^{**a}	-33.51	159.52±7.59	152.28±8.62^{**b}	-4.54^{**c}
Risk factor ((TC/ HDL)	4.99±0.23	3.12±0.13^{**a}	-37.47	4.90±0.16	3.99±0.14^{**b}	-29.35^{**c}
TG (mg/dl)	159.68±6.00	123.70±3.56^{**a}	-22.53	125.15±6.03	110.93±4.42^{**b}	-11.36^{**c}
AST (IU/L)	32.86±0.90	29.27±0.95^{**a}	-10.93	28.97±0.90	25.19±0.60^{**b}	-13.05^{*c}
ALT (IU/L)	50.85±0.79	38.28±0.79^{**a}	-24.72	51.72±0.48	49.80±0.96^{**b}	-3.71^{**c}
DeRitis ratio	0.65± 0.01	0.78±0.02^{**a}	+20	0.56 ±0.02	0.62±0.03	+10.71^{**c}
C peptide (ng/ml)	4.44±0.38	2.37±0.25**a	-46.62	5.22±0.28	3.40±0.18	-34.87^{**c}
M. HOMA-IR	1.67±0.01	1.58±0.01^{**a}	-5.39	1.66±0.01	1.58±0.01	-4.94^{*c}
Cystatin C (ng/ml)	965.25±38.23	728.37±24.11^{**a}	-24.54	957.44±45.99	840.37 ±42.12	-12.23^{**c}

FBG: fasting blood glucose; AST: aspartate transaminase; ALT: alanine transaminase; M.HOMA-IR: modified homeostatic model assessment of insulin resistance *P<0.05 **P<0.01; a: Baseline vs. last visit of group A; b: Baseline vs. last visit of group B; c: % changes group A vs. group B.

Discussion

Obesity is a state of low-grade inflammation and pro-oxidation which lead to vascular dysfunction and altered the metabolic states resulting in an alteration in the liver and kidney functions. The baseline information of this study uncovered that all volunteers had elevated levels of all the anthropometric measurements, lipid profile parameters and the cystatin-C concentration over the recorded reference values that indicated metabolic disarranges. At the beginning of the investigation, the diabetic members had a normal value of the FBG, as they were under medical treatment. Nonetheless, serum C-peptide as a marker of the functional-pancreatic cell mass, showed mild rise (> 3.75 ng/ml). Rosselli et al., (2013) expressed that C-peptide expects a role in early atherogenesis in diabetic patients; also it might be considered a marker to cardiovascular diseases risks in patients without diabetes [20].

Data in this study demonstrated the healthy beneficial effect of the supplement consumed by the patients on the central obesity measurements (waist circumference and WHR) and the blood pressure values. The higher per cent decrease was found in the systolic blood pressure percentage where it was -12.06 & -7.85% group (A) and group (B) respectively, that followed by the decrease in the body fat percentage values (-3.05 & -1.82%) and WHR (-2.53 & -1.23%). The body weight and BMI were significantly decreased more in the low caloric diet group (-4.11 & -4.45%) and (-2.21 & -3.51%) respectively. Furthermore, the improvement of the biochemical parameters was high in its value when compared to the supplements' effect on the anthropometric measurements. The mean level of the FBG decreased significantly (-16.8 & -1.74%) in both groups, but was much more in the group (A). All the biochemical parameters improved significantly in both groups, especially in group A.

Interest in β-glucan increased due to its bioactive and functional properties with no adverse effects following consumption of a diet rich in it as barley, oat or their extracts [21]. The health benefits of these foods arise from their high fibers content which ranges from 9.9-14.9 g for each 100 g serving and lipid-lowering effect of β-glucan, either by bile acid binding, delay in the absorption and/or digestion of fat, filling full, suppress appetite and finally β-glucan depress oxidative stress associated with inflammatory state caused by obesity [22].

Oats additionally contain more lipids (5-9%) than other grain edits and are rich in unsaturated fats, including the basic unsaturated fat linoleic acid. Oats contain antioxidant, called avenanthramides, and also the vitamin E-like compound, tocotrienols and tocopherols (Wursch and Pi-Sunyer, 1997) [23] [24] [25].

Body muscle mass decreased numerically in both groups; this result focuses the importance of consuming supplement enriched on amino acid as soy products, the preservation of body muscle mass is a must in any overweight, losing program [26].

Correlation between obesity and renal impairment has been demonstrated by several types of research. The gradual expansion of renal tubulointerstitial fibrosis by fibrous tissue destroys the normal structure of the kidney. More, the local release of inflammatory substances and active biological factors result in renal impairment. Other theory states that obesity leads to endothelial dysfunction and thickness of the intima-media resulting in vascular damage and leaking of albumin. Obesity, when present since childhood, is associated with a lower glomerular filtration rate even before the appearance of other comorbidities as diabetes and hypertension [27] [28] [29] [30].

Clinical markers used to reflect renal damage incorporate albuminuria and the assessed glomerular filtration rate (GFR). Given the same GFR level, urine albumin may be a superior marker to foresee the progression of chronic kidney disease (CKD) and the future of cardiovascular diseases (CVDs). Serum cystatin-C is rising as another biomarker for early identification of renal damage related to obesity, MetS and cardiovascular disease [31] [32] [33]. Weight control, strict control of blood pressure, glucose and lipid levels decrease renal damage and even the ensuing CVD [34].

In this study, high values of cystatin-C were detected in both groups at the beginning of this study which may indicate early renal injury. After the end of the study, a significant reduction of the cystatin-C concentration was observed among both groups more in the oat supplement group (-24.54 & -12.23%). Animal examinations revealed that oat consumption affects kidney function. In any case, the impacts of oat utilisation have not been completely evaluated in humans. However, Rouhani et al., (2016) conducted a study that investigated fifty-two patients with CKD; the authors reported that admission of oats might beneficially affect both serum albumin and potassium [35].

The results of this study showed the high value of the ALT enzyme and the De Ritis ratio was (< 1.0) at the beginning of the study in both groups, denoting the presence of early nonalcoholic fatty liver disease (NAFLD). At the end of the study, a significant reduction in the levels of both liver enzymes was detected, and the De Ritis ratio was improved in both groups. Improvement of insulin resistance with oat supplement or dietary interventions constitutes an essential step in the treatment of NAFLD. Excessive triglyceride accumulation in the liver in the absence of alcohol consumption results in a disorder known as NAFLD. Insulin sensitisers and antioxidant drugs hold promise for the management of NAFLD [36] [37].

In conclusion, whole wheat flour and oat flour snack prepared supplement have higher nutritive values. Oat is an essential food component used in the modulation of obesity-related metabolic disorders and renal impairment.

Ethics approval

The research was given ethical approval from Ethical Committee of National Research Centre; Signed written informed consents was a must to participate in the research project after they had been given a full explanation of the study.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1382-1386.
https://doi.org/10.3889/oamjms.2018.210
eISSN: 1857-9655
Clinical Science

The Position of Neutrophils-To-Lymphocytes and Lymphocytes-To-Platelets Ratio as Predictive Markers of Progression and Prognosis in Patients with Non-Small Cell Lung Cancer

Plamen Minkov[1*], Maya Gulubova[2], Petar Chilingirov[1], Julian Ananiev[2]

[1]Trakia University, Stara Zagora, Bulgaria; [2]Department of General and Clinical Pathology, Forensic Medicine and Deontology, Trakia University, Stara Zagora, Bulgaria

Citation: Minkov P, Gulubova M, Chilingirov P, Ananiev J. The Position of Neutrophils-To-Lymphocytes and Lymphocytes-To-Platelets Ratio as Predictive Markers of Progression and Prognosis in Patients with Non-Small Cell Lung Cancer. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1382-1386. https://doi.org/10.3889/oamjms.2018.210

Keywords: Lung cancer; NLR; PLR; Prognosis

***Correspondence:** Plamen Minkov. Trakia University, Stara Zagora, Bulgaria. E-mail: uki_duki@abv.bg

Received: 18-Jan-2018; **Revised:** 15-Jun-2018; **Accepted:** 18-Jun-2018; **Online first:** 04-Aug-2018

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Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: Non-small cell lung cancer (NSCLC) is an insidious metastasis condition of the lungs often presenting no symptoms at the onset. Defining markers for quick determination of prognosis is essential for building up a treatment strategy.

AIM: The aim of this study is to define the role of the Neutrophils-to-Lymphocytes ratio (NLR) and Platelets-to-Lymphocytes ratio (PLR) as biomarkers in patients with NSCLC, according to the stage and prognosis of the disease.

METHODS: We investigated 20 patients with NSCLC. NLR and PLR are calculated and are evaluated according to the presence or absence of metastasis, stage of the disease, histological type and survival rate.

RESULTS: We found that thirteen of the patients had low NLR, while the rest 7 had high NLR (mean 3.15). By analysing PLR we found that 11 patients have low and 9 have high level of PLR (mean 1.42). After the correlations have been made we discovered that in 90.1% of the patients with low PLR no lymph metastasises were detected, while in 50% of the patients with high PLR lymph metastasises were observed ($\chi 2 = 3.99$; $P = 0.046$). We also discovered that in 84.6% of the patients with low NLR lymph metastases were absent, while in 42.9% with high NLR lymph metastasises were present ($\chi\ 2 = 1.83$; $P = 0.176$).

CONCLUSION: In conclusion, NLR and PLR were discovered as prominent biomarkers which provide relatively fast determination for prognosis in patients with NSCLC.

Introduction

Lung cancer (LC) is one of the leading causes of cancer related mortality [1]. Non-small cell lung cancer (NSCLC) accounts almost 85% of all LCs, while small cell lung cancer (SCLC) accounts nearly 13% [1]. There are many prognostic factors that are associated with development and progression of LC: TNM status, age, stage, performance, gender, histological variant, serum levels of lactate dehydrogenase LDH, carcinoembryonic antigen CEA and others [2] [3] [4] [5]. Some newer biomarkers like epidermal growth factor receptor EGFR mutations and anaplastic lymphoma kinase ALK rearrangements provide useful information for determining the prognosis and building up a treatment strategy. Unfortunately, these tests are expensive, they can only be evaluated in a small subset of patients and the results take time [6] [7].

The validation of new biomarkers could ease the stratification of high risk patients and could also help make more accurate treatment plan. The relation between the immune response of the patients and the progression and prognosis of the neoplasms is confirmed by multiple analyses [8] [9]. It is known that the tumor microenvironment, which is conditioned by the immune response of the patient and also by the cancer itself, plays a crucial role in the processes of angiogenesis, metastasis and proliferation of the cancer cells [10]. Very important role of the

development of these processes is given to the cells of the extracellular matrix, the cells of the connective tissue and especially to cells such as: lymphocytes, neutrophils, macrophages, dendritic cells, platelets and others.

Lymphocytes are the main cells of the antitumor immune response. Their antitumor capabilities are carried out by cytotoxic T-lymphocytes [11]. The tumor cells are vanished by cytolytic reactions or by induction of apoptosis via membrane receptor of the programmed death. To be effective, the antitumor response requires antigen presentation from the tumor cells or from the antigen presenting cells like macrophages and dendritic cells [12]. Antitumor capabilities of the lymphocytes are ineffective in clinically detectable cancer and are inversely proportional to the tumor size [13]. The cells of the NSCLC escape these immune mechanisms by expression of unstable or bad presented antigens as result of genetic or epigenetic mutations in course of oncogenesis [14].

Neutrophils play main role in the processes of inflammation or antibacterial defense. Chronic inflammation is an established factor that increases the risk of cancer development. Examples are hepatitis B and the inflammatory bowel diseases, which could lead to development of hepatocellular and colorectal carcinoma respectively [15] [16]. The neutrophils take place in the processes of angiogenesis by secretion of pro-angiogenic factors. They directly affect the tumor progression by proteolytic release of epidermal growth factor, transforming growth factor beta and platelet derived growth factor [17]. Furthermore, neutrophils have the capability to influence other tumor promoting cells as T-lymphocytes and NK-cells [18]. Neutrophils have also direct or antibody dependent cytotoxic effect on the cancer cells [17]. It is known that there is neutrophilic polarization, which is caused by different cytokines (TGF-beta, INFs). Polarization defines the development of subpopulations of neutrophils that have antitumor properties as well as subpopulations that support the tumor progression [19]. A high number of neutrophils favor the prognosis according to a number of studies and exactly the opposite effect according to others [20].

Thrombocytes have central place in the processes of growth, progression and metastasis of neoplasms [21]. A hyper coagulation is related to more aggressive cancer disease and even more to thromboembolism, which in turn is one of the leading causes of death in patients with cancer [22] [23]. Platelets release many factors as PDGF, thrombospondin and thrombocytic factor 4 (which favor the hematogenic cancer spread), the adhesion of the tumor cells, invasion, the angiogenesis and in that way the tumor progresses. The prognostic significance of the platelets count in subsets of patients with NSCLC is known for a long time but it has unknown correlation [24] [25] [26] [27] [28].

Many inflammatory indicators attract attention because of their accessibility and prognostic efficacy when determining the prognosis in cancer patients. Such indicators are Neutrophil-to-Lymphocyte ratio (NLR) and Platelets-to-Lymphocyte ratio (PLR). The NLR is an important marker of systemic inflammation. Neutrophils, T- and B-lymphocytes have central role in the antitumor immune response [29]. The disturbance of the normal NLR is considered to be a consequence of the tumor related hypoxia and/or necrosis and is associated with anti- apoptosis effect [30]. The NLR is proven as a prognostic biomarker for determination of the prognosis of patients with different kind of cancer, including colorectal, breast cancer, gastric cancer, pancreatic cancer and esophageal cancer [31] [32] [33] [34] [35]. Many studies try to define the exact place of NLR as a prognostic biomarker in patients with NSCLC. The known evidences show unstable and discrepant results [36]. The prognostic value of the PLR is also associated with some kinds of cancer including gastric, breast, colorectal cancer and NSCLC [37] [38] [39] [40]. The prognostic value of PLR for determining the prognosis of patients with NSCLC is contrary [40] [41]. According to some authors, the high PLR has a negative prognostic value, while others do not succeed to establish clear correlation between prognosis and PLR [42] [43].

The objective of this study is to define the role of the NLR and PLR as biomarkers in patients with NSCLC, according to the stage and prognosis of the disease.

Materials and Methods

This is a retrospective analysis of NLR and PLR in patients with NSCLC at the time of diagnosis and before treatment. Twenty (20) patients with NSCLC were sampled between 2007 and 2016. Their respective NLR and PLR were calculated and evaluated accordingly with emphasis on the presence or absence of metastasis, stage of the disease, histological variants and survival.

NLR and PLR are calculated and are evaluated according to the presence or absence of metastasis, stage of the disease, histological variant and survival.

The study participants comprise of 19 men and 1 woman aged 24 to 75 years (mean 60.7 ± 11.9 years). The patients were initially operated in the thoracic surgery clinic in Stara Zagora between 2007 and 2016. Fifteen percent (15.0%) of the patients were diagnosed in stage T1 and T2; 75% in T3 and T4; lymphatic metastasises were detected in 5 patients; distant metastasises were found in 4 patients (20%). Lung adenocarcinoma was diagnosed in 7 patients, the other 13 patients were diagnosed with

squamous cell lung cancer.

The Statistical Package for the Social Sciences SPSS 16.0 program for Windows was used for statistical analysis. The descriptive statistical tests, including the mean, standard deviation, and median, were calculated according to the standard methods. The frequency of distribution of NLR and PLR and the clinico-pathological parameters in 2x2 contingency tables was analyzed by χ2-test. For all statistical analysis, $p < 0.05$ was considered to be statistically significant.

The study was approved by the local Ethical Committee.

Results

Thirteen of the patients had low NLR, while the rest 7 had high NLR (mean 3.15). By analyzing PLR we found that 11 patients have low and 9 have high level of PLR (mean 1.42). After the correlation has been made we found that in 90.1% of the patients with low PLR no lymph metastases were detected, while in 50% of the patients with high PLR lymph metastasizes were observed (χ2 = 3.99; P = 0.046). In 84.6% of the patients with low NLR lymph metastasizes were absent, while in 42.9% with high NLR lymph metastasizes were present (χ2 = 1.83; P = 0.176) (Table 1).

Table 1: Correlations between NLR, PLR and clinico-morphological factors

Parameter		NLR			PLR		
		Low	High	p	Low	High	p
Age	> 60.7	5	5	0.515	6	4	0.463
	< 60.7	7	3		5	5	
Sex	M	12	7	0.452	10	9	0.381
	F	1	0		1		
Tumor (T)	T1-2	2	1	0.948	2	2	0.737
	T3-4	11	6		9	7	
Nodulus (N)	N0	11	4	0.176	10	4	0.046
	N1-3	2	3		2	4	
Metastsasis (M)	M0	11	5	0.482	10	5	0.134
	M1	2	2		1	3	
Histology type	Adenocarcinoma	4	3	0.589	4	3	0.599
	Squamous cell carcinoma	9	4		7	6	

Discussion

The definition of prognosis is crucial for determination of the treatment strategy in patients with neoplasy. Many studies have tried to discover biomarkers, which could be used for defining the prognosis of patients with NSCLC [44]. The role of development and progression of cancers is the subject of research by many authors. The ratios NLR and PLR are very intensively investigated biomarkers. because of their accessibility and easy interpretation. The important place that NLR and PLR takes in the processes of cancerogenesis is evaluated by comparison of NLR and PLR in healthy persons compared to lung cancer patients. In one of the studies. significantly higher NLR and PLR are found in patients with LC (NLR: 4.42 vs 2.45. PLR: 245.1 vs 148.2) [45].

Our data gained from the small group of patients show that higher NLR and PLR correlate with advanced disease and respectively worse prognosis. Similar results were observed in other studies involving more patients [41] [45] [46] [47] [48] [49].

Increased NLR and PLR calculated from peripheral blood samples are proved as independent predictive marker which is associated with worse prognosis in patients suffering from different kind of cancer including NSCLC [45]. Close to our results were seen in studies which evaluate cancers at different stages. These studies demonstrate that higher NLR and PLR are associated with worse outcome and advanced disease. Increased NLR and PLR determined at the time of diagnosis in non-treated patients are associated with significantly worse survival in a study that includes 94 patients with NSCLC [50].

Even more interesting is the fact that the lower NLR and PLR are associated with better prognosis in patients with NSCLC. Our results show lower NLR and PLR in patients with no lymph metastasis and lack or in smaller degree distant metastasis. A similar conclusion was reported in earlier studies which investigated the combination of both NLR and PLR as prognostic biomarkers as one study that includes 366 patients with NSCLC in advanced stages. The patients were divided in three groups: worse prognosis NLR > 2.68 in the middle NLR < 2.68, PLR > 119.5 and better prognosis NLR < 2.68, PLR < 119.5 [43].

It is observed that the NLR and PLR could change in course of therapy which supposes their estimation in each stage of treatment. In a study that evaluates the change of the values of NLR and PLR show that permanent elevated ratios are associated with worse prognosis and worse survival after treatment [51] [52] [53]. Despite. these observations the place of NLR and PLR is not fully defined. Some authors have not managed to find association of the prognosis and the value of NLR in patients with NSCLC [42]. Some data demonstrated lacks correlation between prognosis and the value of PLR in patients with NSCLC [43].

In conclusion, it can be noted that NLR and PLR are very accessible biomarkers and could be

very useful for relatively fast determination of the prognosis in patients with NSCLC. According to our data, only PLR can be used for prognosis determination, although very small subset of patients was investigated. Usually, higher NLR and PLR are associated with worse prognosis. Unfortunately, the reliability of these biomarkers is not well defined. More investigations are needed to clarify the place of these ratios as biomarkers.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1387-1393.
https://doi.org/10.3889/oamjms.2018.319
eISSN: 1857-9655
Clinical Science

Risk Factors Associated with Neonatal Jaundice: A Cross-Sectional Study from Iran

Sayed Yousef Mojtahedi[1], Anahita Izadi[2], Golnar Seirafi[3], Leila Khedmat[4,5], Reza Tavakolizadeh[6*]

[1]Department of Pediatrics, Ziyaeian Hospital, Tehran University of Medical Sciences, Tehran, Iran; [2]Department of Pediatric Infection Disease, Tehran University of Medical Science, Tehran, Iran; [3]School of Medicine, Ziyaeian Hospital, Tehran University of Medical Sciences, Tehran, Iran; [4]Department of Community Medicine, School of Medicine,Tehran University of Medical Science, Tehran, Iran; [5]Health Management Research Center and Department of Community Medicine, Faculty of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran; [6]Department of Pediatrics, Tehran University of Medical Sciences, Tehran, Iran

Citation: Mojtahedi SY, Izadi A, Seirafi G, Khedmat L, Tavakolizadeh R. Risk Factors Associated With Neonatal Jaundice; a Cross-Sectional Study from Iran. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1387-1393. https://doi.org/10.3889/oamjms.2018.319

Keywords: Neonatal Jaundice; Predisposing factors; Jaundice

***Correspondence:** Reza Tavakolizadeh. Department of Pediatrics, Tehran University of Medical Sciences, Tehran, Iran. E-mail: rezatavakolizadeh@yahoo.com

Received: 06-Jul-2018; **Revised:** 23-Jul-2018; **Accepted:** 07-Jul-2018; **Online first:** 11-Aug-2018

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Neonatal jaundice is one of the main causes of the patient's admission in the neonatal period and is potentially linked to morbidity.

AIM: This study aimed to determine the possible risk factors for neonatal jaundice.

METHODS: We investigated the case of infants who were admitted to the neonatal department of Ziyaeian hospital and Imam Khomeini Hospital for jaundice. Simple random sampling was used to evaluate variables related to maternal and neonatal predisposing factors based on the medical records and clinical profiles. All variables in this study were analysed using SPSS software.

RESULTS: In this study, about 200 mothers and neonates were examined. Our findings depicted that mother's WBC, Hb, PLT, and gestational age were associated with jaundice ($P < 0.05$). Furthermore, there were significant relationships between different degrees of bilirubin with TSH, T4 levels and G6PD ($P < 0.05$). In fact, TSH, T4 levels and G6PD were found to be linked to neonatal hyperbilirubinemia. The risk factors for jaundice in our study population comprise some predisposing factors such as WBC, Hb, PLT, gestational age, TSH, and T4 levels, as well as G6PD. Neonates at risk of jaundice are linked to some maternal and neonatal factors that can provide necessary interventions to reduce the burden of the disease. Therefore, identification of associated factors can facilitate early diagnosis, and reduce subsequent complications.

CONCLUSION: Neonatal jaundice should be considered as the main policy in all health care settings of the country. Therefore, identification of factors affecting the incidence of jaundice can be effective in preventing susceptible predisposing factors in newborns and high-risk mothers.

Introduction

Neonatal jaundice is a common event that occurs especially in the first week of birth [1] [2] [3] and is one of the most common causes of hospitalisation of the term and preterm neonates in neonatal wards [1]. Based on the present evidence, 80% of premature infants have clinical symptoms, including yellowish skin and sclera, caused by serum bilirubin levels [4] [5]. Hyperbilirubinemia is a common disease that occurs especially in the first week of birth [1] [2] [3] and is one of the most common causes of hospitalisation of the term and preterm infants in neonatal hospitals [1]. It usually occurs on the second day of birth and is not usually harmful, and a self-limiting condition, where disease usually improves without treatment after reaching the normal amount of bilirubin [6] [7], but very high levels of bilirubin may lead to kernicterus as permanent brain damage. Nevertheless, diagnosis of newborn jaundice and its management will play an important role in the health of newborns [8]. If jaundice lasts more than 14 days, it is called to be prolonged neonatal jaundice [6].

An imbalance between bilirubin production and conjugation is the main mechanism of jaundice, which leads to an increase in bilirubin levels. This imbalance often occurs due to the immature liver and the rapid breakdown of red blood cells, which may be involved with several factors [9] [10] [11] [12].

The indirect bilirubin value in the physiologic jaundice of the term neonates does not exceed 12 mg/dL on the third day, and, this maximum increase reaches 15 mg/dL in preterm infants on the fifth day [13].

In physiologic jaundice, the maximum indirect bilirubin of infants who fed breast milk may be higher than those fed with *skimmed milk* (15-17 mg/dL versus 12 mg/dL); this higher level is probably due to the lower consumption of fluid by infants who are breastfeeding [14].

Jaundice on the first day of life is always pathologic, and urgent attention is needed to find its cause. Early jaundice is often due to hemolysis and internal haemorrhage (*cephalohematoma*, liver or spleen hematoma) or infection. Furthermore, jaundice is considered to be pathologic after two weeks and suggests direct hyperbilirubinemia [13] Jaundice is usually seen in newborns when the concentration of bilirubin reaches 5-10 mg/dl; however, it is seen as 2-3 mg/dl in adults. If the jaundice is observed, the total bilirubin should be measured to determine its severity. On the other hand, If the concentration in the newborn is more than 5 in the first day of life or higher than 13 mg/dl in the following days, further studies are needed to determine the direct and indirect bilirubin value, blood group, Coombs test, CBC, *Peripheral blood smea*r and reticulocytes count [13].

Identification of predisposing factors in the management of the disease is important [15] [16], there are a number of predisposing factors in the occurrence of this disease, including maternal diabetes, race, prematurity, height, polycythemia, male sex, *cephalohematoma*, medications, Trisomy 21, weight loss, breastfeeding, delayed meconium passage and family history of jaundice [17] [18] [19] [20] [21]. The most common cause of jaundice can be *ABO incompatibility*. Rh incompatibility and type of delivery can be among the controversial factors. Furthermore, some factors may contribute to jaundice, such as congenital infections (Syphilis, CMV, rubella, toxoplasmosis), and age more than 25 years [22]. To the best of our knowledge, there were not many studies on the epidemiology of jaundice in Iran.

On the other hand, there has also been no program for the prevention and management of jaundice. Regarding the importance of irreversible complications of hyperbilirubinemia and the prevention of these complications, the present study was aimed to investigate the predisposing factors (maternal and neonatal risk factors) in the incidence of jaundice in newborn infants admitted to Ziaeean medical centre. Identifying predisposing factors in predicting the occurrence and prevention of such risks in neonates is important to reduce the morbidity and mortality of hyperbilirubinemia.

Material and Methods

This cross-sectional study was conducted on 207 neonates (<15 days) with hyperbilirubinemia (> 15 mg/dL) admitted to Ziaeean and Imam Khomeini hospitals in Tehran from April 2010 to May 2016.

All neonates were examined for neonatal jaundice risk factors. Neonates born with jaundice were selected based on the clinical outcomes of the neonates. Furthermore, data from medical records and interviews with mothers were collected by survey staff. A checklist including demographic information and other information was also provided.

Maternal variables including blood group, RH, *Gestational diabetes mellitus* (*GDM)*, familial history of diabetes, history of anemia and thalassemia minor, history of thyroid disease during and before pregnancy, history of birth of a newborn with jaundice, history of smoking during pregnancy, use of herbal medicines during pregnancy, history of perinatal infections (TORCH: syphilis, rubella, toxoplasmosis), CMV, CBC were evaluated in the current study. Neonatal variables included gender, the age of birth, birth season, birth weight, blood group and Rh.

Hyperbilirubinemia was the criteria for entering this study. Also, exclusion criteria included incomplete medical records. However, 20% of the sample size was considered as additional samples in the current study.

Finally, data were collected from medical records and questionnaires. All data were then analysed by using SPSS software version 19. In this study, all principles of the Helsinki Statement were considered as a statement of ethical *principles* for medical *studies.* It is worth noting that parents were informed about the study.

20% of the extra sample size was added to prevent loss and withdrawal (N = 200). P is considered to be the first pregnancy in the formula, as reported previously (24 and 25). Thus, the sample size was calculated as 200 individuals: alfa = 0.05, $Z_{1-a/2=}$ 1.961150776, d= 0.03, p= 0.96, n= 165.

Data were collected by a questionnaire that was asked by the researcher from the patient. Moreover, a set of data was collected from medical records. Then, the data were analysed by SPSS software. Frequency was calculated for qualitative variables, while the mean, range and standard deviation were calculated for quantitative variables. The chi-square test was used to examine qualitative data, and t-test for non-dependent samples was used to study quantitative data. It should be noted that the *P-value of* < 0.05 was *considered significant*.

Results

The mean age of the pregnancy (weekly) based on the level of bilirubin was shown in Table 1. The result of statistical analysis indicated that the gestational age was significantly related to jaundice (P = 0.003).

Table 1: Evaluation of Blood Factors and Other Neonatal Factors in Different Levels of Bilirubin

t4/n	tsh/n	hct/n	plt/n	mcv/n	hb/n	wbc/n	Retic	bili/d	bili/ total	b/w	g/age	Bilirubin	
0.105	0.003	0.704	0.192	0.107	0.389	0.370	0.079	0.740	0.000	0.105	0.003	p-value	
8.98	4.96	40.22	290.59	98.44	15.14	11.82	0.03	0.50	9.75	2727.84	36.01	Average	10-
2.72	3.52	9.27	95.97	7.92	3.23	4.03	0.02	0.38	2.55	715.43	3.29	Deviation standard	14.9
8.74	3.88	40.85	299.48	99.61	15.67	10.70	0.03	0.50	16.24	2896.29	37.94	Average	15-
3.08	1.62	8.38	64.59	8.58	2.45	3.02	0.02	0.21	0.82	572.05	1.59	Deviation standard	19.9
10.45	1.80	48.24	341.33	102.58	16.83	8.90	0.03	0.38	19.25	3250.00	38.00	Average	20-
1.34	0.71	8.59	115.11	4.30	3.43	2.51	0.01	0.16	1.29	388.59	1.10	Deviation standard	24.9
8.97	4.75	40.56	293.49	98.75	15.28	11.56	0.03	0.49	11.04	2769.62	36.37	Average	Total
2.75	3.31	9.18	92.42	7.96	3.13	3.89	0.02	0.35	3.61	693.32	3.13	Deviation standard	

Moreover, as shown in Table, the findings of the statistical analysis revealed that the birth weight of the infant was not significantly associated with the incidence of jaundice based on the bilirubin levels (P = 0.105).

Assessment of neonatal bilirubin based on the different bilirubin level revealed that total bilirubin had a significant relationship with jaundice (P = 0.000). Furthermore, there was no significant correlation between direct bilirubin with jaundice (P = 0.740).

The average *reticulocyte* count of the infant indicated that the *reticulocyte* was not significantly associated with the incidence of jaundice at the different levels of bilirubin (P = 0.079). The mean of *Hb* regarding bilirubin level exhibited that Hb of neonate was strongly associated with jaundice (P = 0.389). Evaluation of the mean of mcv by the level of bilirubin depicted that there was no significant difference in infant mcv in different levels of bilirubin (P = 0.107).

The mean of *PLT* and *WBC* were markedly associated with jaundice (P = 0.192; (P = 0.370). The results of our study showed that the mean of Hct neonates had a significant correlation with hyperbilirubinemia (P = 0.704).

Based on the findings, it was revealed that the TSH and T4 were significantly associated with jaundice (P = 0.003; P = 0.105).

Table 2: Evaluation of Maternal Blood Groups in Different Ages of Bilirubin (bg/m)

Total	Bilirubin			P = 0.1		
	20-24.9	15-19.9	10-14.9			
54	1	9	44	Number	A	Bg/m
27.0%	16.7%	29.0%	27.0%	Percent		
20	0	7	13	Number	Ab	
10.0%	0%	22.6%	8.0%	Percent		
45	1	8	36	Number	B	
22.5%	16.7%	25.8%	22.1%	Percent		
81	4	7	70	Number	O	
40.5%	66.7%	22.6%	42.9%	Percent		
200	6	31	163	Number		Total
100.0%	100.0%	100.0%	100.0%	Percent		

Also, there was no significant difference in the maternal blood group in the neonates with different levels of bilirubin (P = 0.1; Table 2).

Our findings have revealed that there was no significant difference in maternal hematologic Rh among neonates with different levels of bilirubin (P = 0.8; Table 3).

Table 3: Evaluation of RH Blood Groups in Maternal Different Ages of Bilirubin (Rh/m)

Total	Bilirubin			P = 0.8		
	20-24.9	15-19.9	10-14.9			
154	4	24	126	Number	Positive	Rh/m
77.0%	66.7%	77.4%	77.3%	Percent		
46	2	7	37	Number	Negative	
23.0%	33.3%	22.6%	22.7%	Percent		
200	6	31	163	Number	Total	
100.0%	100.0%	100.0%	100.0%	Percent		

Based on Table 4, out of 163 patients, 41 neonates (25.2%), who their mothers suffered from GDM, exhibited a serum bilirubin level of 10-14.9, followed by a bilirubin level of 20-24.9 (16.7%) and a bilirubin level of 15-19.9 (16.1%). Neonates with different levels of bilirubin exhibited no significant difference regarding gestational diabetes mellitus (P = 0.5).

Table 4: Evaluation of Gestational Diabetes Mellitus in Different Ages of Bilirubin

Total	Bilirubin			P = 0.5		
	20-24.9	15-19.9	10-14.9			
47	1	5	41	Number	Yes	*Gestational diabetes mellitus (GDM)*
23.5%	16.7%	16.1%	25.2%	Percent		
153	5	26	122	Number	No	
76.5%	83.3%	83.9%	74.8%	Percent		
200	6	31	163	Number	Total	
100.0%	100.0%	100.0%	100.0%	Percent		

In the present study, out of 163 neonates, 72 patients (44.2%) with a history of familial diabetes revealed bilirubin levels of 10 to 14.9, following a bilirubin level of 15-19.9 (54.8%) and a bilirubin level of 20-24.9 (16.7%), (Table 5). No significant difference was found in the familiar history of diabetes among neonates with different levels of bilirubin (P = 0.2). As a matter of fact, familial history of diabetes was not found to be correlated with hyperbilirubinemia.

Table 5: Evaluation of Familiar Diabetes Mellitus in Different Ages of Bilirubin

Total	Bilirubin			P=0.2		
	20-24.9	15-19.9	10-14.9			
90	1	17	72	Number	Yes	Familiar/dm
45.0%	16.7%	54.8%	44.2%	Percent		
110	5	14	91	Number	No	
55.0%	83.3%	45.2%	55.8%	Percent		
200	6	31	163	Number	Total	

Table 6 indicated that out of 163 neonates 35 newborns (21.5%) with a maternal history of anaemia showed the bilirubin level of 10-14.9, following a bilirubin level of 15-19.9 (32.3%) and a bilirubin level of 20-24.9 (33.3%). There was no significant difference in the maternal history of anaemia between neonates with different levels of bilirubin (P = 0.3).

Table 6: Evaluation of Maternal Anemia in Different Ages of Bilirubin

Total	Bilirubin			P = 0.3		
	20-24.9	15-19.9	10-14.9			
47	2	10	35	Number	Yes	Anaemia
23.5%	33.3%	32.3%	21.5%	Percent		
153	4	21	128	Number	No	
76.5%	66.7%	67.7%	78.5%	Percent		
200	6	31	163	Number	Total	
100.0%	100.0%	100.0%	100.0%	Percent		

Bilirubin levels of 10-14.9% were seen in 9.2% of neonates with maternal thalassemia, followed by a bilirubin level of 15-19.9 (22.6%) and a bilirubin level of 20-24.9 (0%), (Table 7). The maternal thalassemia was not associated with different levels of bilirubin (P = 0.06).

Table 7: Evaluation of Maternal Thalcemia in Different Ages of Bilirubin

Total	Bilirubin			P = 0.06		
	20-24.9	15-19.9	10-14.9			
22	0	7	15	Number	Yes	Thalassemia
11.0%	0%	22.6%	9.2%	Percent		
178	6	24	148	Number	No	
89.0%	100.0%	77.4%	90.8%	Percent		
200	6	31	163	Number	Total	
100.0%	100.0%	100.0%	100.0%	Percent		

We also found that the baby's blood group was not significantly related to Hyperbilirubinemia (P=0.3l Table 8), followed by a bilirubin level of 15-19.9 in 80.6% of RH^+ infants and bilirubin levels of 20-24.9 in 66.7% of Rh^+ infants.

Table 8: Evaluation of Neonatal Blood Groups in Different Ages of Bilirubin

Total	Bilirubin			P = 0.3	
	20-24.9	15-19.9	10-14.9		
66	1	7	58	Number	A
33.0%	16.7%	22.6%	35.6%	Percent	
27	0	7	20	Number	AB
13.5%	0%	22.6%	12.3%	Percent	
49	2	6	41	Number	B
24.5%	33.3%	19.4%	25.2%	Percent	
58	3	11	44	Number	O
29.0%	50.0%	35.5%	27.0%	Percent	
200	6	31	163	Number	Total
100.0%	100.0%	100.0%	100.0%	Percent	

Based on the data presented in Table 9, it was found that the RH blood group was not related to hyperbilirubinemia (P = 0.7).

Table 9: Evaluation of RH Blood Groups in Neonatal Different Ages of Bilirubin (Rh/m)

Total	Bilirubin			P = 0.7		
	20-24.9	15-19.9	10-14.9			
157	4	25	128	Number	Positive	Rh/n
78.5%	66.7%	80.6%	78.5%	Percent		
43	2	6	35	Number	Negative	
21.5%	33.3%	19.4%	21.5%	Percent		
200	6	31	163	Number	Total	
100.0%	100.0%	100.0%	100.0%	Percent		

As shown in Table 10, out of 163 patients, 50 neonates (30.7%) with asphyxia showed a bilirubin level of 10-14.9, following a bilirubin level of 15-19.9 (3.2%). There was no significant difference in asphyxia among newborns with different levels of bilirubin (P = 0.002). In other words, asphyxia was not associated with hyperbilirubinemia.

Table 10: Evaluation of Asphyxia in Neonatal Different Ages of Bilirubin

Total	Bilirubin			P=0.002		
	20-24.9	15-19.9	10-14.9			
51	0	1	50	Number	Yes	Asphyxia
25.5%	0%	3.2%	30.7%	Percent		
149	6	30	113	Number	No	
74.5%	100.0%	96.8%	69.3%	Percent		
200	6	31	163	Number	Total	
100.0%	100.0%	100.0%	100.0%	Percent		

According to Table 11, 15.5% of neonates showed *cephalohematoma*, while 84.5% of them did not show this complication. Of the total number of 163 neonates, 13.5% of neonates with hematoma showed a bilirubin level of 10-14.9, while bilirubin levels of 19-15 and 20-24.9 were found in 25.8% and 16.7% of neonates, respectively. Findings demonstrated that there was no significant relationship between *cephalohematoma* and disease (P = 0.2, Table 10). In the present study, G6PD was also related to the disease (P = 0.02).

Table 11: Evaluation of Cephalohematoma in Neonatal Different Ages of Bilirubin

Total	Bilirubin			P = 0.2		
	20-24.9	15-19.9	10-14.9			
31	1	8	22	Number	Yes	Cephalohematoma
15.5%	16.7%	25.8%	13.5%	Percent		
169	5	23	141	Number	No	
84.5%	83.3%	74.2%	86.5%	Percent		
200	6	31	163	Number	Total	
100.0%	100.0%	100.0%	100.0%	Percent		

Discussion

Recent studies have demonstrated neonatal jaundice occurrence in more than 60% of term and 80% of premature neonates in the first week, where bilirubin is non-conjugate, *lipid-soluble*, and *non-polar pigment*. Bilirubin is one of the final products of haemoglobin catabolism and its clinical significance in the neonates is due to sedimentation in the skin and mucous membrane and the formation of jaundice.

This complication is also the most common cause of hospitalisation of the neonates in the first month after birth (about 19%). In most cases, jaundice can be transient, usually resolved by the end of the first week after birth, when the total serum bilirubin concentration is not considered to be a harmful condition. Severe hyperbilirubinemia has been described to develop with a potential risk for acute bilirubin encephalopathy and kernicterus [23] [24] [25] [26] [27] [28] [32] [33] [34] [35] [36] [37]. Neonatal jaundice usually starts from the face and progresses with the increase in the serum level to the abdomen and legs. Based data described before, Jaundice is one of the most common neonatal problems [23] [24]

[25] [26] [27] [28] [29].

This complication may lead to death in the first months, and infants who are still alive often suffer from mental retardation, movement and balance disorders, seizures, hearing loss at high frequencies, and speech impairment. Therefore, the timely diagnosis and treatment of neonatal jaundice are very important in preventing its complications. Identifying the predisposing factors of neonatal jaundice is still a serious discussion and can be effective in controlling jaundice and controlling the primary problem. In the natural state, since liver enzymes have not evolved enough, some icterus appears on the second to third day, reaching its maximum on the second to fourth day and decreasing on the fifth to seventh days. This type of jaundice is called physiologic jaundice. Factors such as maternal diabetes, race, premature infant, medication use of mother, male gender, cephalohaematoma, breastfeeding, weight loss, delayed stools in the baby may be correlated with physiologic jaundice [23] [24] [25] [26] [27] [28] [29] [30]. We also evaluated neonates for jaundice -specific risk association such as gestational age, birth weight gestational diabetes, familial history of diabetes, low birth weight, maternal history of anemia, maternal thalassemia, asphyxia, *cephalohematoma,* TSH and T4 *and related blood factors* including, blood count (*Hb*, *Hct*. *MCV*. *WBC* and *PLT)* maternal-fetal blood group and their Rh for potential risks.

The result of our study indicated that the gestational age was significantly linked to jaundice. Consist of our study; It has been described that the risk of hyperbilirubinemia significantly increases with decreasing gestational age [31] [32] [33] [34] [35]. Furthermore, weight loss in the neonatal period is considered as another risk factor for jaundice [35] [36]. Low-calorie intake has been indicated to be associated with increased hepatic circulation of bilirubin and often occurs within the first few days before milk comes in [35] [36] [37]; however, we didn't find a significant association of birth weight with the incidence of jaundice. Based on the evidence present in literature, neonates with low gestational age (less than 37 weeks) and increased level of bilirubin in the first hours of life should be evaluated to confirm and monitor them adequately. Also, rapid diagnosis of low birth weight infants with or without visual evidence of weight loss at admission is needed to be included into clinical guideline for the control of neonatal hyperbilirubinemia [38]. Another known risk factors identified in different investigations for the development of jaundice in neonates such as Asian race, birth weight, exclusive breastfeeding, difficulty feeding, male sex, labour with oxytocin, primiparity, etc., [39] [40] [41] [42] [43] [44].

Risk factors identified in different investigations for the development of jaundice in neonates among blood count variables, the mean of Hb, Hct, PLT and WBC were found to be markedly associated with hyperbilirubinemia in the present study. However, our findings have revealed that maternal and neonatal blood group and RH were not significantly associated with hyperbilirubinemia

A previous study indicated that blood type and Rh incompatibilities had been the important causes of kernicterus [45]. It has been depicted that ABO incompatibility, idiopathic jaundice, G6PD deficiency and Rh incompatibility be the most important predisposing factors for acute kernicterus [45].

Based on the data presented in the current study, G6PD was also related to the disease. While the exact mechanism for linking G6PD deficiency to hyperbilirubinemia is still not fully understood, early diagnosis of G6PD deficiency in infants can adequately reduce the risk of hyperbilirubinemia in affected neonates [40] [46], indicating the importance of prevention and timely treatment due to the incompatibility of ABO and Rh. According to the recommendations of the WHO Working Group, screening for all neonates should be performed in areas with a prevalence of 3-5% for G6PD deficiency [47]. It is worth noting that the incompatibility of the blood group can be managed through daily care and the diagnosis of mothers whose neonates are at risk for these disorders [40][48].

A study indicated that the main causes of the high prevalence of Jaundice complications in icteric newborns include incomplete follow-up in acrylic babies due to ABO incompatibility, physician insensitivity, lack of routine examination of neonatal babies born to mothers with type O (Rh+) and parental insensitivity [49].

Our data demonstrated that gestational diabetes mellitus was not linked to hyperbilirubinemia. Also family history of diabetes was not observed to be associated with hyperbilirubinemia.

A study has reported that gestational diabetes has various and dangerous side effects on the baby, the most common being neonatal jaundice (3.7%), [50]. It is also described that the incidence of neonatal jaundice in diabetic mothers is three times higher than that in the control group. Perhaps the reason for the difference in the prevalence of maternal diabetes associated- jaundice in different studies could be due to differences in study type and sample size [51]. In the current study, the maternal thalassemia and anaemia were not found to be associated with neonatal jaundice. However, further studies are needed to clarify the role of maternal thalassemia and anaemia in the development of hyperbilirubinemia. It is worth noting that anaemia may range from asymptomatic to life-threatening and reported to potentially be linked to severe hyperbilirubinemia [52]. Patients with haemoglobin H has been indicated to usually born with hypochromic anaemia, where may be at high risk for neonatal hyperbilirubinemia [53] [54]. Also, Based on our findings, *cephalohematoma and* asphyxia were not observed to be significantly linked to hyperbilirubinemia.

Neonatal asphyxia can inhibit the activity of uridine diphosphate glucuronyltransferase (UDPGT) in the liver, consequently increasing the level of unconjugated bilirubin. Cephalohematoma or ecchymosis can lead to extravascular hemolysis, consequently increasing the level of bilirubin.

A study believed that infants' asphyxia could inhibit the activity of uridine diphosphate glucuronyltransferase (UDPGT) in the liver, leading to an increase in unconjugated bilirubin. Cephalohematoma may be associated with vascular hemolysis, resulting in elevated levels of bilirubin [55]. A study found no relationship between thyroid hormones (iodine deficiency) and jaundice [56]. However, more detailed studies are needed to evaluate the role of thyroid hormones on jaundice.

In the present study, it was revealed that the TSH and T4 were significantly correlated with the occurrence of jaundice. Our study has shown that high TSH increases the likelihood of jaundice (global statistics also indicate this); therefore, paediatricians are more interested in conducting TSH tests on the fifth day of birth and comparing with the level of bilirubin. The risk factors for jaundice in our study population comprise some predisposing Factors such as WBC, Hb, PLT, gestational age, TSH, and T4 levels, as well as G6PD. Our study may be helpful in explaining the relationship between some of the predisposing factors with newborn jaundice to provide more evidence for managing disease in hospitals. Evaluation of risk factors for neonatal hyperbilirubinemia is important because high risk factors play an important role in neonatal jaundice in a Hospital. Large-scale studies are also needed for further and also by the control group. Since the promotion of neonatal health as a vulnerable group in the health care services has a special place, so the evaluation of neonatal jaundice in all levels of health services should be considered as a fundamental policy.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1394-1398.
https://doi.org/10.3889/oamjms.2018.273
eISSN: 1857-9655
Clinical Science

Evaluation of Gait Speed after Applying Kinesio Tape on Quadriceps Femoris Muscle in Patients with Knee Osteoarthritis

Klejda Tani*, Irena Kola, Vjollca Shpata, Fregen Dhamaj

Faculty of Medical Technical Sciences, University of Medicine, Tirana, Albania

Citation: Tani K, Kola I, Shpata V, Dhamaj F. Evaluation of Gait Speed after Applying Kinesio Tape on Quadriceps Femoris Muscle in Patients with Knee Osteoarthritis. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1394-1398. https://doi.org/10.3889/oamjms.2018.273

Keywords: Knee osteoarthritis; Gait; Kinesio Tape; Rehabilitation; 10-meter walk test; Gait speed

***Correspondence:** Klejda Tani. Faculty of Medical Technical Sciences, University of Medicine, Tirana, Albania. E-mail: klejdatani@gmail.com

Received: 21-Apr-2018; **Revised:** 01-Jun-2018; **Accepted:** 09-Jun-2018; **Online first:** 15-Aug-2018

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Knee osteoarthritis is a chronic degenerative disease, known as the most common cause of difficulty walking in older adults and subsequently is associated with slow walking. Functional decline, increased risk of falls and the presence of pain are, in many studies, related to the muscle weakness caused by osteoarthritis especially weakness of the quadriceps muscles. Many studies have shown that the strength of the quadriceps femoris muscle can affect gait, by improving or weakening it. Kinesio Tape is a physiotherapeutic technique, which reduces pain and increases muscular strength by irritating the skin receptors.

AIM: This study aimed to verify if the application of Kinesio Tape on quadriceps femoris muscle increases gait speed while decreasing the time needed to accomplish the 10-meter walk test in patients with knee osteoarthritis and also in subjects without knee osteoarthritis.

METHOD: In this study, we observed the change of gait speed with the help of the 10-meter walk test before, one day and three days after the application of Kinesio Tape in quadriceps femoris muscle. We compared the results of the time needed to perform the 10-meter walk in two groups. In the first group, the Patients group, participated 102 out-patients with a clinical diagnosis of primary knee osteoarthritis, while in the second group, the Control group, participated 73 subjects with a main excluding criterion a clinical diagnosis of primary knee osteoarthritis.

RESULTS: Our results indicated that there was a significant decrease of time needed to perform the 10-meter walk test in both groups three days after application of Kinesio Tape on quadriceps femoris muscle. However, there was not a significant change one day after the application of Kinesio Tape compared before its application in both groups.

CONCLUSIONS: Our results indicated that there was a significant decrease in time needed to accomplish the 10-meter walk test. Kinesio Tape is a technique that can be used especially when changing walking stereotypes is a long-term goal of the treatment.

Introduction

Osteoarthritis is a widespread, slowly developing disease, with a high prevalence increasing with age, also known as a metabolically active, dynamic disease that includes both destruction and repair mechanisms that may be triggered by biochemical and mechanical insults [1]. In developed countries, knee osteoarthritis (OA) affects between 17% and 30% of the elderly population over 65 years of age, with greater incidence, prevalence and severity in women than in men [2] [3]. Functional decline, increased risk of falls and presence of pain are, in many studies, related to the muscle weakness caused by OA [4] [5] especially the weakness of the quadriceps muscles [6].

Quadriceps weakness is one of the most common and disabling impairments seen in individuals with knee OA [7]. Sufficient quadriceps and hamstrings strength, both isometric and dynamic, is essential for undertaking basic activities of daily living such as standing and walking [8].

Patients with knee osteoarthritis have a problem walking and tend to walk slower than controls. It is shown that knee osteoarthritis is the

most common cause of difficulty walking in older adults [9] [10] and subsequently is associated with slow walking [11] [12]. In knee osteoarthritis, decreased walking speed is associated with joint space narrowing [13], increased concentrations of inflammation mediators [14], pain [15] and also quadriceps muscle weakness [16] [17].

Quadriceps femoris weakness, in particular, has been linked to functional impairment such as increased fall risk and slower walking speed, also is one of the earliest and most common symptoms of osteoarthritis [18] [19] [20]. It is also associated with adaptations in walking patterns that are theorised to put articular cartilage at risk.

For instance, subjects with knee OA who have weaker quadriceps femoris muscles exhibit less stance phase knee motion during walking. At self-selected walking speeds, it is the role of the quadriceps femoris muscles to control knee flexion during weight acceptance while the hamstring and gastrocnemius muscles are typically silent. However, in the presence of quadriceps femoris weakness, which occurs with ageing, and in the presence of knee OA, either the hamstring or the gastrocnemius muscles may be required to assist with knee control [21].

Muscle strength testing has revealed that those with knee OA have a 25% to 45% loss of knee extension strength and a 19% to 25% loss of knee flexion strength compared with similarly aged controls [22] [23] [24]. There are 3 factors thought to contribute to knee extension and flexion weakness in those with knee OA: muscle atrophy, failure of voluntary muscle activity, and apparent weakness from increased antagonist muscle co-contraction [25]. Decreases in muscle cross-sectional area (CSA) have been established in subjects with early OA degenerative changes [26] and those with severe knee OA [27]. Ikeda et al., [26] found that women with early degenerative changes in the knee joint had reductions in quadriceps CSA of up to 12%, compared with age-matched women without radiographic changes.

The aim of this study was to verify if the application of Kinesio Tape (KT) on quadriceps muscle changes gait speed while walking for 10 meters at normal speed, in patients with knee osteoarthritis and also in subjects without knee OA, before the application of KT, a day and three days after the application of KT on quadriceps femoris muscle.

Patients and Methods

In this study, we compared the results of the gait speed in two groups. The first group was the Patients group. The subjects (n = 102), aged 50-73 years (mean age 63.2), 67% of whom were female, were consecutive out-patients with a clinical diagnosis of primary unilateral knee osteoarthritis made by a rheumatologist. The main criterion for the selection of the subjects in this study was the diagnosis of knee osteoarthritis by X-ray. The second group was the Control group. The subjects (n = 73), aged 50-69 years (mean age 59.4), 70% of whom were female, were randomly chosen and the main criterion for their selection was that they were not diagnosed with knee osteoarthritis.

Criteria for excluding subjects from both groups in the study were other musculoskeletal diseases, total knee replacement, significant hip or spinal arthritis, neurological diseases and diseases that affect balance and coordination. The subjects were not in medical treatment. All of the subjects signed a written consent to participate in the study voluntarily.

Kinesio Tape (KT) was applied with a tonus regulation technique also called muscle technique on quadriceps femoris muscle. We measured the tape length in the maximal stretched position of the tissue. The application was done with the patient in this maximal stretched position. The tape was applied without stretch following the course of the muscle borders from one insertion to the opposite one.

In the patient's group, the worse knee as assessed by X-ray was the “index” knee, while in the control group we randomly chose a knee where we applied the KT.

We observed the change of time while walking for 10 meters at normal speed for each patient, before, a day after and three days after the application of KT on quadriceps femoris muscle, with the help of a 10-meter walk test, where we measured and marked a 10-metre walkway adding a mark at 2-metres and 8-metres. The patient performed three trials, and we calculate the average of three trials [28].

Statistical Analysis

Continuous variables were presented as mean and standard deviation: mean ± SD (standard deviation). Categorical variables are presented as actual numbers (n) and percentages (%). Chi-square analysis was used to compare frequencies between groups and Student t-test, one-way ANOVA or non-parametric tests were used when necessary for quantitative analysis of the variables. The analysis was conducted using the SPSS (statistical software statistics package for social scientists) version 15.0. Statistical significance was considered to be the value of $P \leq 0.05$.

Results

In this study, we compared the results of the time needed to perform the 10-meter walk test in two groups. In the first group, the Patients group participated in 102 out-patients with a clinical diagnosis of primary knee osteoarthritis, the mean age of the participants was 63.2 (range: 50-73).

Table 1: Data results on gait speed, of the Control group, in seconds before the application of KT (10 MWT before KT), one day after the application of KT (10 MWT 1 Day after KT) and three days after the application of KT (10 MWT 3 Days after KT) on quadriceps femoris muscle

	Number	Mean value	Standard Deviation	Minimum value	Maximum value
Control Group 10MWT before KT	73	7.223	1.0052	5.247	9.610
Control Group 10MWT 1 day after KT	73	7.082	0.9923	5.167	9.497
Control Group 10MWT 3 days after KT	73	6.199	0.9098	4.567	8.193

The worse knee as assessed by X-ray was the “index” knee. In the second group, the Control group, participated 73 subjects aged 50-69 years (mean age 59.4), with a main excluding criterion a clinical diagnosis of primary knee osteoarthritis.

Table 2: Data results on gait speed, of the Patients group, in seconds before the application of KT (10 MWT before KT), one day after the application of KT (10 MWT 1 Day after KT) and three days after the application of KT (10 MWT 3 Days after KT) on quadriceps femoris muscle

	Number	Mean value	Standard Deviation	Minimum value	Maximum value
Patients 10MWT before KT	102	10.412	2.5025	6.173	16.277
Patients 10MWT 1 day after KT	102	9.976	2.4712	6.160	15.847
Patients 10MWT 3 days after KT	102	6.743	1.8205	4.123	10.807

The results show that 1 day after applying KT on quadriceps femoris muscle there was not a significant change of time needed to perform the 10-meter walk test in a comparison of the time needed a day before application of KT, in both groups: Control group P = 0.405 and in the Patients group P = 0.20.

The time needed to perform the 10-meter walk 3 days after KT's application changed significantly from the time needed before KT's application on quadriceps femoris muscle in both groups: in the Control group P < 0.0001 and the Patients group P < 0.0001.

The time needed to perform the 10-meter walk 3 days after KT's application changed significantly from the time needed 1 day after KT's application on quadriceps femoris muscle in both groups: in the Control group P < 0.0001 and the Patients group P < 0.0001.

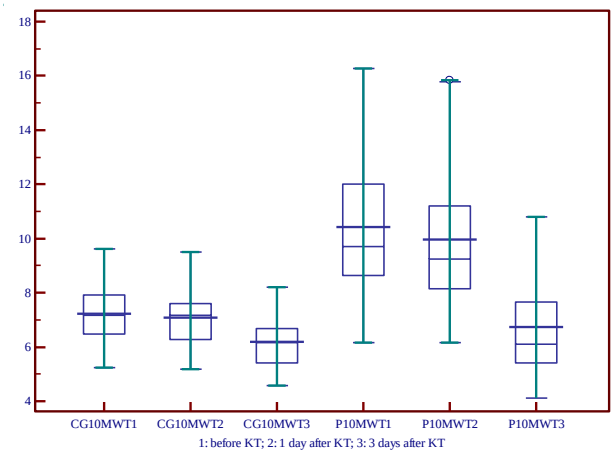

Figure 1: Comparison of values of 10-meter walk test (10MWT) in the control group (CG) and the patient's group (PG), before Kinesio Tape (KT) application (1), 1 day after KT application (2) and 3 days after KT application (3)

Discussion

Lack of information about the impact of elastic therapeutic tape in gait speed in this diagnosis led us to carry out this research. Our objective was to determine whether the application of KT on quadriceps muscle, in patients with knee osteoarthritis and also in subjects without knee OA, will lead on increasing gait speed while walking a 10-meter distance on normal speed.

The results of this study showed a significant decrease of time needed to accomplish the 10-meter walk test three days after applying KT on quadriceps femoris muscle. In graphic 1 is shown that the Patients group and the Control group finish the 10-meter walk test in a shorter time after applying KT on quadriceps muscle three days after the application. However, there was not a significant decrease of time needed to perform the 10-meter walk test one day after application of KT in both groups.

Based on these results, it can be inferred that applying KT facilitated muscle activation in the indexed knee by increasing gait speed and decreasing the time needed to perform the 10-meter walk test three days after applying KT. This suggests that applying KT leads in improving the walking speed through muscle facilitation in the indexed knee [29]. This is because KT effectively stimulated the proprioceptive sense, muscle spindles, Golgi tendons, etc., and strengthened muscles in the affected parts [30]. These results were in agreement with the results of previous studies, which reported that KT increases muscle activity, restricts excessive movement of the joint and increases gait speed [31] [32].

Kase et al., [33] and Thelen et al., [34] also recommend at least three daily actions of elastic therapeutic tape. Kase et al., mentions those three days after the application of KT can occur soft tissue changes, improvement of muscle function, an increase of blood circulation and lymphatic drainage. Thelen et al. found that after three days of KT application, was shown a significant decrease of the functional shoulder joint pain and increase of movement. Similar results as in our study were found in other studies where no significant differences were found immediately after KT application [35] [36]. Also, Chang et al., [37] found no change in grip strength immediately after applying Kinesio tape in healthy people.

The fact that the gait speed also changed in the subjects without knee OA makes us think that the application of KT has a placebo effect. There are anecdotal reports ranging from improvements in circulation to greater strength, but researchers suggest these improvements in performance are due to a placebo effect, not from the KT itself [40]. Ella Ward [41], claims that overall, KT and the placebo effect are relevant in sport and performance, as psychologists can manipulate this intervention to enhance athletic performance; avoiding more intrusive, unethical and expensive treatment methods.

However Słupik et al., [38] evaluated the effects of applying KT over the vastus medialis and found no change in muscle activity 10 minutes post-taping, but they found increased muscle activity 24 hours after Kinesio tape application. Also Lins et al., [39] evaluated the effects of KT application on the activity of the vastus lateral, rectus femoris, and vastus medialis muscles of healthy women who exercised and found no significant effects. The difference between these results may be due to different forms and tensions of Kinesio tape application. Different Kinesio tape techniques can provide different tactile stimulation intensities [30].

Limitations in this study were the sample size, with a greater sample size we could get better results. In this study, the effect of KT in increasing gait speed in knee OA and also in subjects without knee OA was statistically significant. This leads to the fact that the application of KT is effective in all subjects without any significance if they have knee osteoarthritis or not. Further studies are needed to investigate the effect of KT in gait speed on knee osteoarthritis.

In conclusion, there seems to be a significant increase of gait speed and a decrease of time needed to accomplish the 10-meter walk test three days after applying KT on quadriceps femoris muscle in the patient's group and also the control group. However, there was not such a significant decrease of time needed to accomplish the 10-meter walk test one day after application in both groups.

Kinesio Tape is a technique that can be used especially when changing walking stereotypes is a long-term goal of the treatment. More clinical research is needed to investigate the effect of KT on gait speed on knee osteoarthritis.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1399-1404.
https://doi.org/10.3889/oamjms.2018.286
eISSN: 1857-9655
Clinical Science

Early Versus Delayed Umbilical Cord Clamping on Physiologic Anemia of the Term Newborn Infant

Fatma Alzaree[1*], Ahmed Elbohoty[2], Mohamed Abdellatif[2]

[1]National Research Center, Child Health Department, Eltahrir Street, Dokki, Guiza, Cairo 1234, Egypt; [2]Ain Shams University Faculty of Medicine, Cairo, Egypt

Citation: Alzaree F, Elbohoty A, Abdellatif M. Early Versus Delayed Umbilical Cord Clamping on Physiologic Anemia of the Term Newborn Infant. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1399-1404. https://doi.org/10.3889/oamjms.2018.286

Keywords: Delayed cord clamping; Umbilical cord milking; Neonatal physiologic anaemia; Term newborn

***Correspondence:** Fatma Alzaree. National Research Center, Child Health Department, Eltahrir Street, Dokki, Guiza, Cairo 1234, Egypt. E-mail: fatmaalzaree@yahoo.com

Received: 22-May-2018; **Revised:** 22-Jun-2018; **Accepted:** 23-Jun-2018; **Online first:** 15-Aug-2018

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

AIM: Our study aims to make a comparison between the effects of milking of umbilical cord versus delayed cord clamping on Hemoglobin level at 6 weeks from delivery among term neonates and which method is more beneficial for them.

DESIGN: It was a randomised control study. Participants were randomised into 2 groups; Group 1: 125 women were assigned to delay cord clamping; Group 2: 125 women were assigned to milking of the umbilical cord 5 times before cutting. Student t-test was used to compare between the two groups for quantitative data, for qualitative data chi-square test and the Correlation coefficient was done to test the association between variables.

SETTING: This study was at El-Galaa Teaching Hospital, labour suite. Cairo, Egypt.

PARTICIPANTS: A group of 250 pregnant women starting from ≥ 37 weeks' gestational age.

INTERVENTION: In this study, we searched if the mechanism of milking or delayed cord clamping could give some of the positive benefits for neonates or not.

RESULTS: In this study, we found that milking of the umbilical cord five times as in group 1 was associated with higher hemoglobin levels at 6 weeks after birth, at physiological anemia of the fetus and significant but clinically there was no difference between the two groups (10.4 ± 0.5 and 10.6 ± 0.5 respectively, P < 0.001). Also, there was a positive correlation between haemoglobin of the mother and the newborn during the first day and after 6 weeks with r = 0.349 and 0.283 respectively and a P value < 0.001. Furthermore, there was a positive correlation between the haemoglobin of the fetus after the first day and fetus at 6 weeks with r = 0.534 and a P value < 0.001. For most other outcomes (including APGAR score, positive pressure ventilation, poor neonatal outcomes such as respiratory distress syndrome there were no significant differences between the two groups. Our study may recommend the use of umbilical cord milking in term babies when delayed cord clamping is unavailable.

CONCLUSION: Umbilical cord blood milking after its clamping improves some important haematological parameters for newborns, especially in countries with high incidence of anaemia in newborns and children.

Introduction

The umbilical cord is the essential life-keeping connection between fetus and placenta. It represents a strong connection to the fetomaternal interface while permitting fetal mobility that is essential for general fetal development and neuro-motor development in particular [1]. When a baby is born, the umbilical cord is cut, and there is a stump left which should dry and fall off by the time of 5 to 15 days after birth [2]. Delayed cord clamping, which was adopted by American Academy of Pediatrics in all deliveries, is defined as ligation of the umbilical cord 2-3 minutes after birth or on stoppage of cord pulsations, will lead to a huge amount of blood transfused from the placenta than cord clamping done promptly after delivery [3] [4]. However, delayed cord clamping may not be possible, as it could be forgotten by obstetrician or cord may have to be clamped promptly in case of fetal distress or complications at birth [5]. In such cases, we perform umbilical cord milking to transfer the extra blood to decrease blood transfusions and augment haemoglobin in both preterm and term infants**.** Both umbilical cord milking and delayed cord clamping have been related to high

iron stores in neonates [6], but it may strongly affect the cerebral blood flow dynamics [4]. Delayed cord clamping, in which we clamp the cord after 30 to 180 seconds of birth, permits the transfer of blood from placenta to the newborn, makes the hematological values and iron stores in both preterm [7] and term infants better [8] [9], decreases anemia, decreases the need for blood transfusion, improves cerebral oxygenation in earlier born babies [10] and provides considerable amount of placental stem cells to the baby without causing any adverse effects to the mother [11] [12]. A previous study has shown that there is a transfer of about 80ml of blood from the placenta at 1 minute after birth, reaching about 100 ml at 3 minutes after birth.

These additional amounts of blood can supply extra iron reaching 40–50 mg/kg of body weight. When this extra iron is added to the nearly 75 mg/kg of body iron that a full-term neonate is born with, the total volume of iron can reach 115–125 mg/kg of body weight, which may help to avoid iron deficiency anaemia during the first 12 months of life. Stabilization of the circulatory system of the neonate during the first 24 hours of life occurs if we delay cord clamping for minimum 30 seconds, leading to less need for volume therapy, transfusion and inotropic support, decreases the need for given cell transfusions, decreases the occurrence of intraventricular hemorrhage and improves neuro-developmental outcome [13]. Although apparent benefits in cord clamping after delivery 30-45 seconds, it can prevent neonatal resuscitation. So, the delay in clamping of the cord is not preferred in extremely low birth weight newborns [14]. As a substitutive manner, umbilical cord milking towards the neonate before clamping usually lasts < 5 seconds, therefore, will not prevent resuscitation of the baby. Recent studies have found that umbilical cord milking also leads to significant increase in haemoglobin in both premature [15] [16] and term infants with milking being performed either with clamped [6] or unclamped umbilical cord [17]. At the age of 6 months, high body iron stores were found in delayed-clamped babies in comparison to early-clamped infants by about 27 mg of iron. Delayed cord clamping in newborn found to decrease blood values of lead, because of improved iron status during infancy [18]. Delayed cord clamping was found to be related to some adverse reactions; it may augment rates of hyperbilirubinemia, polycythemia, and transient tachypnea in the neonate but, has never been proven to increase the rate of symptomatic neonatal disease or blood loss in mothers [19]. All healthy newborns show a drop in red blood cells values during the first weeks of life which is due to multiple physiological factors. In sick preterm infants, it occurs due to several additional predisposing causes; the most important of them is phlebotomy which is blood loss for the sake of laboratory testing. The nadir haemoglobin value in healthy term newborns rarely decreases below 10 g/dl at 10 to 12 weeks of age [20]. As this decline in haemoglobin value after birth in term infants is well tolerated and no need for therapy; it is generally known as the “physiological anaemia of infancy.” On the contrary, this drop is immediate, and the blood haemoglobin concentration descends to lower levels in premature infants weighed 1.0 to 1.5 kg at delivery to approximately 8 g/dl in infants, and approximately 7 g/dl in infants weighed < 1 kg at birth.

For this reason, because the marked decrease in haemoglobin concentration that found in many extremely low birth weight neonates is usually seen with abnormal clinical signs and need for allogeneic red blood cell transfusions [21]. Iatrogenic anaemia caused by multiple blood sampling for laboratory investigations is not uncommon and with no symptoms in babies who were born prematurely. Signs and symptoms of hypovolemic shock can become marked and life-threatening to the degree that warrants replacement of blood loss in the newborn baby when losses reach 20% of total blood volume. We should record the amount of the collected blood to prevent any unwanted iatrogenic losing of blood. Chronic losing of blood and moderate haemorrhage usually is asymptomatic in babies except for some pallor. Both term and preterm infants should be sent home on iron supplementation, either as formulas fortified with iron or as a supplementation by the mouth of 2-3 mg/kg per day elemental iron especially infants on breastfeeding. Enteral iron supplementation is available and safe for newborns with birth weight <1301 gram. Early transfusion protocols called for infants to be transfused with “fresh” RBCs (less than 7 days old). The target was to increase the life of cells in vivo and decrease the risk of hyperkalemia and acidosis. At approximately 15 milliliters per kilogram body weight, tiny babies require relatively small volumes of blood per transfusion [22]. Also; an erythropoietin is sometimes needed to limit red blood cell transfusion where the initial r-erythropoietin trials in very low birth weight infants demonstrated that administration of the drug resulted in reticulocytosis with an increase in hematocrit. Furthermore, most r-erythropoietin-exposed infants received fewer and lower volumes of red blood cell transfusion during the study period. This finding was strongest in stable, growing preterm infants, most of whom had received multiple blood transfusions prior to study entry [23].

This randomised control study, from June to December 2017, included 250 pregnant women starting from ≥ 37 weeks’ gestational age attending at El Galaa teaching hospital; to compare the effect of milking of umbilical cord versus delayed cord clamping on infant haemoglobin level at 6 weeks after delivery. The mothers were pregnant women at or above 37 weeks of gestation, with a single baby, free of other medical disorder (Diabetes-hypertension-cardiac problems-renal, hepatic, etc.) and free of other obstetric complications, e.g.: (antepartum haemorrhage, Preeclampsia, etc.). Mothers with twins’ pregnancy, preterm delivery (< 37 weeks, patient should be sure of date and date confirmed by ultrasound, e.g. biparietal diameter and femur length), prolonged rupture of membranes (> 18 hours), fever

or foul smelling liquor, antepartum hemorrhage, pregnancy-induced hypertension or diabetes mellitus and history of maternal liver or kidney disease or any other systemic illness were excluded from the study. Written informed consent was obtained from the parents and was included in the medical record. The Ethical Committee of both the National Research Centre and Faculty of Medicine, Ain Shams University have approved the study. Each woman was subjected to the following:

- General examination: pulse, blood pressure and temperature.

- Systemic examination: head and neck, cardiac, chest, abdominal and neurological examination.

- Abdominal: Fundal, umbilical and pelvic grips were done to assess gestational age by fundal height, assess fetal lie, and assess fetal presentation.

- Ultrasound evaluation to confirm fetal presentation, assess fetal growth status and amniotic fluid index. Fetal measurements include biparietal diameter, head circumference and femur length.

- After labour records include a neonatal requirement for resuscitation, admission in a neonatal intensive-care unit (NICU) and Apgar score of the fetus at 1 minutes and 5 minutes.

Neonatal haemoglobin measurement (A sample of cord blood was taken immediately after delivery of haemoglobin) by Easy Touch GCHb device. The measuring range for haemoglobin: 7-26 g/dl (1.1-33.3 mmol/L). Minimal sample volume for haemoglobin analysis: 2.6 µl.

- The EasyTouch®GCHb system is made for self-testing of Glucose, Cholesterol and Hemoglobin levels in the blood.

- Data were analysed using Statistical Program for Social Science (SPSS) version 20.0. Quantitative data were expressed as the mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage.

Non parametric data was represented by median and range.

- Data were analysed to test the statistically significant difference between groups.

1. For quantitative data (mean ± SD), student t-test was used to compare between 2 groups.

2. For qualitative data (frequency and proportion), the chi-square test was used.

3. The correlation coefficient was done to test the association between variables.

P is significant if ≤ 0.05 at a confidence interval of 95%.

Based on the previous study; comparing early versus delayed clamping of the cord and comparing early clamping versus milking of the cord [19], the expected effect size is 0.5. At an alpha level of 0.05 and a power of the study of 0.9, a total sample of 248 cases is required to elicit the difference between groups. Two cases will be added to each group to guard against fallacies in laboratory measurements. Thus, each group will include 125 women delivering a singleton fetus at term vaginally.

Results

The current study was conducted at EL Galaa Teaching Hospital during the period between June and December 2017. A total of 250 pregnant women were included in the current study. The demographic and obstetric characteristics of the study population are shown in (Table 1)**.**

Table 1: Demographic data of included women

	Group 1 N = 125	Group 2 N = 125	t/Z*	P value
Age (years)	20-37 25.6 ± 3.2	20-38 26.2 ± 4.4	-1.195	0.233
Parity (years)	0-4 1 ± 1	0-4 1 ± 1	7190.500*	0.258
Gestational Age (weeks)	37-40 38.93 ± 0.90	37.14-40 38.99 ± 0.96	-0.516	0.606
Gravidity(median)	3 (1-8)	2 (1-6)	6833.500*	0.078
Hemoglobin of mother	12.1-8.7 10.4 ± 0.6	11.7-8.5 10.3 ± 0.7	1.855	0.065

Group 1: delayed umbilical cord; Group 2: milking umbilical cord; SD standard deviation Data presented as a range, mean ± SD; range, median Z*: Mann-Whitney All mothers' data taken within one week before delivery. And mother haemoglobin is taken within one week.

Regarding the mode of umbilical cord clamping, there was a significant statistical difference between cases delivered by milking umbilical cord and those delivered by a delayed umbilical cord in haemoglobin after 6weeks, but this deference is not important clinically, regarding the haemoglobin level as shown by (Table 2).

Table 2: Comparison regarding fetal haemoglobin between the first day and 6 weeks after delivery

	Group 1 (n = 125)	Group 2 (n = 125)	t	P value
HB F1	14.3-17.3 15.9 ± 0.6	13.9-18.0 15.8 ± 0.7	1.926	0.055
HB F6W	8.9-11.7 10.4 ± 0.5	9.6-11.9 10.6 ± 0.5	-3.804	0.001*

SD: standard deviation; HBF1: Hemoglobin of fetus during first-day p<0.001*; Hbf6w: haemoglobin of fetus after 6 weeks Data presented as a range, mean ± SD.

There was a significant positive correlation between haemoglobin of mother and haemoglobin of fetus during the first day, and 6 weeks, anaemic mother affected fetus after delivery (Table 3).

Table 3: Agreement between the mode of delivery of umbilical cord and positive pressure ventilation

	Group (250)		X^2	*P-value*
	G1 (125)	G2 (125)		
PPv	9 (7.2%)	9 (7.2%)	1.211	0.271

Ppv: positive pressure ventilation. Chi-square (X^2) test was used. Data presented as a probability (p-value).

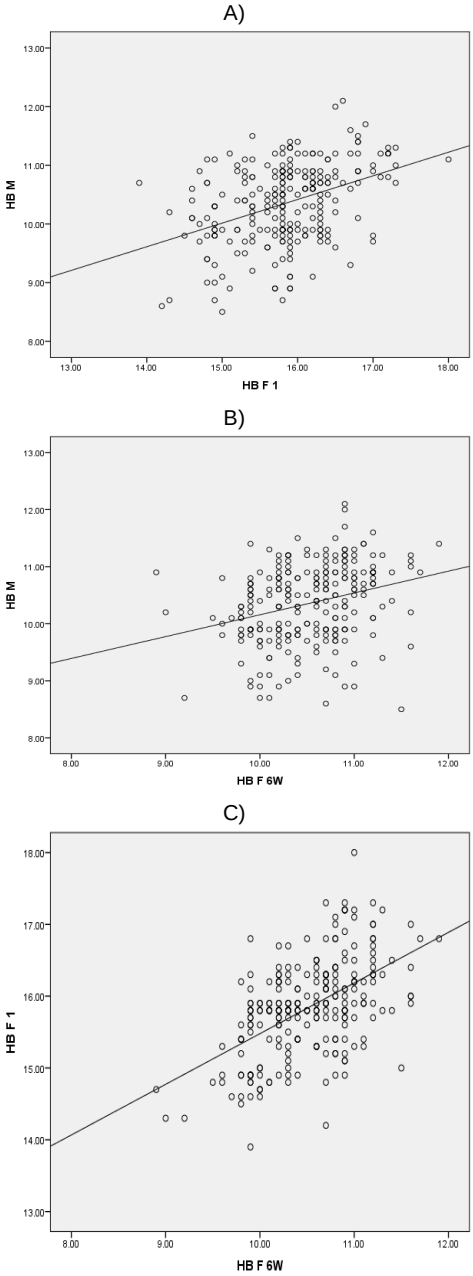

Figure 1: A) Positive correlation and significant between Hb M and Hb F1; P<0.001; B) Positive correlation and significant between Hb M and Hb F6w; P<0.001*; C) Positive correlation and significant between Hb F1 and Hb F6w; P<0.001**

There was a significant positive correlation between haemoglobin of fetus after first day and haemoglobin of the fetus after 6 weeks (Table 4).

Table 4: Correlation between haemoglobin of fetus after first day and fetus at 6 weeks

		HB F 1
HB F 6W	r	0.534
	p	<0.001*

Pearson's correlation coefficient p<0.001*.

Discussion

This study was done at EL Galaa teaching hospital in the period from June till December 2017. Some 250 pregnant women (20-40) years participated in our study. They were divided into two groups to make a comparison between the effects of Milking of umbilical cord versus delayed cord clamping on infant Hemoglobin level at 6 weeks after birth. The first group (delayed umbilical cord clamping) 125 patients aged (20-37) years with (mean ± SD 25.6 ± 3.2) with gestational age (37-40) weeks with (mean±SD 38.93 ± 0.9). The second group (milking umbilical cord) 125 patients aged (20-38) years with (mean ± SD 26.2 ± 4.4) with gestational age (37.14-40) weeks with (mean ± SD 38.99 ± 0.96). According to fetal haemoglobin on the first day, there was no statistical significance between the two groups that agree with other studies [23] [17]. Concerning the fetal haemoglobin at 6 weeks, there was a strongly elevated haemoglobin level with umbilical cord milking rather than delayed cord clamping. Different results were observed in a study had done by Yadav et al., 2015 to estimate hemodynamic parameters between 3 groups. The first group, they adopted umbilical cord milking, the second group delayed cord clamping was performed, the third one delayed cord milking was done, there was no statistical difference between the two groups concerning the haemoglobin levels at 6 weeks. The number of women in each group was 93 in their study, and in our study, each group has 125 participants [24]. Although milking was done 3 times in their study, we milked the cord 5 times in ours**.** Our study showed that milking of umbilical cord for 5 times improved haemoglobin level at 6 weeks of age in term infants in comparison to delayed cord clamping. However, haemoglobin levels on the first day were the same as in the two other groups. However, no significant change in hemodynamic parameters or clinical adverse effects in the first 24 h of life were found, haemoglobin and hematocrit were markedly elevated in both delayed cord clamping with milking cut cord group in the first 48 h of life [24]. In the process of delayed cord clamping, another 15 to 40 ml/kg of blood is passed to the baby by permitting transfusion through the placenta to be finished [25], which is recommended by a decline in umbilical venous pressure and pulsating contractions of uterus just after birth.

Milking of the cord after finishing of delayed cord clamping may invite extra blood stored in the non-pulsatile cord in addition to the normal placental transfusion and make haematological parameters better [4] [6] [17]. Our findings were similar to other studies as they did not notice a marked difference in the mean haemoglobin in umbilical cord milking and delayed cord clamping group in the period after birth promptly [17] [24]. However, in both groups in our study, we found lower mean haemoglobin levels in comparison to the mean haemoglobin values in a previous study [17]. Different from our results, a study examined 58

preterm infants between 24 and 32 weeks of gestation where clamping of the cord was postponed 30 seconds in delayed cord clamping group, and they milked the cord four times at a similar rate of 10cm/s [17]. In our study, the haemoglobin immediately after birth was lower than those shown in other studies [15] [17]. This can be attributed to non-healthy eating habits and noncompliance with oral iron therapy during the period of pregnancy among mothers in our population in comparison to developed countries. This might explain elevated mean haemoglobin value in umbilical cord milking group in a previous trial [17]. Our study also showed that haemoglobin in the first 48 h was the same as in umbilical cord milking and delayed cord clamping groups in full-term infants [4]. Another study showed that 3min of delayed cord clamping in term infants caused a marked improvement in serum ferritin level at the age of 6 months with no marked change in haemoglobin value that was different from our initial results at the age of 6 weeks in delayed cord clamping group [26].

Although, this outcome of delayed cord clamping did not continue until the age of 12 months [27]. One of the studies showed the place of the baby before clamping of the cord does not cause any effect on the amount of transfusion through the placenta [28]. We performed the umbilical cord milking five times at a speed of 10cm/sec.. However, other studies milked the umbilical cord 4 times at similar speed [17]. In our study, we perform umbilical cord milking before clamping the umbilical cord from the mother's clitoris to the umbilicus of the baby; similar to other studies where the milking of the cord was done during its attachment to the placental end [29][30]. However, the comparison between milking before and after clamping of the cord was not done, more blood is normally passing if umbilical cord milking is done before clamping. Different from Yadav et al., umbilical cord milking was done after clamping umbilical cord at a distance of 25cm from the neonate end [24]. Concerning the gestational age, there was no statistical difference between the two groups which is similar to Yadav et al., [24]. In this study, we found that no statistical difference between the two groups according to the mean of hemoglobin of the mother that is similar to other studies which showed also no statistical difference between the three groups according to the mean hemoglobin of the mother [14] [24]. Concerning Apgar score at 1 minute and 5 minute, we found no statistical difference between the two groups. The same results were found by other study [4]. There were no marked statistical differences between women of 3 groups regarding neonatal measures of birth weight, 1-min Apgar score and 5-min Apgar score [31]. In our study; concerning maternal blood transfusion, postpartum hemorrhage, and retained placenta after delivery there was no marked statistical difference between the two groups. Also, there was no great difference between the two groups according to positive pressure ventilation of fetus after delivery. The clear strength of our study, that it was a randomized controlled trial with a proper sample size, has few points of weakness and a short duration of follow up. A longer follow up till 6 to 12 months of age is needed to confirm if the primary advantage in hemoglobin remains later during infancy and early childhood. The weakness of our study is that this method may not be suitable for increasing the amount of placental transfusion for newborns with a short cord. We did not measure the real amount of blood transfused in each newborn. Nowadays, there is no direct, correct, easy and rapid way to measure blood volume. We had relied on clinical history and examination to exclude infection at 6 month of age rather than on lab investigation, such as CRP. Another restriction of our study is that we did not measured serum ferritin as it reflects real iron stores of the infant along with hemoglobin.

In conclusion, milking of umbilical cord blood after its clamping improves some important haematological parameters for neonates, particularly in countries characterised by increased rates of neonatal and childhood anaemia.

Recommendations: Umbilical cord milking can be used in term newborns as a routine or in cases when delayed cord clamping is not suitable, future trials with more follow-up are recommended to construct the continuity of haemoglobin benefit and serum ferritin after that in infancy. Other measures as oxygenation of the brain and amount of cerebral blood could also have been more evaluated. Also, superior vena-cava flow values & ECHO can be demonstrated to see the effect of the extra amount of blood passed on the cardiac function of the baby and finally, further studies of other methods of cord clamping perfectly define the future procedures to be done. That will strengthen the correct interpretation of the outcome, supports further meta-analysis, and will lead markedly to the definition of appropriate practice in this new unknown area.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1405-1409.
https://doi.org/10.3889/oamjms.2018.247
eISSN: 1857-9655
Clinical Science

The Relationship between Serum Vitamin D Levels with Allergic Rhinitis Incidence and Total Nasal Symptom Score in Allergic Rhinitis Patients

Lia Restimulia*, Dwi Reno Pawarti, Haris Mayaguyang Ekorini

Department of Otorhinolaryngology Head and Neck Surgery, Faculty of Medicine, Airlangga University, Jl. Prof. Dr Moestopo No. 47, Surabaya 60132, Indonesia

Citation: Restimulia L, Pawarti DR, Ekorini HM. The Relationship between Serum Vitamin D Levels with Allergic Rhinitis Incidence and Total Nasal Symptom Score in Allergic Rhinitis Patients. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1405-1409. https://doi.org/10.3889/oamjms.2018.247

Keywords: Allergic rhinitis; 25-hydroxyvitamin D; Allergic diseases; Total nasal symptom score; Cut-off points

***Correspondence:** Lia Restimulia. Department of Otorhinolaryngology Head and Neck Surgery, Faculty of Medicine, Airlangga University, Jl. Prof. Dr Moestopo No. 47, Surabaya 60132, Indonesia. E-mail: lia_resti@yahoo.com

Received: 16-Apr-2018; **Revised:** 23-May-2018; **Accepted:** 25-May-2018; **Online first:** 10-Aug-2018

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Allergic diseases and vitamin D deficiency were found to have a relationship. However, there was limited number of studies on the relationship between vitamin D with allergic rhinitis (AR) and total nasal symptom scores (TNSS), particularly in determining the cut-off points of serum vitamin D levels which correlated to AR.

AIM: As this particular study has never been conducted in Indonesia, the main objective of this study was to investigate this issue.

METHODS: The research was conducted at Dr Soetomo Hospital, Surabaya in January 2017. A group of 30 subjects were recruited using consecutive sampling. Levels of serum vitamin D were measured using electrochemiluminescence immunoassay (ECLIA) method while the total nasal symptom scores were obtained by accumulating all the nasal symptoms. Data of serum vitamin D levels and TNSS were analysed statistically with the Pearson correlation test.

RESULTS: It was found that the mean of serum 25(OH) vitamin D levels (9.13 ng/mL) of the AR group was significantly lower than the non-AR group (26.22 ng/mL) (P = 0.000). The vitamin D cut-off points which correlated to AR was about 12.83 ng/mL (sensitivity = 80%; specificity = 100%). A Pearson correlation test found a strong, negative correlation between vitamin D levels and TNSS (P = 0.000; r = -0.800).

CONCLUSION: There was a strong, negative correlation between serum vitamin D levels with AR and TNSS. The cut-off points of serum vitamin D levels correlated to AR were approximately12.83 ng/mL. Thus, further research needs to be conducted.

Introduction

Allergic Rhinitis (AR) is one of the inflammation diseases of the nasal mucous, caused by immunoglobulin E (IgE) after allergens exposure, which affects 10-20% of total population and keeps increasing [1]. Severity measurement of the AR symptoms can be conducted subjectively by counting the total nasal symptom score (TNSS) and objectively by counting the serum IgE levels. Moderate to severe AR present in around 67.5% of the AR population and affects the quality of life [2].

Related to that, recent studies point out the relationship between allergic diseases and vitamin D deficiency. Vitamin D deficiency has been widely discussed as one of the world health problems which can lead to acute and chronic illnesses [1] [2]. Also, recently, new research was developed over a period of three years to deal with the function of the immunomodulatory role of calcitriol (the active form of vitamin D) that correlated with AR [3]. Vitamin D can regulate the body's immune cells which work on the pathophysiology of AR. Previous research in India discovered that there was vitamin D deficiency in 91% of AR samples; however, the incidence of vitamin D deficiency in AR in Indonesia has not been found yet [4].

Nevertheless, previous findings of the relationship between serum vitamin D levels and AR were still controversial. Most studies claimed that there was a significant difference in the levels of serum vitamin D between AR patients and normal people; thus, there was a relationship found between serum vitamin D levels and TNSS [5] [6] [7]. In contrast, some other studies showed different results in the response of interleukin IL-4 and IL-13 in mice bronchoalveolar secrete after being given vitamin D [8] [9]. In other words, the relationship between serum vitamin D levels with AR and TNSS is still unclear; hence, further study is needed to obtain more accurate results.

Although Indonesia is a tropical country that sunlit throughout the year, some research found that the percentage of vitamin D deficiency was 60% in young adult women, 35% in women aged over 65 years, and 78.3% in children [10] [11]. High rates of vitamin D deficiency can be occurred due to many factors affecting serum vitamin D levels, such as age, gender, skin elasticity, and others [10].

Based on the above explanation, the relationship between allergic diseases and vitamin D deficiency was predicted to be associated with immune-modulatory effects of the derivatives of vitamin D. It has been revealed that the active form of vitamin D has some direct effects on antigen-presenting cell (APC), on mast cell, on T helper Th-2, on B-cell, and on proinflammatory interleukin**.** Vitamin D may modulate innate and adaptive immune response component functions played by T lymphocytes, both T helper Th1 and Th2 cells. Dendritic cells as an antigen-presenting cell (APC) can synthesise vitamin D by expressing vitamin D3-25-hydroxylase enzyme (CYP27A1) [12]. Vitamin D mechanism of action regulated the performance of macrophages, toll-like receptors (TLR) and natural killer cells (NK), as well as most of the Th2 cell-mediated components. The reaction of type I hypersensitivity of AR was characterised by releasing various mast cell mediators [1] [12]. Inhibition process of AR pathophysiology by vitamin D may reduce the clinical nasal symptoms [7] [13] [14]. Based on the description, this research was aimed to prove the relationship between serum vitamin D levels with AR incidence and TNSS.

Methods

This study was observational analytic with a cross-sectional approach. The subjects of the current study were AR and non-AR patients at ORL-HNS outpatient division of Allergy-Immunology at Dr Soetomo Hospital in January 2017. The AR patients were clinically diagnosed according to the criteria of *Allergic Rhinitis and its Impact on Asthma* (ARIA) [15]. Based on the formula to determine the minimum sample size, the result showed a minimum of 20 subjects: 10 subjects for each group. However, this study recruited 15 subjects in each group to find more reliable results which can be generalised to the population. Fifteen AR and fifteen non-AR patients in the age of 21-60 years, both gender, were included in the study. All patients were interviewed and undergone a complete *Ear Nose and Throat* (ENT) examination. The total nasal symptom scores of the AR patients were recorded. Also, the levels of serum vitamin D in all patients were also measured. Previously, the study has been approved by the Regional Committee of Medical Research Ethics. All subjects gave their informed consent before the study.

The exclusion criteria included acute respiratory tract infections, acute and chronic paranasal sinusitis, severe septal deviation, obstructive nasal disease, upper respiratory infection, asthma under treatment, hypercalcemia, severe hypertension, anaemia, coronary heart disease, renal and liver impairment, pregnant and lactating conditions, damaged blood preparation, or examination failure.

The total nasal symptom score (a runny nose, nasal congestion, sneezing and itchy nose) was assessed based on the severity of the symptoms. The severity degree of each symptom was based on the following scores: 0 = no symptom; 1 = mild, unobtrusive symptoms; 2 = moderate, disturbing but tolerable symptoms; and 3 = severe, disturbing, perceived to interfere with daily activities/sleep and difficult to tolerate. The maximum total nasal symptom score was 12.

The serum vitamin D levels were measured by employing the electrochemiluminescence immunoassays (ECLIA) method using Cobas E411 (fully automated) hormone-immunoassay analyser. Normal vitamin D is defined when 25(OH)D level ranges between 30-60ng/mL while vitamin D insufficiency is defined to be between 20 and 30ng/mL and vitamin D deficiency is defined to be under 20 ng/mL.

Data were analysed using the Software Package for the Social Sciences (SPSS). Pearson correlation test was used to analyse the relationship between serum vitamin D levels with AR and TNSS. The cut-off points were determined by ROC curve.

Results

Among 15 AR patients, there were 11 female patients (73.33%) and 4 male patients (26.67%) (Table 1). The AR patients were mostly found in the age group of 21-30 years, with the mean age of AR

patients was 28.87 (9.01) years. The distribution of patients according to age is summarised in Table 1. Severe AR was the most common classification found in 9 patients (60%).

Table 1: Demographic Characteristics

Variable	RA (*n* = 15)		Non-RA (*n* = 15)		*p*
	Mean	SD	Mean	SD	
Age	28.87	9.01	35.33	10.05	0.000*
	N	%	N	%	
Gender					
Male	4	26.67	11	73.33	
Female	11	73.33	4	26.67	
AR Classification	Mean	SD			
Mild inter-mittent	1	6.67			
Moderate-severe inter-mittent	1	6.67			
Mild persistent	4	26.66			
Moderate-severe persistent	9	60.00			

**P* <0.05 with independent t-test.

As shown in Table 2, the mean value of serum vitamin D levels in the AR group was 9.13 (5.06) ng/mL. While the minimum value was 3.64 ng/mL, the maximum value was 20.23 ng/mL. On the other hand, the mean value of serum vitamin D levels in the non-AR group was 26.22 (8.45) ng/mL.

Table 2: Relationship Between Serum Vitamin D Levels and Allergic Rhinitis

Group	*n*	Vitamin D		*p*
		Mean	SD	
AR	15	9.13	5.06	0.000*
Non-AR	15	26.22	8.45	

**p* <0.05 with independent t-test.

The analysis results found a significant difference in the serum vitamin D levels between AR and non-AR patients (P = 0.000). The examination techniques of serum vitamin D levels were using ECLIA method. The ROC analysis curve in Table 3 and Figure 1 showed that the cut-off points related to AR were 12.83 ng/mL with 100% sensitivity and 80% specificity.

Table 3: Vitamin D Cut-Off Points That Correlated to AR

	Cut-off Points	Sensitivity (%)	Specificity (%)	*p*
Vitamin D of AR	</ 12.830	100	80	0.000*

**P* <0.05 with ROC.

The results of this study showed a significant negative relationship between serum vitamin D levels and TNSS of the AR patients (*p*=0.000). The correlation coefficient (*r*) between the two variables was -0.8 which indicated that the two variables had a strong negative correlation (Table 4).

Table 4: The Relationship Between Serum Vitamin D levels and TNSS

TNSS	*n*	Vitamin D		Statistical analysis	*p*
		Mean	SD		
4-6	5	11.87	7.14		
7-9	6	8.74	3.56	*r*=-0.80	0.000*
10-12	4	6.29	2.61		

**p* <0.05 with Pearson correlation test.

It also indicates that vitamin D has an important role in AR symptoms because the level could be detected in the blood of AR patients and the value was inversely proportional to TNSS.

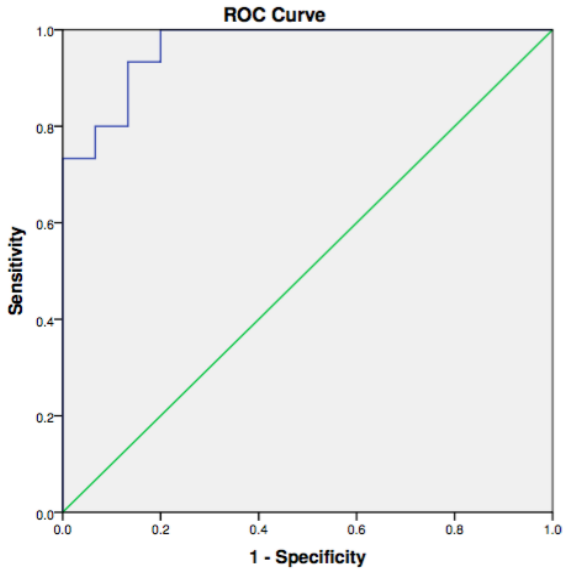

Figure 1: Receiver Operating Characteristic Curve

Discussion

This study found a significant difference between the serum vitamin D levels of the AR and non-AR patients. Similarly, a study by Yalcinkaya et al. showed that the serum vitamin D levels of AR patients were lower than the non-AR group. The mean value of serum vitamin D levels in the AR group was 15.39 ng/mL whereas the mean value in the non-AR group was 53.80 ng/mL. Furthermore, a study in India found that vitamin D deficiency occurred in 91% of the total AR samples and a significant improvement shown in TNSS after vitamin D supplementation [4] [16].

Regarding the above findings, the improvement of AR symptoms was occurred due to immunomodulatory effects of vitamin D on the immune system. Milovanovic et al. stated that there was a significant negative correlation between serum vitamin D levels and IgE [17]. That study result was from the study conducted by Yip et al., which found that vitamin D could suppress the activity of IgE-mediated mast cells [6]. Besides, Vasiliou et al. also found a similar finding: serum vitamin D levels were associated with another allergic disease, asthma [18]. Also, an increase in serum vitamin D levels after given UV-B to mice was associated with a decrease in airway inflammatory and hyperresponsive reactions compared to the samples which were not exposed to UV-B [5].

The cut-off points from the ROC analysis curve obtained in this study with chi-square were insignificant ($P > 0.05$), but the symmetric measure was significant ($P = 0.000$). The result of cut-off points is considered as valid if the test result shows an insignificant chi-square and significant symmetric measure. Therefore, the cut-off points in this study were valid [19].

Even though the previous paradigm claimed that vitamin D was exclusively produced from kidney as the result of pre-vitamin D metabolism from the sun and food intake, the new paradigm stated that vitamin D could be produced from some types of immune cells with vitamin D receptor (VDR), namely APC and T-cells. Vitamin D receptor itself is expressed by T-cells, B-cells, APC, and mast cells. The levels of vitamin D produced by these cells varied by genetic influence. This vitamin regulates two thousand different genes, one of which is a gene that plays a role in the pathophysiology of allergies [20]. Vitamin D encodes chromosome 12 which is closely related to allergic rhinitis and asthma. This vitamin regulates chromosomes played by IgE, 13Q14 and 7Q14 [5] [21].

This study found evidence of a strong, negative relationship between serum vitamin D levels with AR incidence and TNSS. It indicates that vitamin D plays an important role in the AR symptoms because the level could be detected in the AR patients serum and the value was inversely proportional to TNSS. Furthermore, the results of this study were by the study by Thakkar et al., which also found a negative relationship between serum vitamin D levels with TNSS with moderate correlation strength [7].

The action mechanism of vitamin D can be explained by its ability to control Th2-mediated cell regulation. It controls the APC by decreasing lipopolysaccharide activity (LPS), enhancing the tolerogenic phenotype of dendritic cells, and inhibiting APC differentiation [22]. Vitamin D inhibits mast cell differentiation and can cause mast cell apoptosis within 30-40 days. The inhibitory pathway of other mast cells was by inhibiting IgE and IL-4 [18]. Research in Australia with mice samples suggested the suppression of degranulation of IgE-mediated mast cells after vitamin D administration [6]. The study by Hypponen et al. found that serum vitamin D levels had a significantly negative correlation with IgE [23]. Inhibition of histamine binding to its receptor in the nasal mucosa and the induction IL-10 activity leads to suppression of Th2 activity in the initial phase [6]. Anti-inflammatory IL-10 can be produced by APC, Th2 cells, and mast cells [23].

Still, in controlling the Th2-mediated cell regulation, vitamin D can inhibit the proliferation and differentiation of B-cells into plasma cells by inducing IL-10 and inhibiting the action of IgE, IL-2, IL-4, IL-6 and pro-inflammatory chemokines [22]. In the late phase, vitamin D can inhibit the recruitment of blood eosinophils into mucose, thus inhibiting the differentiation of B-cells into plasma cells. Interleukin 5 inhibition by vitamin D causes resistance to the process of differentiation, maturation, migration, and infiltration of eosinophils into the nasal mucosa [6].

In addition to working on Th2, it also has a role in enhancing the Th1-mediated cell regulation, such as NK cells, TGF-β and IL-10, resulting in the suppression of inflammatory responses [6]. Vitamin D may also inhibit Th1-mediated cells, such as IFN-γ which decrease MHC class II activation and inhibit TLR. This process leads to decreased regulation of proinflammatory cytokines [23].

In conclusion, a strong, negative correlation was found between serum vitamin D levels with AR and TNSS. The cut-off points of serum vitamin D levels related to AR were 12.83 ng/mL. However, further randomised controlled trials are needed. Vitamin D as a potential therapeutic regimen for allergic rhinitis treatment may reduce the severity of the disease and control the frequent attacks of allergic rhinitis.

Acknowledgement

The authors would like to thank the Chair of Allergy Immunology Division at Dr Soetomo Hospital in Surabaya, East Java, Indonesia for the opportunity to conduct this research. The acknowledgement would also be given to the Research Ethics Committee at Dr Soetomo Hospital in Surabaya, East Java, Indonesia.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1410-1412.
https://doi.org/10.3889/oamjms.2018.305
eISSN: 1857-9655
Clinical Science

Quality of Life-Repeated Measurements Are Needed In Dialysis Patients

Lada Trajceska, Daniela Mladenovska, Pavlina Dzekova-Vidimliski, Aleksandar Sikole*

University Clinic of Nephrology, Ss. Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia

Citation: Trajceska L, Mladenovska D, Dzekova-Vidimliski P, Sikole A. Quality of Life-Repeated Measurements Are Needed In Dialysis Patients. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1410-1412. https://doi.org/10.3889/oamjms.2018.305

Keywords: Hemodialysis; Mortality; Quality of life; Predictors; Deterioration

***Correspondence:** Aleksandar Sikole. University Clinic of Nephrology, Ss. Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia. E-mail: asikole@hotmail.com

Received: 08-Jun-2018; **Revised:** 23-Jul-2018; **Accepted:** 26-Jul-2018; **Online first:** 15-Aug-2018

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: There is a general agreement that, besides survival, the quality of life is a highly relevant outcome in the evaluation of treatment in patients with the end-stage renal disease. Moreover, it is very important to determine whether the quality of life impacts survival.

AIM: This study aims to assess whether changes or absolute scores of the quality of life (QOL) measurements better predict mortality in dialysis patients.

MATERIAL AND METHODS: In a longitudinal study comprising 162 prevalent hemodialysis patients QOL was assessed with the 36-item - Short Form Health Survey Questionnaire (SF-36) at baseline and after 12 months. Patients were followed for 60 months. Mortality risk was assessed using Cox proportional hazards analysis for patients with below and above median levels of both physical and mental QOL component scores (PCS and MCS, respectively).

RESULTS: At the beginning of the study the mean Physical Component score was 47.43 ± 26.94 and mean Mental Component Score was slightly higher 50.57 ± 24.39. Comparative analysis of the changes during the first year showed a marked deterioration of all quality of life scores in surviving patients. The 5-point decline for PCS was noted in 39 (24%) patients and 42 (26%) for MCS. In the follow-up period of 60 months, 69 (43%) patients died. In the Cox analysis, mortality was significantly associated with lower PCS: HR = 2.554 [95% confidence interval (CI): 1.533-4.258], (P < 0.000) and lower MCS: 2.452 (95%CI: 1.478-4.065), P < 0.001. The patients who had lower levels of PCS and MCS in the second QOL survey 1 year later, had similarly high mortality risk: 3.570 (95%CI: 1.896-6.727, P < 0.000); 2.972 (95%CI: 1.622-5.490, P < 0.000), respectively. The hazard ratios for mortality across categories for the change of PCS and MCS were not significant. In the multivariate model categorising the first and second scores as predictors and adjusted for age, only the second PCS and MCS score were associated with mortality.

CONCLUSION: Low QOL scores are associated with mortality in repeated measurements, but only the more recent overwhelmed the power of the decline.

Introduction

Several studies have investigated the associations of longitudinal changes of quality of life (QOL) with mortality in hemodialysis patients. There is still controversy if the absolute values or relative changes are stronger predictors of mortality [1][2][3].

This study aims to assess whether changes or absolute scores of life quality measurements better predict mortality.

Material and Methods

We conducted a longitudinal study comprising 162 prevalent hemodialysis patients in one dialysis centre. Patients with age above 18 years and regular HD treatment for at least 3 months were included in the study. The lack of mental or physical capacity to communicate were exclusion criteria. The hemodialysis (HD) prescription in our study included 4 to 5 hours of HD thrice weekly for all patients with flow rates of 280 to 300 mL/min using a standard bicarbonate dialysis solution and low flux synthetic membranes with a surface area of 1.3 to 1.8 m^2. Data

was collected using medical histories and interviews for demographic and clinical indices.

QOL was assessed with the 36-item-Short Form Health Survey Questionnaire (SF-36) [4]. The SF-36 is a generic multidimensional instrument consisting of eight multi-item scales representing physical functioning, social functioning, role limitations caused by physical problems, and role limitations caused by emotional problems, mental health, vitality, bodily pain, and general health perceptions. The scale scores were transformed to a 0 to 100 scale, with a higher score indicating a better QOL. Finally, the physical and mental components of the eight scales were combined into a physical (PCS) and mental (MCS) component summary score. The PCS primarily reflects the dimensions of physical functioning, role limitations caused by physical health problems, pain, and general health perception. The MCS reflects primarily mental health, role limitations caused by emotional problems, social functioning, and vitality.

At the beginning of the study, during a regularly scheduled mid-week HD treatment, after being given a brief explanation, for each patient, QOL assessment using a 36-Item Short Form Health Survey Questionnaire (SF-36) was performed by a full-time employed psychologist. Assistance was available for patients who were illiterate, with an interactive approach. The SF-36 questionnaire was translated and adapted for the Macedonian population. Informed consent was provided.

Patients were followed for 60 months, until a change of dialysis modality, transplantation or death. Those transferred to other dialysis centres were continued to be followed. At 12 months of the study, the second SF-36 measurement was performed.

Statistical analysis was performed with SPSS 16.0 for Windows. Descriptive data were presented as mean±standard deviation (SD), or a the median. Percentages are given for categorical variables. Mortality risk was assessed using Cox proportional hazards analysis for patients with below and above median levels of both QOL component scores. A P-value of 0.05 or less was considered significant.

Results

The mean age of study participants was 56 years and mean dialysis vintage was 100 months. 53% of patients were male, and 24% were diabetics. Most of the patients were dialysed for 4 hours, and had good anaemia management, as shown in Table 1.

At the beginning of the study, the mean component physical score was 47.43 ± 26.94 and mean mental component score was slightly higher at 50.57 ± 24.39.

Table 1: Patients characteristics (N = 162)

N = 162	Mean ± SD/%
Age (years)	56,15 ± 13.35
Male (%)	85 (53%)
Dialysis vintage (months)	100.69 ± 76.08
Diabetes (%)	40 (24%)
Hemoglobin (g/L)	116.5 ± 8.5
Albumin (g/L)	38.8 ± 2.5
C-reactive protein (mg/L)	6.95 ± 8.46
Dialysis session time (hours)	4.07 ± 0.21
spKt/v	1.38 ± 0.20
Body mass index (kg/m²)	23.86 ± 4.6

Comparative analysis of the changes during the first year showed a marked deterioration of all quality of life scores in surviving patients (Table 2). The median level of PCS scores at twelve months was 43, and for MCS the value was 51. The 5-point decline for PCS was noted in 39 (24%) patients and 42 (26%) for MCS. Improvement of both scores was noted in 8 (6%) patients. The mean delta values varied in different QOL domains from minus 0.2 to minus 7.9 (Physical functioning: -4.2, Role-physical: -4.2, bodily pain: -7.2, general health: - 2.0, Role-emotional: -3.0, Social functioning: -4.9, Vitality: -7.9, Mental health: -0.2). Overall delta for PCS was -3.8 and for MCS was -3.7 points. The median of changes for both scores was 0.

Table 2: Comparison of Quality of life scores in two annual measurements

SF-36 scores	Patients at the start of the study (N = 140)	Survived after 12 months (N = 140)	*P*
Physical functioning	53.87 ± 32.58	49.60 ± 32.89	0.003
Role-physical	42.26 ± 42.25	39.86 ± 41.10	0.204
Bodily Pain	63.45 ± 56.17	56.17 ± 30.69	0.0001
General health	36.48 ± 18.00	34.47 ± 19.23	0.052
Role-emotional	48.57 ± 24.98	45.57 ± 24.19	0.010
Social functioning	61.96 ± 31.83	57.12 ± 33.27	0.0001
Vitality	55.64 ± 20.16	49.65 ± 44.93	0.002
Mental health	52.15 ± 23.50	55.40 ± 19.47	0.833
Physical component (PCS) score	48.93 ± 26.10	45.13 ± 26.38	0.0001
Mental component (MCS) score	52.50 ± 23.50	48.44 ± 24.61	0.0001

In the follow-up period of 60 months, 69 (43%) patients died. We evaluated associations between mortality and the absolute PCS scores, and also the different grades of scores declinations. In the univariate analysis the basal PCS values (45 < PCS > 45), PCS values at twelve months (43 < PCS > 43), delta PCS (declination more than 5 points) < ΔPCS > (declination more than 5 points), delta PCS (declination more than 10 points) < ΔPCS > (declination more than 10 points), were included. In the Cox analysis, mortality was significantly associated with lower PCS: HR = 2.554 [95% confidence interval (CI): 1.533-4.258], (P < 0.000) and lower MCS: 2.452 (95%CI: 1.478-4.065), P < 0.001. The patients who had lower levels of PCS and MCS in the second QOL survey 1 year later had similarly high risk of mortality: 3.570 (95%CI: 1.896-6.727), (P < 0.000); 2.972 (95%CI: 1.622-5.490, P < 0.000), respectively. The Hazard Ratios for mortality across

categories for PCS and MCS change were: 1.164 (95%CI: 0.840-2.551), P < 0.178 and 0.706 (95%CI: 0.404-1.233), P < 0.221 for a change -5 points; 1.205; (95%CI 0.606-2.395), P < 0.595 and 1.202 (95%CI: 0.567-2.546), P < 0.632 for >10 points decline.

Table 3: Cox proportional hazard multivariate analysis on mortality and Physical Component Score (PCS)

Variable	P	Hr	95% CI Lower upper
PCS at baseline (< 45)	0.685	1.189	0.514-2.751
Age (> 65)	0.021	2.030	1.111-3.708
PCS second survey (< 43)	0.001	2.982	1.556-5.715

In the multivariate model categorising the first and second scores as predictors, adjusted for age, only the second PCS and MCS score were associated with mortality (Table 3 and 4).

Table 4: Cox proportional hazard multivariate analysis of mortality and Mental Component Score (MCS)

Variable	p	HR	95% CI lower upper
MCS at baseline (<52)	0.802	1.101	0.520-2.328
Age (>65)	0.007	2.255	1.245-4.086
MCS second survey (<50)	0.002	2.583	1.398-4.772

Discussion

As published before, the study presented here found that low physical and mental scores predicted mortality [5] [6]. There are few studies addressing the impact of the annual change of the quality of life scores on mortality. Liebmans group compared the 1-year mortality rates in patients whose PCS and MCS increased or decreased ± 5 points *vs* those who did not. This retrospective study demonstrated an increased risk in mortality only for MCS score [7]. In the HEMO study, patients older than 70 years showed no substantial mean declines in both component scores over 3 years [3]. The clinical importance of the changes in QOL scores is still under investigation [8] [9]. DOPPS study data did not find the score change clinically relevant. Only the second QOL score predicted outcomes, which implicates the importance of frequent measurements and not the sharpness of decline. Our study observation is in agreement with those findings. Even the decline of 10 points did not achieve significance in predicting life quality scores. We speculate that marked individual variations of change among patients and slightly higher mean scores in our dialysis population when compared to European patients'-data [7], could contribute to these findings.

In conclusion, deteriorated physical and mental aspects of quality of life in hemodialysis patients are accompanied by further deterioration. Low QOL scores are associated with mortality in repeated measurements, but only the more recent overwhelmed the power of the decline. Medical, psychological and social interventions of preventable factors are expected to improve the well-being of dialysis patients. Particularly important is the increased awareness for the need for regular QOL assessments.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1413-1418.
https://doi.org/10.3889/oamjms.2018.287
eISSN: 1857-9655
Clinical Science

Quality of Life in Children with Asthma versus Healthy Children

Samaneh Kouzegaran[1], Parisa Samimi[2], Hamid Ahanchian[1], Maryam Khoshkhui[1*], Fatemeh Behmanesh[1*]

[1]Allergy Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran; [2]School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Citation: Kouzegaran S, Samimi P, Ahanchian H, Khoshkhui M, Behmanesh F. Quality of Life in Children with Asthma versus Healthy Children. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1413-1418. https://doi.org/10.3889/oamjms.2018.287

Keywords: Asthma; Children; Physical Function; Emotional Function; Social Function

***Correspondence:** Maryam Khoshkhui and Fatemeh Behmanesh, Allergy Research Center, Ghaem Hospital, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran. Tel/Fax: 00985118458769. E-mail: KhoshkhuiM@mums.ac.ir and BehmaneshF@mums.ac.ir

Received: 19-Apr-2018; **Revised:** 25-Jun-2018; **Accepted:** 29-Jun-2018; **Online first:** 16-Aug-2018

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Asthma is the most prevalent chronic disease in the pediatric age group. The disease affects different aspects of the children's lives, such as physical, emotional, social and educational aspects. Thus, more focus has been on the quality of life in these patients rather than the duration of their illness in recent years.

AIM: This study examined the different aspects of quality of life in asthmatic children for the first time in this geographic area.

METHODS: The study was cross-sectional conducted in 2015-2016. The asthmatic group was 100 patients aged 8 to 12 admitted to the Asthma and Allergy Clinic of Ghaem Hospital (as) in Mashhad with the control group composed of 100 healthy children of the same age and gender. The standard questionnaire pedsQLTM was used for comparing the quality of life of children in the two groups. Statistical analysis was SPSS23 with P-value less than 0.05, which was statistically significant.

RESULTS: In each group, 58 patients were boys, and 42 were girls. In a comparison of the quality of life of children, the asthma group with a mean total score of Peds QL 20.99 ± 12.54 compared to the healthy children with a mean total score of Peds QL of 8.8 ± 5.41 had a lower quality of life ($P < 0.001$). Moreover, regarding various aspects of quality of life asthma group had a lower quality of life in physical performance, emotional performance and performance in school ($P < 0.001$). Nonetheless, there was no significant difference between the two groups considering social function ($P = 0.267$). Examining the relationship between Peds QL score of patients with asthma with various variables was indicative of the fact that Peds QL scores were significantly correlated with the gender of the patients, showing better quality of life in the girls ($P = 0.001$).

CONCLUSION: The results indicated that children with asthma have a significantly lower quality of life compared with healthy children of the same age. Also, in examining the different aspects of quality of life, these children had a lower quality of life in physical performance, emotional performance, and performance at school, and were at the level as that of healthy children only in social performance.

Introduction

Asthma is a respiratory condition marked by attacks of spasm in the bronchi of the lungs, causing difficulty in breathing. It is usually connected to an allergic reaction or other forms of hypersensitivity [1].

Asthma management for reducing inflammation of the air passage is through minimising pre-inflammatory environmental contacts using daily anti-inflammatory drugs and controlling the condition of the onset of the condition that makes asthma worse [2]. Less inflammation usually leads to better control of asthma, with fewer attacks, and reduction of the need for fast-paced asthma medications, but the attacks keep happening yet [3]. Early intervention with systemic corticosteroids greatly reduces the severity of such attacks [4]. The progress in asthma management, especially in pharmacotherapy, enables everyone, except the child with severe asthma, to live naturally [5].

Even though the reason behind childhood asthma is not known, concurrent research showed a combination of environmental contacts and inherited biological, genetic talents [6]. Pulmonary contacts of its environmental causes are inhaled allergens, viral respiratory infections and chemical contaminants, viral respiratory infections and chemical and biological air pollutants like tobacco smoke in the environment [7]. In a prone host, immune responses to these common

contacts can be a stimulant for pathogenic, long-term inflammation and undesired repair of damaged airways. In the early life of the growing lung, these pathological trends cause adverse effects on the growth and differentiation of airways, resulting in altered adult paths [5].

Asthma is a common chronic disease with significant pathogenesis. In 2011, more than 10 million children (14% of American children) had been diagnosed with asthma throughout their lives, 70% of the group had asthma in 2015. Being a male and life in poverty are the risk factors for childhood asthma: 15% of boys had asthma compared to 13% of girls, and 18% of all children living in low-income families compared to 12% of children who were not poor [5].

Childhood asthma is among the commonest causes of emergency admission, hospitalisation and absenteeism from school [8]. The high prevalence of hospitalisation and death from asthma has to do with poverty, ethnic minority and urban living [9]. In the past two decades, admission to emergency rooms, hospitalisation, and death from asthma were 2 to 7 times higher in African American children compared with white children. For ethnic minority asthmatic patients living in low-income urban communities, it is believed that a combination of biological, environmental, economic and psychosocial risk factors increases the likelihood of severe asthma attacks [10].

Several studies in many countries have shown a prevalence of around 50% for childhood asthma. Overall, the prevalence of childhood asthma varies greatly from one area to another. A major international study on the prevalence of Childhood Asthma, 233 centres from 97 countries (International Study on Asthma and Allergies in Childhood, Phase 3) revealed a wide range of current outbreaks at 6-7-year-old (2.4 to 27.6%) and 13-14-year-old children (from 0.8 to 32.6%) [11].

Almost 80% of all asthmatic patients report the disease onset before age 6. However, from among all young children with recurrent wheeze, only a few have stable asthma at the end of their childhood. Early childhood asthma risk factors are defined for stable asthma. Asthma prognosis includes major (parent asthma, eczema, inhaler allergy) and minor risk factors (allergic rhinitis, cold wheeze, more than 4% eosinophil, food allergen allergy). Allergies in young children with a frequent cough or wheeze are the strongest risk factor for childhood asthma. The prevalence of asthma is well-connected with the incidence of allergic rhinoconjunctivitis and atopic eczema [5] [12].

Various studies have shown that quality of life varies in chronic diseases, and as asthma is considered as one of the common chronic diseases, it is important to study the quality of life of these patients. Additionally, the prevalence, symptoms, complications and effects of asthma on growth and development and other aspects of the life of school-age children point to the importance of research in this age group [13]. Thus, by determining the quality of life of these patients, one can ease decision making in treatment and propose new solutions to the treatment team.

Considering this, we can determine the different aspects of quality of life in asthmatic children for the first time in this geographic area and designed interventional plans to improve the quality of life of these children according to the results.

Methods

This cross-sectional study was conducted in 2015-2016 to evaluate the quality of life of asthma patients aged 8-12. One part of the population was the patients aged 8-12 admitted to childhood asthma and Allergy Clinics of Ghaem Hospital (as) Mashhad diagnosed with asthma by their children's allergy specialist. The purpose and content of the questionnaires were described to the parents of the child by the GP present at the clinic, and they were given a questionnaire to complete in case of agreement. The questionnaire was given to the control group as well, selected from the two parts of the city and various schools, completed by children and their parents. Finally, the data was collected with statistical analysis was performed on them.

The study used a pedsQLTM standard questionnaire to assess the quality of life of children with asthma. The questionnaire examines the quality of life of children in four aspects including physical, emotional, social and educational performance, whose validity and reliability are confirmed in various studies.

In addition to the standard questionnaire, a questionnaire prepared by the researchers containing information such as age, gender, parental education, family income, etc. was also provided to parents and completed.

Considering the professors' opinions and the paper by Jafari et al., [14], with an alpha of 0.05 and a beta of 0.2, the sample size in each group was considered 100. Thus, 100 children were selected with asthma and 100 healthy children as the sample size.

Data analysis was done after data collection and initial processing in two parts. First, all observations were performed using descriptive statistics method including frequency tables, frequency distribution and columnar graphs, and statistical indices. Central tendency and dispersion were described.

Examining the goals and hypotheses of the plan, the Kolmogorov-Smirnov test was used to test the normality of the data. For comparison of quantitative variables between groups, a t-test was used in the case of normal distribution of data, and Mann-Whitney-U test was used in the case of a normal distribution. The relationship between qualitative variables was also evaluated using chi-square and Fisher's exact test. Statistical analysis was performed using SPSS22, with a P value less than 0.05 was considered statistically significant.

In this study, no intervention was performed for patients, but prior to entering the study, the goals and method of the study, as well as the contents of the questionnaires were completely explained to patients and their parents, and then informed consent form was given to the participants, so that in case of consent to be completed. Information about each patient was recorded using the code assigned to the patient, and access to information was only available to project implementers to provide the complete safety and patient information protection.

Results

This study examined 100 children aged 8-12 years with asthma and 100 healthy children, compared in this age range. Fifty-eight patients were boys, and 42 were girls. The mean age of the patients was 9.37 ± 1.34 in the asthmatic group and 9.29 ± 1.24 years in the control group. Mann-Whitney test showed no significant differences between the two groups (P = 0.781). Moreover, children in asthmatic and control groups were divided and compared according to different living conditions like parenting life (P = 0.304), living with parents (P = 0.234), father's education (P = 0.957), maternal education (P = 0.87), maternal occupation (P = 0.508), family income level (P = 0.953), parental smoking (P = 0.852) and the mean number of family members (P = 0.307). None of these cases showed a significant difference between the two groups. Chi-square test was used to compare the two groups.

Comparison of children's quality of life using Peds QL questionnaire in two groups showed that the asthmatic group with a mean total score of Peds QL was 20.99 ± 15.54 compared with healthy children with a mean total score of Peds QL of 8.8 ± 5.41% had a lower quality of life (P < 0.001).

Moreover, compared with different aspects of life quality in these two groups, asthmatic patients showed a lower level of quality of life in physical performance, emotional performance and performance in school (P < 0.001). However, in the case of social performance, the difference in score from the questionnaires was insignificant groups (P = 0.267) (Table 1).

Table 1: Comparison of the life quality of the subjects in the control and asthma groups

Characteristics	Control group N = 100		Asthma group N = 100		p-value
	Mean	SD	Mean	SD	
Physical performance	2.12	2.11	7.63	4.82	<0.001
Emotional performance	2.43	2.39	5.62	4.09	<0.001
Social Performance	2.13	1.84	3.57	4.08	0.267
Performance at school	2.12	2.5	4.17	3.75	<0.001
Total score of Peds QL	8.8	5.41	20.99	12.54	<0.001

*Mann-Whitney test was used to compare the two groups.

Comparing the quality of life between the two genders showed the group observed had no significant differences between boys and girls genders in the control group (P > 0.05) neither in the overall Peds QL score nor any of the aspects of quality of life. However, in the group of children with asthma, the physical function (P < 0.001) and social function (P = 0.015) had a higher quality of life than boys. Moreover, the mean total score of Peds QL in boys with asthma was 24.57 ± 13.32 and in girls was 16.23 ± 9.68, which shows the higher quality of life in asthmatic girls (P < 0.001) (Table 2).

Table 2: Comparison of quality of life between boys and girls in two groups: control and asthma

Characteristics	Control group N = 100		P value	Asthma group N = 100		P value
	Boy	Girl		Boy	Girl	
Physical performance	2.05±1.75	2.21±2.55	0.952	9.19±4.96	5.55±3.76	<0.001
Emotional performance	2.36±2.07	2.52±2.8	0.907	6.21±4.24	4.83±3.79	0.104
Social Performance	1.91±1.51	2.42±2.21	0.421	4.45±4.5	2.39±3.12	0.015
Performance at school	1.56±1.41	2.88±3.36	0.16	4.71±4.11	3.44±3.11	0.174
Total score of Peds QL	7.89±3.34	10.04±7.25	0.673	24.57±13.32	16.23±9.68	0.001

*Mann-Whitney test was used to compare the two groups.

As shown in Table 3, comparing different aspects of quality of life in patients based on the severity of the disease using Kruskal-Wallis statistical test showed that only physical function (P = 0.01) was affected by the severity of the disease. Patients with a higher grade of step had a higher average score in this aspect of quality of life and a lower quality of life later on. However, there were no significant differences between the different stages of the disease (P > 0.05) (Table 3 and Figure 1).

Table 3: Comparison of the life quality of patients with asthma according to the severity of the disease

	Step I N = 28	Step II N = 35	Step III N = 33	Step IV N = 4	P-value
Physical performance	6.17±5.23	7.3±5.55	8.88±3.56	9.5±1.73	0.01
Emotional performance	5.17±3.84	5.72±4.59	6.08±3.94	3.75±2.87	0.5
Social Performance	5.1±4.87	2.93±4.28	2.77±2.93	5±2.44	0.065
Performance at school	4.17±4.58	3.75±3.4	4.37±3.39	5.75±3.94	0.616
Total score of Peds QL	20.64±14.79	19.72±14.12	22.11±9.5	24±5.09	0.263

*Mann-Whitney test was used to compare the two groups.

The relationship between Peds QL scores of patients with asthma with different variables related to the characteristics of the patients as well as the severity of their disease was examined using statistical regression test.

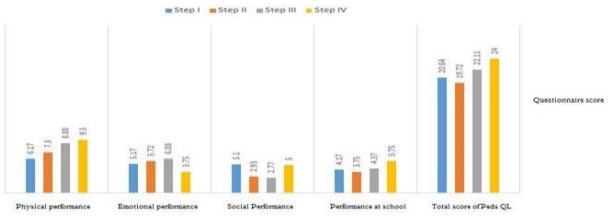

Figure 1: Comparison of the life quality of patients with asthma according to the severity of the disease

In this evaluation, it was found that Peds QL scores were significantly correlated with the gender of the patients (B = -0.928; standard error 2.58 and P = 0.001). Girls with asthma have a better quality of life compared to boys with asthma (Table 4).

Table 4: The relationship between Peds QL score and different characteristics of patients with asthma in the regression test

Independent variable	B	Standard error	P-value*
Age	-0.246	1.16	0.833
Gender	-9.28	2.58	0.001
Number of children	2.27	1.32	0.091
The parents being alive	-1.78	9.71	0.855
Life with parents	2.64	8.43	0.755
Father's education	-3.09	2.71	0.257
Mother's education	5.85	2.55	0.025
Mother's employment	-7.41	3.78	0.053
Income	-0.51	2.68	0.85
The onset of the disease	0.552	1.56	0.726
History of asthma	-0.29	1.63	0.859
Parental smoking	1.69	3.75	0.653
Infant eczema	4.33	3.98	0.281
Allergic rhinitis	-4.67	2.91	0.112
Hospitalization	-5.42	3.9	0.168
Disease step	1.27	1.51	0.402

Discussion

Asthma is an allergic disease affecting air passage (bronchus). When an allergic reaction occurs, the bronchial are constricted, narrowed, and blocked by the mucus, making it difficult to breathe. An asthma attack can be very frightening for a child as the feeling of choking makes fears more difficult to breathe [15]. Life quality in patients with asthma is a process affected by the interaction between different physiological and psychological variables. Psychological variables directly affect the life quality of patients, whereas physiological variables indirectly affect the life quality of these patients due to psychological variables [16].

In this study, compared with children's quality of life, the asthma group with a mean total score of Peds QL 20.99 ± 12.54 compared to healthy children with a mean total score of Peds QL of 8.8 ± 5.41 had a lower quality of life. Moreover, comparing the different aspects of quality of life shows that in these two groups, asthmatic patients showed a lower level of quality of life in physical performance, emotional performance, and performance in school. However, in the case of social performance, the difference in scores obtained from the questionnaire was insignificant between the two groups. Comparing different aspects of patients' quality of life-based on the severity of the disease, only the physical function of the patients significantly decreased with increasing severity of the disease. The relationship between Peds QL score of patients with asthma with different variables using regression analysis showed that Peds QL scores had a significant relationship only with the patients' gender so that girls with asthma had a better quality of life than boys

The study found that children with asthma had a lower quality of life compared to healthy children of the same age. Previous studies have shown that due to the chronicity of asthma, this disease can have a profound effect on the lifestyle and function of the affected patients, and negatively affects group activities, social functioning, mental performance, and academic achievement [17].

In previous studies, the drop of quality of life of patients with asthma of different ages has been reported, including Miadich et al., 2015 [18] who examined the quality of life associated with asthma using a questionnaire and eventually reported asthma as a chronic disease affecting the quality of life of children with asthma greatly influences asthma.

Kiotseridis et al., 2018 [19] reviewed the quality of life of 399 patients (236 adults and 163 children) with asthma using HRQL questionnaire and reported that children with asthma have the significantly lower quality of life than the other communities of children.

Annett et al., 2001 examined 339 children aged 4-8 with asthma in a study. In this study, the life quality of children with asthma and their relationship with the symptoms of patients has evaluated: 63% of these children had moderate asthma, and 37% had mild asthma. The study stressed the negative effects of asthma on children's quality of life [20].

Reichenberg et al., 2000 [21] examined 61 children aged 7-9 with asthma in Sweden, of whom 36 were boys and 25 were girls, 11 had mild asthma, 40 moderate asthma and 10 severe asthma. Asthma showed a significant negative effect on the quality of life of these patients using the PAQLQ questionnaire, which wears more pronounced in young children. The study reported that gender, eczema and rhinoconjunctivitis had no significant effects on the life quality of these patients. However, our study showed that gender affects the quality of life of children, this difference can be due to the type of attitude in both genders, girls and boys are different from the limitations of the disease and how to deal with the

disease as well as the attitudes of others in this regard. Another point is the difference in the type of independence and access to academic goals. Hence, the physical and mental differences between the two genders change their view of the disease, its acceptance, and its treatment.

Hallstrand et al., 2003 [22] studied the life quality of 160 adolescents with mild asthma using Peds QL questionnaire. They reported that these patients have a lower quality of life than healthy children regarding their life quality related to health. Also, these patients had lower levels of physical, emotional and school performance than the healthy children of the same age did. These results are totally in line with these results because in our study, in addition to the overall quality of life of these patients, in examining different aspects of quality of life similar to this study, the quality of life was lower in physical, emotional, and school performance. However, there were no significant differences in social function in healthy children.

Everhart et al., 2008 [23] examined 14 review studies for the relationship between the quality of life of children with asthma and their severity, reporting that 9 out of 14 studies showed that the severity of asthma had a significant effect on the quality of life of patients. They also stated that the type of questionnaire used to assess life quality affected the outcomes of these studies. The study showed that factors like the economic level of the family and the number of children in the family affected the life quality of children with asthma. However, in the study by Salman Yazdi et al., [24], there was a significant relationship between the number of children in the family and the quality of life of two-year-old children. Moreover, it was reported that an increase in the number of children in the family reduced the quality of life of children. This is probably because although the number of family members increases the level of independence in children and adolescents, due to the possibility of reducing family health care and increasing the risk factors for asthma (e.g. allergens, infections, inappropriate nutrition, stress and competition among children) quality of life in children decreases.

However, childhood asthma leads to different problems and suffering for the child and his family, so determining the quality of life of these patients can help identify the specific needs of these patients and their families. After this, the adoption of suitable therapeutic and educational methods for these patients and their families, because of difficulty in many aspects of life quality, could be related to the reduction of illness and its harm, the improvement of the physical and psychological health of children. Finally, they helped improve their quality of life.

In conclusion, the results indicated that Peds QL questionnaire score for children aged 8-12 with asthma was significantly higher than that of healthy children matched by age and gender.

Hence, children with asthma had the significantly lower quality of life than their peers. Also, in an exclusive study of different aspects of life quality, these children had lower levels of quality of life in physical performance, emotional performance, and performance in school and had the same level of quality to that of healthy children only in social performance. Another important issue was that of children with asthma, girls have a higher quality of life than boys that may be due to the cultural issues of society.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1419-1422.
https://doi.org/10.3889/oamjms.2018.329
eISSN: 1857-9655
Clinical Science

Effect of Ceftriaxone versus Amoxicillin + Clavulanic Acid for Treatment of Acute Bacterial Rhino Sinusitis: Short Course Therapy

Malath Azeez Al-Saadi[1]*, Safaa Sahib Naji Sultan[2]

[1]Pharmacology and Toxicology, College of Dentistry, University of Babylon, Hilla, Iraq; [2]Surgery Department, College of Medicine, University of Babylon, Hilla, Iraq

Citation: Al-Saadi MA, Sultan SSN. Effect of Ceftriaxone versus Amoxicillin+ Clavulanic Acid for Treatment of Acute Bacterial Rhino Sinusitis: Short Course Therapy. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1419-1422. https://doi.org/10.3889/oamjms.2018.329

Keywords: Acute bacterial rhinosinusitis; Amoxicillin+ clavulanic acid; Ceftriaxone

***Correspondence:** Malath Azeez Al-Saadi. Pharmacology and Toxicology, College of Dentistry, University of Babylon, Hillah, Iraq. E-mail: malathazeez1122@gmail.com

Received: 27-Jul-2018; **Revised:** 22-Jul-2018; **Accepted:** 27-Jul-2018; **Online first:** 16-Aug-2018

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Acute bacterial rhinosinusitis is one of upper respiratory tract infection that disturbs patient life and requires special consideration.

AIM: To evaluate the efficiency of Ceftriaxone versus a high dose of Amoxicillin-clavulanic acid for the treatment of acute bacterial rhinosinusitis.

PATIENTS AND METHOD: Observational retrospective study include120 patients of both sex classified into two groups equally conducted. G1 treated with Ceftriaxone 1 g intramuscular injection once daily while, G2 treated with oral Amoxicillin-clavulanic acid (875 mg/125 mg) twice daily for 3-4 days then, the outcome of treatment evaluated as a cure or failed at the fifth or fourth day of treatment.

RESULTS: Significant cure response observed in Ceftriaxone treated patient's $P \leq 0.05$ and significant failure response observed in Amoxicillin-clavulanic acid-treated patients when groups compared with each other. About gender and age groups, no significant differences in number between group 1 and 2 $P \geq 0.05$.

CONCLUSION: Ceftriaxone found more effective in the treatment of acute bacterial sinusitis than Amoxicillin+ clavulanic acid. Amoxicillin+clavulanic acid associated with more male failure cases recorded than female.

Introduction

Rhinosinusitis is an inflammation of the lining epithelia of paranasal sinuses and nasal cavity associated with nasal purulent secretion [1] [2]. Rhinosinusitis classified to acute and chronic infection according to the duration of symptoms and to viral and bacterial rhinosinusitis depending on the causative source [3]. Symptoms are nasal congestion, purulent secretion, facial pain or pressure, pain on bending forward, upper teeth pain, fever and headache [4][5]. Acute rhinosinusitis among the principal cause to millions of physician visits every year [5]. The common cold is the common upper respiratory tract disease that could be complicated with bacterial infection leading to acute bacterial rhinosinusitis [2] [6].

The inability of clinical criteria to distinguish between viral and bacterial rhinosinusitis cause inappropriate, excessive antibiotics prescription [6] [7].

Although most of the rhinosinusitis is of viral cause and self-limiting if symptoms persist for more than 10 days or worsen within 7 days regarded as acute bacterial rhinosinusitis [8]. According to that strategy of treatment changed to involve antibiotic treatments, topical corticosteroids, analgesics and/or decongestants [9].

However, proper antibiotics choice and duration are of great interest. Chose antibiotic prescription to depend on bacterial species, including *Streptococcus pneumoniae*, *Haemophilus influenzae* and, particularly in children, *Moraxella catarrhalis* [10].

Guidelines of treatment suggest Amoxicillin-clavulanate as a first-line empiric antibiotic for mild rhino sinusitis short course treatment [11] [12]. In case of no improvement or penicillin sensitivity Amoxicillin-clavulanate dose either increased or another antibiotic selected like respiratory fluoroquinolone such as moxifloxacin, levofloxacin or cephalosporin's [12] [13].

Ceftriaxone is third-generation cephalosporin with strong wide range antibacterial profile, its β-lactam antibiotic act by inhibition of bacterial cell wall synthesis. Ceftriaxone half-life about eight hours allows single daily dose for treatment of infections caused by various bacterial species like Klebsiella, Providencia, Serratia, and Haemophilus species. It is the drug of choice for meningitis caused by *Haemophilus influenza*. Adverse reaction of Ceftriaxone is hypersensitivity responses that are indistinguishable from those of penicillin [14].

Previous studies show that Ceftriaxone is good replacements for amoxicillin in the treatment of acute tonsillopharyngitis due to its safety [15].

The current study aims to evaluate the efficiency of Ceftriaxone versus oral Amoxicillin-clavulanic acid for the treatment of acute bacterial rhinosinusitis.

Patients and Method

A retrospective observational study conducted in (ear, nose and throat) ENT consultant clinic of our teaching hospital at a period from October 2016 to December 2017 for patients diagnosed with acute bacterial rhinosinusitis under the approval of a responsible, ethical committee of surgical department

A group of 120 patients of both sexes with age 15-52 years attend ENT consultant clinic suffering from facial pain and pressure, nasal obstruction with purulent discharge (rhinorrhoea) and fever after flu-like reaction for more than 10 days included according to Infectious Disease Society of America (IDSA) guidelines [12].

Patients with a history of flu-like illness without complication within the first 7 days, patients with previous surgery on nasal sinuses, patients with diabetes mellitus or renal impairment, patients with allergy to penicillin groups or ceftriaxone, patients with traumatic nasal injury, and symptoms duration more than 12 weeks excluded.

All patients included clinically examined by nasal endoscope finding the followings:

Nasal septal deviation, bilateral hypertrophy of inferior turbinate, pus discharge from middle meatus with red nasal mucosa and postnasal drip (mucus pus), then sent to computerised coronal tomography to ensure no complications.

Patients with a positive sign and symptoms of acute bacterial rhinosinusitis treated as the following:

Group 1: 60 patients prescribed with Ceftriaxone 1g intramuscular injection every 24 hours.

Group 2: 60 patients prescribed with Amoxicillin-clavulanic acid orally 1000 mg (875 mg/125 mg) every 12 hours for 3-4 days.

Xylometazoline nasal decongestant and analgesia prescribed for both groups.

After antibiotic regimen, patients followed up on the fifth day to evaluate the following:

1 - cure: no sign and symptoms of acute bacterial rhinosinusitis clinically.

2 - failure: persistence of sign and symptoms of acute bacterial rhinosinusitis clinically or complicated.

3 - an adverse reaction to the antibiotic regimen.

(Amoxicillin + clavulanic acid) oral tablet 1000 mg (875 mg/125 mg) (Comox Acino, Acino Pharma., Switzerland).

Ceftriaxone vial for intravenous /intramuscular injection 1 g (TORLAN, Spain).

Data presented by the proportion of the total number. Statistical significant assessed by Fisher exact test at the level of ≤ 0.05 using SPSS version 21 from IBM.

Results

The current study results show patients treated with Ceftriaxone for short course treatment have significantly higher cure proportion than those treated with (Amoxicillin + clavulanic acid) during follow up period, P ≤ 0.05, Table 1.

Table 1: Response to treatments among groups

Treatments	Patients number	Gender		Cure cases	Failure cases
		Male	Female		
Ceftriaxone G1	60	34 (55.9%)	26 (44.06%)	50 (83.33%)*	10 (16.66%)
(Amoxillin/clavulanic acid) G2	60	32 (53.3%)	28 (46.6%)	39 (65%)	21 (35%)*

*p ≤ 0.05.

By gender, no significant differences in number between group 1 and 2, P ≥ 0.05. Cure cases about the gender of Ceftriaxone treated group show higher proportion among male than female, Table 2.

While in-group 2 the proportions of male and female were comparable in cure response, Table 3.

On the other hand, failure of treatment was more in male than female in group 2, Table 3.

About age group, no differences in numbers of patients observed between treatments groups, Table 2 and 3.

Table 2: Response to treatments of group 1 about gender and age group

Age group	Male	Female
20-30 years	25 (42.3%)	19 (31.66%)
30-40	5 (8.47%)	5 (8.47%)
40-60	3 (5%)	3 (5%)
Cure cases	27 (81.8%)	23 (84.61%)
Failure cases	6 (18.18%)	4 (15.38%)

Three patients develop skin rash adverse reaction to Ceftriaxone while only one patient develop a skin reaction to Amoxicillin+ clavulanic acid treatment recorded during the study period.

Failure cases treated by the change of antibiotic regimen or surgical treatment for those complicated during the study period according to clinical examination at follow up visit.

Table 3: Response to treatments of group 2 about gender and age group

Age group	Male	Female
20-30 years	21 (35%)	19(31%)
30-40	8 (13.3%)	6(10%)
40-60	3 (5%)	3(5%)
Cure cases	19 (59.37%)	20(71.4%)
Failure cases	13 (40.6%)	8(28.57%)

Discussion

Acute bacterial rhinosinusitis among the higher complaints of patients attends ENT outpatient clinics in our region. It disturbs the patient's normal life with serious complication if not treated properly [16].

The current study was a retrospective observational study that aimed to evaluate the response of patients with acute bacterial rhinosinusitis to two regimens of antibiotics including first-line oral (Amoxicillin + clavulanic acid) drug in high dose and Ceftriaxone.

Short antibiotic treatment decided to avoid an adverse reaction, development of bacterial resistance and decrease cost; depending on previous studies that found no dependable differences in response between short and long antibiotic treatment [12] [17].

The choice of Ceftriaxone was following recent guidelines that suggest β-lactam agent rather than a respiratory fluoroquinolone for empiric antimicrobial therapy [12].

Beside to Ceftriaxone efficiency against *Haemophilus* species and other pathogenic bacteria which are mostly isolated species from swaps of acute bacterial rhinosinusitis patients and its recorded safety for short course treatments that decrease patients hospitalisation and cost [18].

The study results show significant high cure response to Ceftriaxone than (Amoxicillin + clavulanic acid) as observed at the end of 3-4 days of treatments according to guidelines of short course antibiotic treatments. This result proves the efficiency of Ceftriaxone for acute bacterial rhinosinusitis. Although its parenteral drug, it's well tolerated with broad spectrum efficiency that made Ceftriaxone good alternative antibiotic. This explanation goes with Seaton and Barr [18] study that evaluate outpatient parenteral antibiotic therapy to decrease hospital admission and cost. Also, agree with Duncan and colleague study [15] that found Ceftriaxone excellent broad-spectrum antibiotic for various clinical infection states including upper respiratory tract infection.

Moreover, high failure response to (Amoxicillin + clavulanic acid) observed in this study even though it regarded as the first-line drug for acute bacterial rhinosinusitis could be explained by the development of non B- lactamase type of resistance in certain pathogenic species like *Streptococcus pneumonia* making Ceftriaxone more efficient replacement [19] [20].

However, it required future microbiological study to look for the most common causative microorganism for acute bacterial rhinosinusitis in our region.

This finding unlike Muhammad and colleague [21] finding that demonstrate the effectiveness of high dose Amoxicillin + clavulanic acid in the treatment of acute bacterial rhinosinusitis as compared to levofloxacin for 10 days treatment, Which may be due to the dissimilarity of treatment period that lasts for 3-4 days in the current study.

About gender response to treatment, male treated with Ceftriaxone show more resolution of symptoms (cure) as compared to female patients, that probably due to differences in some included cases. While, in group 2, male patients show more failure response than the female of the same group that could be due to the variation of an inhabitant of a respiratory microorganism or due to variation in immune response and tolerance between male and female [22] [23].

The antibiotic-related adverse reaction was predicted for the types of antibiotic used with more adverse reaction recorded in group 1 [24] although, it is not serious reactions.

Nasal decongestant and analgesics prescribed for both treatment groups to decrease nasal obstruction and relief painful symptoms associated with acute bacterial rhinosinusitis.

In conclusion, Ceftriaxone found to be more effective than Amoxicillin + clavulanic acid in the treatment of acute bacterial sinusitis. Amoxicillin +

clavulanic acid associated with more male failure cases than female.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1423-1430.
https://doi.org/10.3889/oamjms.2018.310
eISSN: 1857-9655
Clinical Science

Effect of Combination Therapy of Ceftazidime/Amikacin and Monotherapy with Imipenem on the Treatment of Fever and Neutropenia in Patients with Cancers

Ali Arash Anoshirvani[1], Nader Zarinfar[2*], Mohammad Rafiee[3], Zahra Zamani[4]

[1]Department of Hematology and Medical Oncology, Arak University of Medical Sciences, Arak, Iran; [2]Department of Infectious Disease, Arak University of Medical Sciences, Arak, Iran; [3]Department of Statistics and Epidemiology, Arak University of Medical Sciences, Arak, Iran; [4]Department of Internal Medicine, Arak University of Medical Sciences, Arak, Iran

Citation: Anoshirvani AA, Zarinfar N, Rafiee M, Zamani Z. Effect of Combination Therapy of Ceftazidime/Amikacin and Monotherapy with Imipenem on the Treatment of Fever and Neutropenia in Patients with Cancers. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1423-1430. https://doi.org/10.3889/oamjms.2018.310

Keywords: Imipenem; Ceftazidime; Amikacin; Fever; Neutropenia

***Correspondence:** Nader Zarinfar. Department of Hematology and Medical Oncology, Arak University of Medical Sciences, Arak, Iran. E-mail: nzarinfar@yahoo.com

Received: 25-Jun-2018; **Revised:** 23-Jul-2018; **Accepted:** 26-Jul-2018; **Online first:** 18-Aug-2018

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

AIM: This study aimed to compare the effect of Imipenem monotherapy and combination therapy with Ceftazidime/Amikacin in febrile episodes in neutropenic cancer patients.

MATERIALS AND METHODS: In this double-blind randomised trial, 122 adult patients with cancer, neutropenia and fever who were treated by chemotherapy were gathered by simple sampling method and were divided randomly to two equal Imipenem (IP) and Ceftazidime/Amikacin (CA) groups. 500 mg of Imipenem was administered every 6 hours IP group and 2 g of Ceftazidimeplus 15 mg/kg/day in 2 equally divided doses of Amikacin was administered in the CA group. The treatment was continued for 72 hours in both groups. Data were analysed with SPSS19.

RESULTS: There was a significant difference between the mean temperatures of three days in each group ($P < 0.001$). There was no significant difference between the two groups regarding microbial response to antibiotics. There was no significant difference between 19 patients of IP and 13 patients of CA groups regarding bacteriologically documented infection ($P = 0.3$).

CONCLUSION: Unmodified therapy by Imipenem is as effective as combinational therapy by Ceftazidime/Amikacin in clinically and bacteriologically documented infection.

Introduction

Cancer is the second leading cause of death after heart disease [1] [2]. Cancer patients are susceptible to neutropenia following chemotherapy. One of the complications of chemotherapy is a fever in neutropenic patients. Neutropenia is a medical emergency [3] [4] [5]. Fever occurs in 10-50% of patients with solid tumours and more than 80% of patients with haematological malignancies during chemotherapy-induced neutropenia [6] [7]. Bacteremia can occur in 10-25% of all patients, especially at neutrophil levels below 100 cells/μl. Gram-negative bacteria resistant to the drug increase the risk of infection in patients with febrile neutropenia [9]. In the initial assessment of fever, blood collection of all catheter lumens (if present) is important as a culture of the peritoneal vein in the patient with neutropenic cancer [11]. Choosing the right antibiotic in patients with neutropenic fever can effectively improve survival and quality of life and reduce the cost of treatment in health centres. No specific drug or drug combination cannot be recommended to all patients for the treatment of neutropenic fever.

There is no consensus to manage fever and neutropenia in cancers, which can be due to several factors including the emergence of new risk factors in hosts with reduced immunity, changes in the epidemiology of infections, increased bacterial

resistance, and treatment costs [12] [13]. The combination of amikacin and ceftazidime is considered as a standard treatment. Ceftazidime is widely used in this field, and the effect of its combination with aminoglycosides has been applied in the treatment of neutropenic fever [11]. Imipenem can be used as a single drug and has been very effective in controlling infection in neutropenic patients due to its wide-ranging bactericidal properties. Imipenem is an intravenous β-lactam antibiotic that has a nephrotoxic potential. Today, imipenem is widely used as a broad-spectrum antibiotic in the first line of treating fever and neutropenia in patients with cancer [14].

Previous studies have reported that the efficacy of ceftazidime in the treatment of fever and neutropenic was 44% and 41% respectively [15], while in other studies, the efficacy of ceftazidime and amikacin were determined to be similar [16] [17] [18] [19] [20] [21] [22]. However, it has been demonstrated that the use of imipenem is superior to ceftazidime in the treatment of chemotherapy-induced febrile neutropenia [15]. It has been revealed that the efficacy of meropenem in comparison with the combination of ceftazidime and amikacin did not have any significant difference [20] [23]. While a similar study depicted that, the use of meropenem in the treatment of fever and neutropenia was more effective than combination therapy of ceftazidime and amikacin [21] [22] [23]. Since our country is different regarding climate, economic, antibiotic availability and possibly the type of microbial flora with other countries, revision of the studies undertaken by other countries is necessary because the selection of these regimes in each region and even in each hospital is unique. Few studies have been done on the therapeutic effect of imipenem, ceftazidime/amikacin. Imipenem also has a high price compared to ceftazidime/amikacin. Reducing the monetary burden on patients and the state is important in treating the disease. Furthermore, the continued use of a high-value, broad-spectrum antibiotic by the most physician is a factor in the development of drug resistance in organisms that give the drug a good response.

This study aimed to determine predominant germs in patients with febrile neutropenia and to determine the antibiotic resistance in febrile neutropenic cancer patients. Also, patients' responses to these two drug regimens were investigated to help clinicians decide on appropriate treatment for this condition [23].

Material and Methods

In this interventional study, 122 patients with neutropenic fever who referred to Khansari Hospital in Arak were enrolled in the study. Inclusion criteria include a person less than or equal to 18 years old with cancer (a hematologic or a solid tumour), Patients undergoing chemotherapy, Oral temperature >38.3°C or > 38°C for one hour, neutrophil count below 1500 μL, satisfaction to participate in the study. Exclusion criteria include pregnancy, lactation, and antibiotic intake at least three days before the study, history of pneumonia or anaphylaxis with antibiotics studied, patients with history of seizure, localized leukemia and central nervous system infection, cystic fibrosis, liver and kidney failure, aplastic crisis, coma, septic shock, bone marrow transplantation, acquired immunodeficiency virus, fever with a specific origin.

To validate the hypotheses and analysis of the symptoms, if patients were diagnosed with another episode of fever and neutropenia, patients have not re-entered the study. Patients were randomly divided into two groups. The first group received 500 milligrams of imipenem (built in Iran) intravenously every 6 hours (at 100 ccs normal saline diluted) in 30 minutes. For the second group, 2 g of ceftazidime (made in Iran) every 8 hours (in 100 ml of normal saline) was intravenously used over 30 minutes and immediately followed by amikacin therapy (15 mg) twice a day.

Before administration of antibiotics, all eligible patients underwent an initial assessment including history, physical examination, and culture from any potential site of infection (e.g., blood culture, oral and urinary tract). Laboratory tests included complete blood count (CBC), Na, K, blood urea nitrogen *(*BUN*),* Cr, Ca, aspartate aminotransferase (*AST*), Alanine aminotransferase (*ALT*), alkaline phosphatase (Alk-p), Bilirubin. Patients were checked daily for the presence of fever, and the patient's body temperature was recorded accurately by using a mercury thermometer. All patients in both groups were evaluated for response to treatment after 72 hours of antibiotic therapy. Clinical confirmation of infection is defined as Clinical evidence of infection with or without microbiological confirmation. Bacteria are defined as fever and at least one positive blood culture. Clinical response to treatment included fever in the patient.

If the symptoms of the infection were resolved without modification in the diet, it was considered an improvement. Moreover, the condition was classified as a treatment failure, if the symptoms were not changed or worsened, or even the signs of infection improved by changing the antibiotic regimen or the occurrence of death following infection.

On the third day, blood and urine culture was performed from patients to assess the bacteriological response to treatment. The bacteriological response for all infections was measured by comparing the cultures before treatment with post-treatment cultures. The positive bacteriological response is defined as 1: Eradicating which pathogens are eliminated on the treatment lines. 2: Eradicating with potentially strong, where appropriate principles for cultivation are not

available, but the patient has a satisfactory clinical response. The failure of the bacteriological response is defined as 1 - Continuity means the removal of some or all of the pathogens before treatment. 2 - Failure with a high probability where positive culture is unavailable, but the clinical signs of infection in the patient persist or worsen. In patients who responded to treatment, we continued treatment for up to 7 days or 4 days after the fever. If after 72 hours, the fever did not stop, another drug was added for treatment, and the patient was excluded from the study.

Death in the first three days of treatment was considered as treatment failure for clinical and bacteriological responses. Patients were regularly evaluated for adverse drug-related side effects. Before starting an antibiotic, three blood samples, urine culture, stool culture, and catheter tip were obtained from each patient. Mac Conkey agar and blood agar were used in this study, followed by urine culture (Chocolate agar), blood cultures (Mac Conkey agar and xld agar), stool culture (Thioglycolate), and catheter tip (elemental environment) all of which were made by CONDA.

An antibiogram test was performed, where antibiotic disks containing imipenem (with 10 µg per disk) and ceftazidime (30 µg per disk) and amikacin (30 µg per disk) (antibody medicine, Iran) were placed on the medium, followed by incubation in 37°C for 24 hours.

After 24 hours, the diameter of the inhibition zone was measured. The susceptibility and resistance of isolated microbes from culture media were determined based on the diameter of the inhibition zone (mm).

Data were analysed by SPSS 19 software. The Independent Samples t-Test was applied to compare the means of two independent groups for data with normal distribution, and the Mann-Whitney test was used in the absence of normal distribution.

Results

In this study, 122 patients with neutropenic fever were studied in cancer patients. Of these, 68 (55.7%) were male, and 54 (44.3%) were female. The mean age of the imipenem group was 49.9 (SD: 11.73), and the mean age of the Ceftazidime / Amikacin group was 43.36 (SD: 11.65), with a significant difference between the two groups (P = 0.002). The underlying disorders of the patients were as follows; leukaemia: 50 subjects (41%), lymphoma: 24 (19.7%), solid tumour: 38 subjects (31.1%), myelodysplastic syndrome: 7 subjects (5.7%). There was no significant difference between the two groups regarding the distribution of underlying disease (P = 0.93). All patients entering the study had a temperature of more than 38.3°C on the first day. The average body temperature on the first day in the imipenem group was 39.08 (SD: 0.22), while it was determined to be 39.2 in the ceftazidime/amikacin group (SD: 0.25), which depicted a significant difference between the two groups in terms of mean fever on the second and third days (P = 0.004). Of all patients entering the study, 17 (13.9%) were febrile on the third day, 7 (11.5%) were in the imipenem group and 10 (16.4%) in the ceftazidime/amikacin group. There was no significant difference between the two groups regarding the number of discontinuation of fever in the two groups (P-value = 0.60). In this study, the neutrophil count was mild in 19 patients (15.6%), followed by medium neutrophilia in 80 subjects (65.6%), severe neutrophilia in 23 patients (18.9%). There was no significant difference between the levels of neutrophil in the first day (P = 0.20) and the third day (P = 0.13) between the two groups. On the third day, 13 patients (10.7%) had neutrophil levels below 500 cells/µl. Twelve patients (52.2%) with severe neutropenia remained febrile on the third day, of which 5 subjects (38.5%) belonged to the imipenem group, and 7 subjects (70%) were assigned into ceftazidime/amikacin group. There was no significant difference in fever on the third day in both groups (P = 0.21). Furthermore, 21 patients (17.2%) had positive blood culture on the first day. No significant difference was found between the two groups regarding positive blood culture (P-value = 0.63).

Seventy patients (33.3%) had positive blood culture on the third day, where 3 (25%) patients belonged to the imipenem group, while 4 subjects (44.4%) belonged to ceftazidime/amikacin group. No significant difference was found between the two groups regarding positive blood culture on day 3 (p=0.39). Moreover, sixteen patients (13.1%) had a positive urine culture on the first day. There was no significant difference between the two groups regarding positive urine culture in the first day (p=0.78), while 100% of patients had a negative urine culture on day 3. No patient showed any positive stool culture. One patient (1.6%) had a positive catheter tip culture, where it was observed in the imipenem group. There was no significant difference between the two groups regarding the catheter tip culture at day 1 (P = 1.00). The catheter culture of this patient remained positive on the third day.

Out of 21 patients who had positive blood culture on the first day, 5 patients (23.8%) showed Staphylococcus epidermidis, Followed by the presence of Staphylococcus aureus (9.5%; 2 patients), Escherichia coli (57.1%; 12 patients) and Pseudomonas aeruginosa (9.5%; 2 patients). There was no significant difference between the two groups regarding bacterial isolation from the blood on the first day (P = 0.77). Blood culture in the third day of patients with gram-negative organisms (E. coli and Pseudomonas spp.) was negative. Blood cultures of all patients with Gram-positive organisms (*S. aureus*

and *S. epidermis*) remained positive on day 3. No significant difference was observed between the two groups regarding the type of bacteria isolated from blood culture on day 3 (P = 0.90).

It is worth noting that the only bacterial species isolated from urine culture was *Escherichia coli*. We did not observe a significant difference between the two groups regarding bacterial isolation from the urine (P-value = 0.78). The only bacteria isolated from the catheter tip culture were *S. aureus*. In 16 patients, we had to modify the type of antibiotic. Vancomycin was added to the regimen of 14 patients (87.5%), of which 5 patients (83.3%) belonged to the imipenem group and 9 subjects (90%) was observed in the ceftazidime/amikacin group. Two patients (12.5%) also received amphotericin, of which 1 patient (16.7%) was determined to belong to the Imipenem group, and 1 patient (10%) was observed in the group of ceftazidime/amikacin. Therefore, no significant difference was found between the two groups regarding the need for drug modification (P = 1.00). Bacteriologic infection was confirmed in 32 patients (26.2%) during episodes of fever, of which 19 patients (31.1%) were in the imipenem group and 13 patients (21.3%) in the ceftazidime/amikacin group. There was no significant difference in bacteriological infection between the two groups (P = 0.30).

The clinical response of patients to antibiotics was divided into three groups: 1) fever reduction occurred in 105 patients (86.1%), of which 54 patients (88.5%) in the imipenem group and 51 patients (83.6%) in the ceftazidime/Amikacin; 2) improvement with antibiotic adjustment occurred in 16 patients (13.1%), of which 6 subjects (9.8%) were in the Imipenem group, and 10 patients (16.4%) were observed in the group of ceftazidime/amikacin; and 3) death occurred in one patient (1.6%) in the imipenem group.

There was no significant difference between the two groups regarding clinical response to antibiotics (P-value = 0.35).

Of the 38 patients with positive microbial culture, 29 (80.6%) had a positive microbial response to antibiotics.

The results were negative on the third day, of which 18 patients (85.7%) were in the Imipenem group, and 11 patients (73.3%) were in the ceftazidime/amikacin group. There was no significant difference between the two groups regarding microbial culture response to an antibiotic (P = 0.41). Imipenem was more effective than ceftazidime/amikacin reducing fever and improving clinical symptoms of patients with and without bacteriological confirmation. However, there was no significant difference between the two groups. Both drugs were more effective in patients with bacteriological confirmation than non-bacteriologic confirmation.

Nevertheless, there was no significant difference between the two groups. No side effects were seen in any of the patients. Because the fever values were measured in three consecutive periods in two groups, advanced analysis of the repeated measurements analysis has been used. Using this analysis, there was a statistically significant difference between the mean fever in three periods (P < 0.001), but there is no significant difference between the mean fever in the three consecutive periods in the two groups.

Imipenem was depicted to be more effective than ceftazidime/amikacin in clinical improvement and fever reduction. However, no significant difference was observed in the two groups. Also, ampicillin was more effective than ceftazidime/amikacin in clinical improvement and fever reduction in patients with severe neutropenia, but no significant difference was found between the two groups.

Table 1: Demographic and statistical information in two intervention groups

Group / variable		Imipenem	*Ceftazidime* and *amikacin*	P-value
Body temperature of the first day (SD)		39.08(0.22)	39.20(0.25)	0.004
Body temperature of the second day (SD)		37.63 (0.93)	37.64 (1.01)	0.14
Body temperature of the third day (SD)		37.19(0.72)	37.40(0.96)	0.09
Neutrophil counts on the first day	1000-1500	(%9.8)	13(%21.3)	0.20
	500-1000	42(%68.9)	38(%62.3)	
	<500	13(%21.3)	10(%16.4)	
Neutrophil counts on the third day	1000-1500	8(%13.1)	51(%24.6)	0.13
	500-1000	48(%78.7)	38(%62.3)	
	<500	5(%8.2)	8(%13.1)	
Body temperature on day 3 in patients with neutrophil counts below 500	38.3<	5(%38.5)	7(%70)	0.21
	37.2>	8(%61.5)	3(%30)	
Blood culture on the first day	Positive	12(%19.7)	9(%80.3)	0.63
	Negative	49(%14.8)	52(%85.2)	
Blood culture on the third day	Positive	3(%25)	4(%44.4)	0.39
	Negative	9(%75)	5(%55.6)	
Urinary culture on the first day	Positive	9(%14.8)	7(%11.5)	0.78
	Negative	52(%85.2)	54(%88.5)	
Urinary culture on the third day	Negative	9(%100)	7(%100)	_
First day stool culture	Negative	61(%100)	61(%100)	_
The first day of the catheter tip culture	Positive	1(%1.6)	0(%0.0)	1.00
	Negative	60(%98.4)	61(%100)	
The third day of the catheter tip culture	Positive	1(%100)	_	_
Tabs episode classification	With bacteriological confirmation	22(%36.06)	16(%26.22)	0.30
	No bacteriological confirmation	39(%63.93)	45(%73.77)	
Clinical response to antibiotics	Stop the fever	54(%88.5)	51(%83.6)	0.35
	Improve with antibiotic change	6(%9.8)	10(%16.4)	
	Death	1(%1.6)	0(%0.0)	
Microbial culture response to antibiotics	Positive	18(%85.7)	11(%73.3)	0.41
	Negative	3(%14.3)	4(%26.7)	
Body temperature on day 3 in patients without bacteriological confirmation	38.3<	4(%11.42)	6(%15.38)	0.77
	37.2>	31(%88.57)	33(%84.61)	

None of the Gram-positive bacteria was susceptible to three antibiotics. One hundred per cent of the gram-negative bacteria were sensitive to imipenem and ceftazidime, and 86.6% were sensitive to amikacin. Both *P. aeruginosa* and two *E. coli* blood cultures were resistant to amikacin.

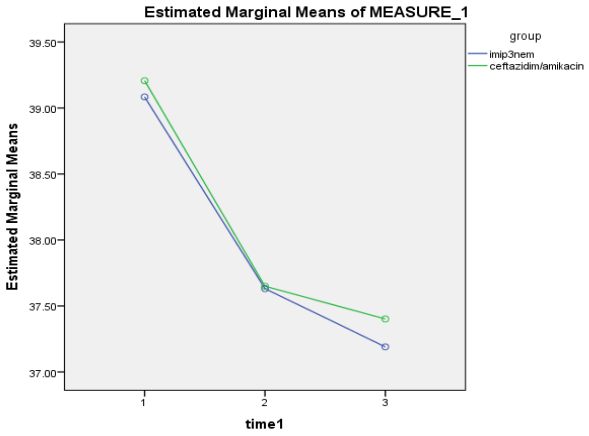

Figure 1: The effect of imipenem and ceftazidime/amikacin on discontinuation of fever for 3 consecutive days

Discussion

The present study compared the effect of imipenem with the standard drug combination of ceftazidime/amikacin as an experimental therapy in controlling fever and improving clinical and microbiological outcomes for confirmed infections and unspecified fever in neutropenic cancer patients. There was no significant difference between the two groups in the rate of discontinuation of fever, unmodulated treatment time, or the response of patients to bacteremia. After 72 hours from the beginning of experimental therapy and body temperature measurement and experimental results analysis, we found that the clinical and therapeutic effect of monotherapy with imipenem as an experimental therapy in febrile episodes in patients with neutropenic cancers, which was similar to combination therapy with ceftazidime/amikacin. It is worth noting that imipenem was more effective than ceftazidime/amikacin to reduce fever and to improve clinical efficacy in cancers with severe fever and neutropenia (approximately two times), but there was no significant difference between groups due to the small number of these patients.

The death of one patient (1.6%) occurred on imipenem group on the third day. The patient had a background of large diffuse b-cell lymphoma. There was no positive culture in this patient. It should be noted that the patient had severe neutropenia at admission. On the third day, the patient suffered from abdominal pain and tenderness. During the diagnostic evaluations, the patient had an intestinal rupture. Furthermore, the patient suffered from a severe fall in cardiac blood pressure during surgery, following underlying ischemic heart disease and then died. The patient's death did not correlate with the drugs used in the present study.

In the present study, after 3 days and checking the patient, it was determined that the clinical effect of imipenem in the experimental treatment of febrile episodes in patients with neutropenic cancer is similar to that of ceftazidime/amikacin. The level of improvement at the end of treatment was similar in both groups. Similarly, there was no difference between the two groups in the rate of discontinuation of the fever. Finally, unlike the available studies, which noted that the gram-positive bacteria are the common cause of fever in patients with neutropenic cancer, gram-negative organisms were more common in the current study.

Our positive bacterial cultures in 78.9% of cases included gram-negative bacteria and in 21% of them were gram-positive bacteria. The dominant organism was gram negative. *E. coli* was the most commonly isolated organism. All isolated gram-positive bacteria were resistant to our antibiotics. All Gram-negative bacteria were sensitive to imipenem and ceftazidime, while 28.5% of gram-negative bacteria isolated from the blood were resistant to amikacin. Both *P. aeruginosa* and two *E. coli* blood cultures were resistant to amikacin. No urine culture showed resistance to amikacin. Imipenem was more effective than ceftazidime/amikacin in reducing fever and improving clinical symptoms of patients with and without bacteriological confirmation. However, there was no significant difference between the two groups. Both drugs were more effective in patients with bacteriological confirmation than patients without bacteriological confirmation. However, there was no significant difference between the two groups.

There was no difference in drug tolerance in the two groups, and no drug complications were seen in the two groups. It is very important that in the present study, none of the patients died due to gram-negative sepsis, which indicated the strength of both drug regimens including imipenem monotherapy and ceftazidime/amikacin combination therapy against gram-negative bacteria. All gram-negative bacteremia responded to our drugs without adding additional antibacterial agents. However, the bacteremia induced by gram-positive pathogens showed a very poor response to treatment in both groups.

There was no significant difference in drug tolerance in the two groups, and no side effects were seen in the two groups. On the other hand, the poor clinical effect of imipenem and ceftazidime/amikacin on gram-positive bacteremia in recent studies suggests that there is no reason to delay the addition of glycopeptides such as vancomycin to imipenem and ceftazidime/amikacin following a lack of clinical response after 72 hours of treatment.

Medication modification has occurred in 9.8% of the imipenem group and 16.3% of the ceftazidime/amikacin group. The higher response to imipenem compared to ceftazidime/amikacin may be due to a wider range of coverage against uncommon

pathogens or resistant pathogens; however, more studies are needed for this. Various studies have been conducted since 1992, with conflicting results from the effects of these drugs. A study has shown that the use of imipenem in the treatment of fever and neutropenia has been more effective than combination therapy of ceftazidime/amikacin [23].

Ronald and colleagues have shown that the rate of successful clinical response at the end of unmodified treatment was higher in the imipenem group (43%) than ceftazidime group (32%). Meropenem was significantly more effective in neutropenia below 100 cells/µl than ceftazidime [27]. It has been indicated that meropenem monotherapy has been well tolerated and produced response rates similar to those obtained with ceftazidime/amikacin. The least success rate in both methods was consistent with other recent studies and was probably associated with a combination of several factors, including the adoption of strict evaluation criteria [20]. Concerning the efficacy of meropenem in comparison with combination therapy with ceftazidime and amikacin, there has been no statistically significant difference between the two groups in the efficacy of these drugs [23].

It has been shown that single-agent therapy with ceftazidime or imipenem can be effectively suitable for the experimental treatment of febrile episodes in patients with neutropenia and solid tumours. Early addition of amikacin or vancomycin could provide an opportunity for treatment in the first step [25].

Another study has reported that monotherapy with meropenem, can lead to suitable treatment of fever in patients with granulocytopenia cancer, as effective as ceftazidime plus amikacin, where both regimens have been indicated to be tolerated in the mentioned study [28]. Another study found that Imipenem/cilastatin and ceftazidime/amikacin combination have been shown to be effective in treating episodes in neutropenic patients [26]. It has been depicted that the patient's response to an antibiotic with fever in patients receiving imipenem (77%) is significantly better than those receiving ceftazidime (56%), especially in patients with confirmed microbial infection [24]. A study also revealed that there had been no significant difference between ceftazidime and Imipenem regimens in febrile and neutropenic episodes in patients with cancer. It has been suggested that ceftazidime can be used as an experimental treatment for fever and neutropenia in cancer patients, due to lower prices and availability [23]. In one study, it was concluded that Gram-negative organisms were more common in cancer patients with neutropenic fever undergoing chemotherapy, unlike most of the available sources that indicated the most commonly reported Gram-positive agents are causes of fever in these patients [29] [30].

About the above, the combination regimen of ceftazidime/amikacin in the treatment of febrile episodes of neutropenic patients can have the same effect as imipenem monotherapy. Given the lower cost of this therapeutic regimen, it can be a cost-effective alternative to imipenem. Considering the two-fold effect of imipenem on the reduction of fever and the improvement of clinical symptoms in patients with severe fever and neutropenia compared to the combination of ceftazidime/amikacin and no significant difference between the two groups, it seems that the low number of these patients were associated with current results. It is recommended that a study is conducted with a larger sample size or a study in which only patients with severe neutropenia be studied. The current was performed for 3 days, and the patients were followed up for 4 days after the discontinuation of the fever or 7 days. Patients were not followed up after the study period, and no information was available on the return of the fever, the incidence of fatalities, the improvement of severe neutropenia, and the death rate of the patients after the Study period. It is therefore recommended that a similar study is conducted with a longer follow-up of the patients. We recommend that the standardisation of laboratory and microbiological tools be considered for better results. Considering the limited studies in this area and different microbial flora in each region, it can be recommended that similar studies be carried out in each region, followed by determination of the predominant microbial flora in neutropenic febrile patients and measurement of the sensitivity and resistance of the organism to this antibiotic. Because of the contradiction in the definition of "response" in various studies, comparing the results of this study with other studies in the evaluation of experimental antibiotic therapy in febrile neutropenia is difficult. However, the results of neutropenic studies are affected by the definition of "response", especially when the response rate of two or more antibiotic regimens is compared. The final choice of experimental antibiotic regimen for use in the treatment centre should be based on the antimicrobial resistance patterns of each region.

In conclusion, the results of our study showed that imipenem and ceftazidime/amikacin are both effective in the initial experimental treatment of febrile neutropenic patients, and both are well tolerated. Results should be interpreted with caution in the absence of confirmatory studies in a specific subgroup of patients.

Acknowledgements

This study has been obtained from doctoral dissertations (code of ethics: 6_133 _91, Arak University of Medical Sciences) with a clinical practice

code (IRCT2012121511756N1). Finally, we thank all the people who participated in this study and the deputy director of research and technology in Arak University of Medical Sciences.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1431-1434.
https://doi.org/10.3889/oamjms.2018.256
eISSN: 1857-9655
Clinical Science

Influence of Flexible Insulin Dosing with Carbohydrate Counting Method on Metabolic and Clinical Parameters in Type 1 Diabetes Patients

Feyzi Gokosmanoglu[1], Attila Onmez[2*]

[1]Department of Endocrinology, Sakarya University Medicine Faculty, Sakarya, Turkey; [2]Department of Internal Medicine, Duzce University Medicine Faculty, Duzce, Turkey

Citation: Gokosmanoglu F, Onmez A. Influence of Flexible Insulin Dosing with Carbohydrate Counting Method on Metabolic and Clinical Parameters in Type 1 Diabetes Patients. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1431-1434. https://doi.org/10.3889/oamjms.2018.256

Keywords: Type 1 DM; Carbohydrate counting; Metabolic control

***Correspondence:** Attila Onmez. Department of Internal Medicine, Duzce University, Medicine Faculty, Duzce, Turkey. E-mail: attilaonmez@gmail.com

Received: 17-May-2018; **Revised:** 31-Jul-2018; **Accepted:** 05-Aug-2018; **Online first:** 19-Aug-2018

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

OBJECTIVE: The purpose of providing and maintaining a proper metabolic control is to prevent the development of chronic complications. In this study, we aimed to determine the influence of flexible insulin dosing with carbohydrate counting method on metabolic and clinical parameters in type 1 diabetes patients.

MATERIAL AND METHODS: This study was conducted with patients following up at the Endocrinology Clinic with a diagnosis of type 1 diabetes mellitus between 2012 and 2015. Metabolic and clinical parameters before and after carbohydrate counting were compared.

RESULTS: Forty patients were included in the study. Of the patients, 40% (n = 16) were female, and 60% (n = 24) were male, and mean age was 21.5 ± 7 year at the time of diagnosis. Statistically significant differences were not detected when haemoglobin A1c, fasting plasma glucose, post-prandial glucose, LDL-cholesterol, and HDL-cholesterol levels were compared at standard dose insulin use and after carbohydrate counting ($P < 0.005$). Among the parameters measured when the patients received standard dose of insulin without counting carbohydrate and flexible insulin dosing by counting carbohydrate, statistically, significant differences were not detected for baseline insulin dose, bolus insulin dose, triglyceride level, body mass index, or monthly hypoglycemia episodes ($P > 0.05$).

CONCLUSION: Flexible insulin dosing with carbohydrate counting provides significant improvements in clinical and metabolic control. We detected improvements in lipid profiles and glycemic control. Additionally, patients generally did not gain weight despite flexible nutrition, and frequency of hypoglycemia remained unchanged despite strict glycemic control.

Introduction

Diabetes mellitus is a critical health problem. Its prevalence is gradually increasing, leading to higher morbidity and mortality. Approximately 5 to 10% of diabetic patients have type 1 DM [1]. Diabetes prevalence has been determined to range from 7.2 to 11.4% worldwide according to data from the International Diabetes Federation. In Turkey, the prevalence of diabetes among individuals 20 years and above is 13.7% [2].

The goal of providing and maintaining proper metabolic control is to prevent the development of chronic complications. Various treatment methods have been investigated for this purpose [1] [2] [3]. Reducing HbA1c levels through effective insulin treatment was shown to reduce the risk of microvascular complications of diabetes. Carbohydrate counting enables diabetic patients to pursue flexible nutrition and flexible insulin dosing [4]. In this study, we aimed to investigate the influence of flexible insulin dosing with carbohydrate counting method on metabolic and clinical parameters in type 1 diabetes patients.

Material and Methods

This study was conducted with 40 patients who are presented at the Endocrinology Clinic with a diagnosis of type 1 diabetes mellitus between 2012 and 2015. Definition of type 1 DM followed the criteria defined by the World Health Organization. Subjects were evaluated regarding age, gender, clinical signs and symptoms at the time of diagnosis, duration of symptoms, and blood and urine biochemistry. Glycosylated haemoglobin concentrations (HB A1c%) were measured using the tribometric inhibition immunoassay method with a Cobas Integra analyser. Biochemical tests-fasting plasma glucose, postprandial plasma glucose, total cholesterol, triglyceride, LDL-cholesterol, HDL-cholesterol levels were measured using an Olympus AU600 (Olympus Optical Co. Ltd., Japan) auto-analyser following manufacturer instructions.

Patients who participated in the study completed three stages of carbohydrate counting. The patients who have been using flexible insulin dosing with carbohydrate counting were doing this for 6 to 24 months. A total of 56 patients with type 1 DM younger than 18 years were assessed for eligibility in the study, or those who were pregnant (n = 4), lactating (n = 2), or unaware of carbohydrate counting (n = 6) and had not completed all three stages of carbohydrate counting (n = 4) were excluded from the study. Body composition was determined using Tanita BC-418 via height, weight, bioelectric impedance analysis, and body mass index (BMI) calculations. Data about insulin dosing before carbohydrate counting, hypoglycemic event count, biochemical and metabolic parameters were obtained from patient files. Those patients who had missing data were not included in the study.

Subjects were compared regarding metabolic and biochemical parameters before and after carbohydrate counting.

All data are given as mean±standard deviation (SD). Statistical analyses were done with SPSS 16.0 (SPSS Inc., Chicago, IL). Correlation analyses were performed using Pearson correlation analysis. Statistical analyses of averages were done using independent paired t-test and Wilcoxon test. A p level of < 0.05 was accepted as statistically significant.

Results

A total of 40 patients completed the study. Of the patients, 40% (n = 16) were female, 60% (n = 24) were male. Mean age was 21.5 ± 7 years at the time of diagnosis. A statistically significant difference was not detected between groups regarding gender distribution (P > 0.05). Socio-demographic characteristics of the subjects are shown in Table 1. Clinical and laboratory outcomes of the patients when using standard insulin dosing and flexible insulin dosing with carbohydrate counting are shown in Table 2.

Table 1: Socio-demographic characteristics of the patients

Parameters	
Age (year), median	21.5 ± 7
Body mass index (BMI, kg/m^2)	22
Diabetes age (year)	12.8 ± 9.1
Gender (M/F)	n:24.60%/n:16.40%
Duration of carbohydrate counting (month) median	18.4 (6-24)

Median haemoglobin A1c values with standard insulin dosing and flexible insulin dosing were 8.0% and 7.30%, respectively (P = 0.007). Fasting plasma glucose was found to be 165.90 mg/dl before carbohydrate counting and 140.70 mg/dl after carbohydrate counting (P = 0.049).

Table 2: Clinical and laboratory outcomes of the patients when using standard insulin dosing before carbohydrate counting and when using flexible insulin dosing according to carbohydrate counting

Parameters	Standard insulin dosing before carbohydrate counting	Flexible insulin dosing according to carbohydrate counting	P value
BMI (kg/m^2)	22	22	0.121
Hemoglobin A1c (%)	8.1	7,3	0.007
Fasting plasma glucose (mg/dL)	165.9	140.7	0.049
Post-prandial glucose (mg/dL)	241.1	149.43	0.001
Triglyceride (mg/dL)	85	94	0.863
HDL-cholesterol (mg/dL)	55.5	66.5	0.039
LDL-cholesterol (mg/dL)	85.5	78.5	0.036
Hypoglycemia (episodes/month)	4	4	0.124
Total insulin dose (IU/day)	38.5	37	0.738
Basal dose (IU/day)	16	20	0.056
Bolus dose (IU/day)	19	18	0.224

Post-prandial plasma glucose was 241.10 mg/dl before carbohydrate counting and 149.43 mg/dl after carbohydrate counting (P = 0.001). LDL-cholesterol level was detected to be 85.50 mg/dl when patients used a standard dose of insulin and 78.50 mg/dl when they used flexible insulin dosing (P = 0.036). HDL-cholesterol levels were 55.50 mg/dl before carbohydrate counting; they reached 65.50 mg/dl with flexible insulin dosing (P = 0.038). However, a statistically significant difference was not detected between the periods before and after carbohydrate counting regarding basal insulin dose, bolus insulin dose, triglyceride level, body mass index, or monthly hypoglycemia episode count (P > 0.05).

Discussion

Very strict low carbohydrate intake was a method used to treat type 1 DM before the discovery of insulin. Lower glucose levels are obtained with a flexible dose of multiple insulin injections with

carbohydrate counting. Therefore; actual guidelines recommend intensive insulin treatment paired with a flexible diet [5]. The target of carbohydrate counting is to promote glycemic control by implementing a consistent pattern of carbohydrate consumption with meals and snacks from day to day. Since carbohydrate intake directly identify postprandial blood glucose, management of carbohydrate consumption and suitable insulin adjustments for identified amount of carbohydrate can improve glycemic control [6]. In our study, patients are applying short-acting insulin with carbohydrate counting for 18.4 (6-24) months. This method provides flexibility in carbohydrate intake. Many patients may not optimally follow this method due to the high carbohydrate content of foods or being unable to estimate the proper dose [7]. In follow-ups with our patients, they were seen to match short-acting insulin and carbohydrates according to fasting and post-prandial plasma glucose. This may be explained by close monitoring of the patients and long-lasting education about carbohydrate counting.

Studies are available indicating that carbohydrate-restricted diet may be a lifestyle option in patients wanting to lose weight. A carbohydrate-restricted diet leads to weight loss in patients with type 1 DM [8] [9]. Our patients did not have a change in weight when they used flexible insulin dosing with carbohydrate counting compared to a standard dose of insulin use with a conventional diet ($P > 0.05$). Some studies showed that the patients eating with carbohydrate counting had higher BMI compared to the patients eating a conventional diet [10]. These studies reported that intensive insulin treatment and a flexible eating plan might lead to an increase in BMI of patients with type 1 DM [11].

Studies have shown that the A1c level was lower in patients who used flexible insulin dosing with carbohydrate counting [12]. Prandial insulin dose was shown to lead to 1-1.5 units of a decrease in A1c value, as it is performed according to total insulin dose [13]. The appearance of chronic complications is delayed when glycemic control and A1c control are achieved with intensive insulin treatment. Complication development slows down between 30% and 75% [14] [15]. In our study, we detected that patients could achieve a better glycemic control when they used flexible insulin dosing with carbohydrate counting. We detected a 9.8% decrease in HbA1c value ($P = 0.007$), a 15% decrease in fasting plasma glucose ($P = 0.049$), and a 37.9% decrease in post-prandial plasma glucose ($P = 0.001$). These differences are statistically significant.

Hypertriglyceridemia correlated with hyperglycemia and low HDL-cholesterol is seen in patients with type 1 DM. This impaired lipid profile may improve with active insulin treatment [16]. Studies have shown that while triglyceride, HDL-cholesterol decrease, LDL-cholesterol was shown to increase in patients using flexible insulin dosing [13]. In our study, triglyceride level was seen to increase 11.7% ($P = 0.863$), HDL-cholesterol level was seen to increase 19.8% ($P = 0.039$), LDL-cholesterol level was seen to decrease 8.1% ($P = 0.036$) when treatment was switched to flexible eating, flexible insulin dosing from conventional eating. This lipid profile may be explained by better glycemic control through flexible eating and intensive, flexible insulin dosing.

Studies have not detected a difference between patients who use flexible insulin dosing with carbohydrate counting and who use standard insulin dosing regarding insulin dose and hypoglycemia frequency [10] [11] [12] [13] [14] [15] [16]. Meta-analysis could not be done due to inconsistency between hypoglycemia measurement and reporting; however, the frequency of hypoglycemia was estimated to decrease [17]. In our study, a statistically significant difference could not be detected about basal and bolus insulin dose or some hypoglycemic episodes when treatment was switched to flexible insulin dosing from standard insulin dosing ($P > 0.05$). Daily total insulin dose decreased by 3.8% ($P = 0.738$), bolus insulin dose decreased by 5.2% ($P = 0.224$), and basal insulin dose increased by 25% ($P = 0.056$). Monthly median hypoglycemic episode count was equal in both conditions at 4 hypoglycemic episodes/month. HbA1c levels would be normal in all diabetic patients if hypoglycemia did not occur. Hypoglycemia limits long-term benefits of glycemic control in patients with type 1 DM [18] [19]. Flexible insulin dosing, carbohydrate counting, and flexible eating provide better glycemic control without increasing hypoglycemia risk in patients with type 1 DM.

In conclusion, flexible insulin dosing with carbohydrate counting provides a significant improvement in clinical and metabolic control. We detected that frequency of hypoglycemia did not change despite improvements in lipid profile, glycemic control, lack of weight gain despite flexible eating, and strict glycemic control. This method enables the patients to enjoy a more flexible life and is more sustainable. Patients should be provided with a more flexible lifestyle through better nutrition education and more active participation in the treatment of their diseases.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1435-1437.
https://doi.org/10.3889/oamjms.2018.203
eISSN: 1857-9655
Case Report

Eruptive Basaliomas: "Why we have to Perform Surgery? " Or Said Otherwise: "Catch The Metatypical! "

Ivanka Temelkova[1], Hristo Mangarov[1], Michael Tronnier[2], Ivan Terziev[3], Georgi Tchernev[1,4*]

[1]Medical Institute of Ministry of Interior (MVR), Department of Dermatology, Venereology and Dermatologic Surgery, Sofia, Bulgaria; [2]Helios Klinikum GMBH, Dermatology, Venereology and Allergology, Hildesheim, Germany; [3]Universitetska Mnogoprofilno Bolnitsa za Aktivno Lechenie Tsaritsa Yoanna, Common and Clinical Pathology, Sofia, Bulgaria; [4]Onkoderma, Policlinic for Dermatology and Dermatologic Surgery General Skobelev 26, Sofia, Bulgaria

Citation: Temelkova I, Mangarov H, Tronnier M, Terziev I, Tchernev G. Eruptive Basaliomas: "Why we have to Perform Surgery?" Or Said Otherwise: "Catch The Metatypical! ". Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1435-1437. https://doi.org/10.3889/oamjms.2018.203

Keywords: Basal cell carcinoma; Metatypical basal cell carcinoma; Radiation; Surgery

***Correspondence:** Georgi Tchernev. Medical Institute of Ministry of Interior (MVR), Department of Dermatology, Venereology and Dermatologic Surgery, Sofia, Bulgaria; Onkoderma-Policlinic for Dermatology and Dermatologic Surgery General Skobelev 26, Sofia, Bulgaria. E-mail: georgi_tchernev@yahoo.de

Received: 10-Jul-2018; **Revised:** 17-Jul-2018; **Accepted:** 18-Jul-2018; **Online first:** 30-Jul-2018

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Keratinocyte cancers are malignant diseases with a broad incidence of spread which tends to increase during the last couple of decades. The solar radiation plays a dominant role in the occurrence of BCC, but certain genetic phenotypes appear to be risky from an etiological point of view. Metatypical basal cell carcinoma (MTBCC) is a rare variant of BCC which combines the clinical and histological characteristics of BCC and SCC. Clinically they are indistinguishable from the conventional BCC, and only the histological examination can differentiate them. The MTBCC is a histological subtype which is considered more aggressive due to its ability to produce local recurrences or distant metastases.

CASE REPORT: We present a 44-year old patient with multiple BCCs disseminated on the face and body. The biopsy established mixed type histology: three metatypical and four solid BCCs. The lesions were removed via elliptical excision with a field of operational security of 0.5 cm in all directions.

CONCLUSIONS: The eruptive (multiple) BCCs are a challenge about the choice of a therapy option. This is because clinically completely identical tumours show different histopathological characteristics, namely those with a tendency to metastasise. Having in mind one of the hypotheses of metatypical BCC emergence - the improper or inadequate radiotherapy (as a choice of therapy) could trigger the transition of a conventional tumour to a metastasising one, the surgical treatment appears to be the most secure treatment method.

Introduction

The BCC is the most common malignant skin disease [1]. The solar radiation, skin phototype 1, as well as certain genetic diseases (albinism, xeroderma pigmentosa, Bazex's syndrome and Gorlin's syndrome), are considered as main risk factors about BCC development [1][2]. From the few existing histological subtypes of BCC, the ones of particular interest are the metatypical MTBCCs which combine the clinical and histological characteristics of BCC and SCC [3]. MTBCC is regarded as the most aggressive due to its ability to cause local recurrences and distant metastases [4]. It is believed that the inappropriate/inadequate radiotherapy of non-melanoma skin tumours could lead to their transition to a metastatic metatypical variant of BCC [5]. Due to this reason, the first choice of BCC treatment should be the surgical removal [6].

Case report

A 44-year old man is presented; phototype 1 and many years exposure to solar radiation. Anamnestic data showed 25 years old state of the disseminated lesions on the head and body. Multiple skin lesions of different location and size were seen on examination. In the area of shoulders, face, chest and nape were observed ulcerative lesions (Figure

1a-f) while on the back were set erythema plaques covered with crusty exudate (Figure 2c).

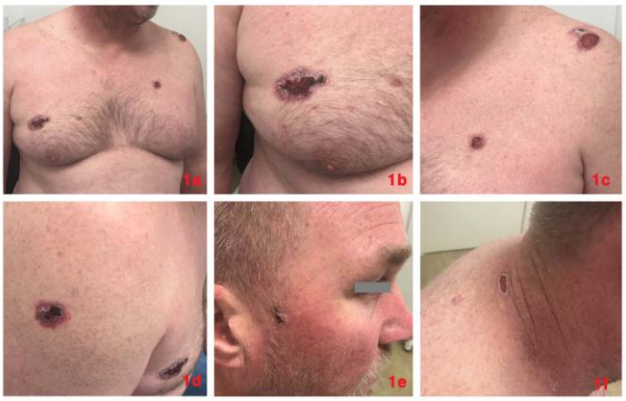

Figure 1: a-f) Multiple BCC with relative similar clinical appearance. Clinical observation during the first medical examination

Seven of the lesions were removed by elliptical excision with a field of operational security of 0.5 cm in all directions (Figure 2a-d). The histological examination showed a mixed histological picture: three multicentric BCCs with clean resection lines and four solid BCCs with free resection lines.

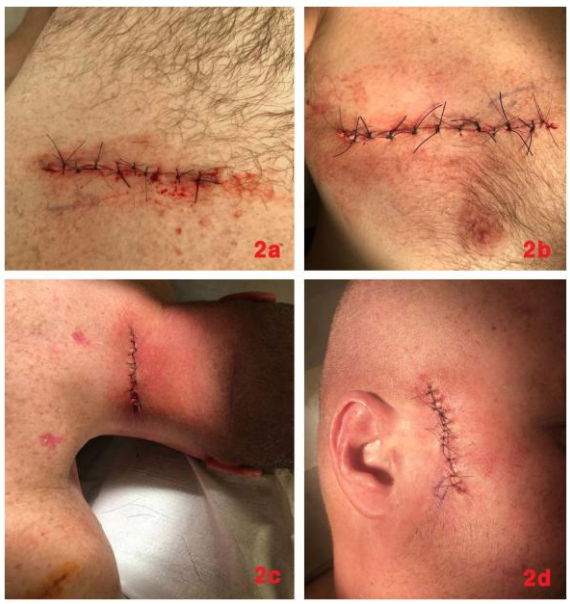

Figure 2: a-d) Clinical status postoperatively

By the histological data was set the diagnosis of metatypical BCCs for two of the surgically removed lesions (Figures 3a-c; 4a-c).

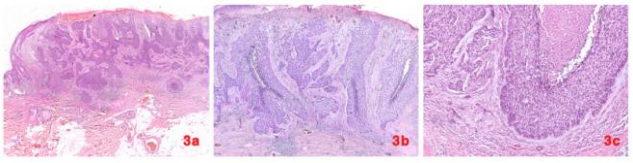

Figure 3: a-c) Metatypical basal cell carcinoma with typical histopathological features

The clinical examination did not find the presence of lymphadenopathy, the lung and heart radiography detected no focal and infiltrative changes, the ultrasound of cervical, axillary and inguinal area did not register enlarged lymph nodes, paraclinical data-with no specificities.

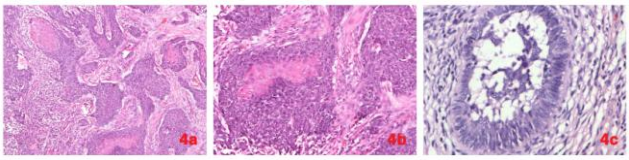

Figure 4: a-c) Metatypical basal cell carcinoma with typical histopathological features

Discussion

The basal cell carcinoma is the neoplasia with the highest incidence rate among the population worldwide which exhibits the trend to increase in the last couple of decades with an average 10% per annum [1]. The solar radiation is considered as the main etiological factor for BCC occurrence [2]. The other risk factors for the development of basal cell carcinoma include skin phototype 1, blond or red hair, blue or green eyes, sunburnt from childhood, family history of cancer or immunosuppressive treatment [1]. Certain genetic diseases are also associated with a higher risk of basal cell carcinoma development, namely: albinism, xeroderma pigmentosa, Bazex's syndrome and Gorlin's syndrome [1]. Interesting are two phenotypes: 1) patients with clusters of BCC which have between two and five clinically manifested basal cell carcinoma, i.e. multiple presentation phenotypes and 2) patients with basal cell carcinoma on the trunk [7]. In both cases is considered that a genetic predisposition exists [7]. The patients with BCC on the trunk are interesting because it is believed that the development of basal cell carcinoma in this localisation is mediated via different mechanisms in comparison with those that are involved in affecting other parts of the body [8]. Male gender, young age and the number of sunburns are regarded as risk factors for the development of BCC on the trunk [9]. The identification of genes that are linked to the basal cell carcinoma development has great importance for better understanding of their pathogenesis [10]. According to recent research, the changed activation of the Hedgehog pathway is a key step in the carcinogenesis of BCC [10]. It has been established that mutations in the genes PTCH1, SMO as well as LATS1 and PTPN14, from the Hippo-YAP pathway are linked to BCC development [10].

The patients with BCC show differences in the tumours' location on the body, the lesions' number as well as the histological subtype [11]. Histologically the BCC is divided into nodular, superficial, morpheaform,

infiltrating, metatypic, and fibroepithelioma of Pinkus [12]. The metatypical BCC is a histological variant which combines the histological characteristics of BCC and SCC [3]. MTBCC is divided into two histological subtypes: intermediate and mixed [3]. Clinically the metatypical BCC cannot be distinguished from the other variants, and only the histological examination could differentiate them [13]. It is believed that MTBCC shows more aggressive behaviour and apart from local recurrences could also lead to distant metastases [14]. The available literature data points that the metastatic potential of MTBCC is around 7.4% [15]. BCC most commonly metastasise via lymphogenic or haematogenic pathway affecting lymph nodes, lungs or bones [16]. This requires the necessity of a more careful approach and specific treatment of the metatypical BCC.

The standard approach of treatment of basal cell carcinoma is surgical excision, curettage, curettage with electrodesiccation, Mohs micrographic surgery and radiotherapy [17]. Superficial radiation therapy is an alternative treatment of non-melanoma skin tumours, such as BCC and SCC [17]. In that case are considered tumour size, location, histological subtype and the patient's age; the often applied radiotherapy regimen is of a total dose of 4,500cGY (300cGy/fractionx15 fractions) [17]. However, according to clinical research data, few factors are risky for metatypical BCC development: 1) history of BCC for many years, 2) no response to conventional methods of treatment and 3) conducted radiotherapy in the past [5]. Because the radiation therapy is a risk factor for transitioning to the metastatic variant of BCC (MTBCC), the full surgical excision of the basal cell carcinoma is of utmost importance and should be the first choice of the treatment method of those carcinoma [5] [6]. The recommended margins of surgical security, which provide for up to 96% optimal elimination of BCC, are on average 4 mm (3 mm for the face and 5mm for the other body parts) [15]. It is necessary that patients with BCC, especially stage T3 or T4, as well as histologically confirmed MTBCC, to be monitored in the course of 10 or more years for the presence of local recurrences or distant metastases [5] [6].

In conclusion, metatypical BCC is an aggressive variant of BCC which is associated with a higher risk of local recurrences and possibility of developing distant metastases. The diagnosis of MTBCC could be determined only by histological examination. Because radiotherapy of skin tumours poses a risk of transitioning to metatypical BCC, surgical treatment of that carcinoma should be a priority.

The surgical approach is the adequate one as clinically often there are no differences, but often the histology is different. On that basis, BCC could be determined, analogically to other diseases in the dermatology, like a chameleon.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1438-1441.
https://doi.org/10.3889/oamjms.2018.267
eISSN: 1857-9655
Case Report

A Rare Dermatologic Disease in Pregnancy: Rosacea Fulminans- Case Report and Review of the Literature

Omer Demir[1]*, Inci Sema Tas[2], Berrin Gunay[3], Funda Gungor Ugurlucan[2]

[1]Clinic of Obstetrics and Gynecology, Erzurum Karayazı Turkish Pharmacists Union, County State Hospital, Erzurum, Turkey; [2]Clinic of Obstetrics and Gynecology, Istanbul School of Medicine, Istanbul University, Istanbul, Turkey; [3]Clinic of Dermatology, Cizre Dr Selahattin Cizrelioglu County State Hospital, Sırnak, Turkey

Citation: Demir O, Tas IS, Gunay B, Gungor Ugurlucan F. A Rare Dermatologic Disease in Pregnancy: Rosacea Fulminans- Case Report and Review of the Literature. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1438-1441. https://doi.org/10.3889/oamjms.2018.267

Keywords: Rosacea; Pregnancy; Rare disease

***Correspondence:** Omer Demir. Clinic of Obstetrics and Gynecology, Erzurum Karayazı Turkish Pharmacists Union County State Hospital, Erzurum, Turkey. E-mail: itf.omerdemir@gmail.com

Received: 07-Apr-2018; **Revised:** 26-May-2018; **Accepted:** 30-May-2018; **Online first:** 04-Aug-2018

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Rosacea is a common, chronic disorder that can present with a variety of cutaneous or ocular manifestations. Skin involvement primarily affects the central face, with findings such as persistent centrofacial redness, papules, pustules, flushing, telangiectasia, and phymatous skin changes. The pathways that lead to the development of rosacea are not well understood. The relationship of pyoderma faciale (also known as rosacea fulminans) to rosacea also is uncertain. We aimed to write this article with the aim of showing how a pregnant patient who has been aggravated by the degree of lesions on the face during the first trimester of pregnancy is treated and to show what is in the literature in this issue.

CASE REPORT: A 22-year-old woman complained of painful erythema, papules and pustules on the face. She had fever and malaise during the sixth week of her first pregnancy and a history of the mild eruption and seborrhea before her pregnancy with flaring over the preceding 4 weeks. Dermatologic examination revealed red erythema of all involved facial areas; the lesions consisted of papules, pustules and nodules. The case was diagnosed as rosacea fulminans (pyoderma faciale) by these findings. In the literature, there are some effective therapeutic options such as retinoids, tetracyclines, antiandrogenic contraceptives, and dapsone and these were not used because they are contraindicated in pregnancy. Amoxicillin-clavulanic acid 1 gr/day, wet compresses, and a fusidic acid cream were started. After the activity of the disease had been suppressed for 10 days, antibiotic was stopped, and the other treatment options were applied topically for the next month. One month after cessation of treatment, the lesions had disappeared with only mild erythema remaining. There was minimally flushing on the face and no telangiectasia.

CONCLUSION: In conclusion, there is no substantial evidence as to the mechanism by which pregnancy may trigger this conditioner whether the gender of the fetus influences the development of rosacea fulminans, but is generally accepted that hormonal changes in pregnancy play an important role. The pathogenesis of rosacea fulminans remains uncertain, but it is obvious that the further basic and clinical research is required to optimise the management of this rare facial dermatosis.

Introduction

Rosacea is a common, chronic disorder that can present with a variety of cutaneous or ocular manifestations.

Skin involvement primarily affects the central face, with findings such as persistent centrofacial redness, papules, pustules, flushing, telangiectasia, and phymatous skin changes. The pathways that lead to the development of rosacea are not well understood [1] [2].

Proposed contributing factors include abnormalities in innate immunity, inflammatory reactions to cutaneous microorganisms, ultraviolet damage, and vascular dysfunction.

The relationship of pyoderma faciale (also known as rosacea fulminans) to rosacea also is uncertain [3]. Patients present with intensely inflammatory, purulent facial plaques and nodules with draining sinuses on a background of erythema. A history of rosacea may or may not be present. Young women are most commonly affected.

Because pregnancy is also a process in which

the immune system is weakened, cases of rosacea exacerbated in pregnancy, namely rosacea fulminans, have been reported in the literature.

We aimed to write this article to show how a pregnant patient who has been aggravated by the degree of lesions on the face during the first trimester of pregnancy is treated and to show what is in the literature in this issue.

Case Report

A 22-year-old woman complained of painful erythema, papules and pustules on the face. She had fever and malaise during the sixth week of her first pregnancy and a history of the mild eruption and seborrhea before her pregnancy with flaring over the preceding 4 weeks. Dermatologic examination revealed red erythema of all involved facial areas; the lesions consisted of papules, pustules and nodules (Figure 1A, 1B).

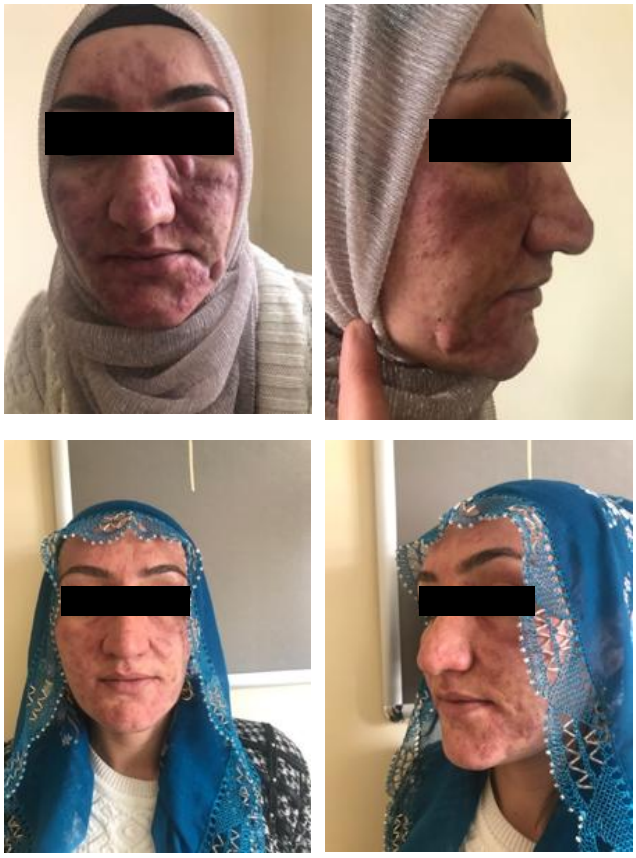

Figure 1: Patient with rosacea fulminans (pyoderma faciale). A, B = before the treatment; C, D = after the treatment

There were no comedons or telangiectasia and no pathologic findings upon systemic examination. Laboratory studies revealed a white blood cell count of 14600/mm, haemoglobin level of 13.8 g/dl, CRP level of 6 mg/dl. Cultures for bacterial pathogens were negative. Hormonal tests were not performed. The case was diagnosed as rosacea fulminans (pyoderma faciale) by these findings. In the literature, there are some effective therapeutic options such as retinoids, tetracyclines, antiandrogenic contraceptives, and dapsone and these were not used because they are contraindicated in pregnancy. Amoxicillin-clavulanic acid 1 gr/day, wet compresses, and a fusidic acid cream were started. After the activity of the disease had been suppressed for 10 days, antibiotic was stopped, and the other treatment options were applied topically for the next month.

One month after cessation of treatment, the lesions had disappeared with only mild erythema remaining. There was minimally flushing on the face and no telangiectasia (Figure 1C, 1D).

Acknowledgement

To use the pictures for scientific purposes, permission was obtained from the patient.

Discussion

In 2002, the National Rosacea Society assembled an expert committee to develop a standard classification system for rosacea.

The committee established four distinct subtypes of rosacea: erythematotelangiectatic, papulopustular, phymatous, and ocular rosacea [3]. Since then, increasing knowledge of the pathophysiology of rosacea has favoured a view of rosacea as a consistent multivariate disease process with multiple clinical manifestations rather than distinct subtypes of disease [4].

Following recommendations from the Global ROSacea COnsensus (ROSCO) Panel supporting the use of phenotype-based, rather than a subtype-based, approach to the diagnosis and classification of rosacea, the National Rosacea Society expert committee released an update supporting a similar approach [4] [5].

The cutaneous histopathologic findings in rosacea are nonspecific, and skin biopsies are rarely indicated. When the diagnosis is uncertain, biopsies may be performed to rule out other disorders or to provide support for a diagnosis of granulomatous rosacea. No serologic studies are useful for diagnosis.

Specimens from erythematous facial skin exhibiting centrofacial erythema and telangiectasias usually show dilation of superficial blood vessels and

a low-grade perivascular, lymphohistiocytic, inflammatory infiltrate with occasional plasma cells. Solar elastosis is often present. Histopathologic examination of papular lesions usually reveals prominent perivascular and perifollicular inflammatory infiltrates in the superficial and mid-dermis composed of lymphocytes, neutrophils, and plasma cells. Superficial accumulations of neutrophils are present in pustules. In contrast to acne vulgaris, inflammation often is more perivascular and extends well beyond the follicle [6].

Rosacea fulminans was first described by O'Leary and Kierland in 1940 as ‘pyoderma faciale [7]. Plewig et al., Regarded the condition as an extreme form of rosacea and suggested that it be renamed ‘rosacea fulminans’ in their report of twenty cases had been reported in the literature [8].

Rosacea fulminans is characterised by rapid onset, a fulminating course, strict localisation to the face, absence of comedones and absence of acneiform lesions on the chest or back. The characteristic lesions are superficial papulopustules and nodules combined with cyanotic erythema.

The pathogenesis of rosacea fulminans is unknown, but severe emotional trauma (such as the death of a family member, divorce or an accident) is often blamed [7] [8].

Dermatologic conditions that may be exacerbated perimenstrually include acne vulgaris, rosacea, psoriasis, atopic eczema and urticaria. Hormonal changes resulting in increased cutaneous vascularity, seborrhea, and dermal oedema during the perimenstrual period resemble those of pregnancy and may be related to eruption or exacerbation of these diseases [9].

Massa and Su reported that 6 of 29 rosacea fulminans cases were associated with pregnancy [10]. In the series published by Plewig et al., 3 of 20 patients had developed the disease during pregnancy [8]. Haugstvedt and Bjerke reported a flare-up of rosacea during pregnancy in 1998 [11].

In 2004, Lewis and colleagues first described a case of rosacea fulminans in pregnancy, and treatment options were mentioned for the first time in this publication [12].

As we did not perform hormonal tests in our patient and there was no history of oral contraceptive use, it is not possible to state that pregnancy or oral contraceptive us may have been a triggering factor for rosacea fulminans in our patient. However, we agree with Plewig et al., [7]. That pregnancy can be considered an exacerbating factor for rosacea fulminans, and that hormonal factors may be a trigger for rosacea fulminans during pregnancy and in females taking oral contraceptive pills.

In 2006, Ferahbaş and colleagues from Turkey, they issued a rosacea fulminans cases occurring in pregnancy and compiled the literature up to that time. They have treated this case with systemic corticosteroids and have continued to treat with topical metronidazole cream and have reported successful treatment [13].

In 2008, Cisse and colleagues reported that rosacea fulminans developed during the early period of a pregnancy obtained with IVF. In this case, hormonal treatments in IVF treatment have been shown to be the major cause of rosacea fulminans development [14].

In 2010, Jarrett and his colleagues identified three cases of rosacea fulminans in pregnancy and explained how this dermatological problem affected pregnancy outcomes [15].

In 2011, Morais e Silva FA and her colleagues published a case of rosacea fulminans in pregnancy that caused ocular perforation, and it states how serious the consequences may be [16]. In 2011, Fuentelsaz and colleagues treated rosacea fulminans case of pregnancy with azithromycin and achieved success. And they stated that the use of systemic corticosteroids is not an untreatable treatment [17]. In 2014, Wollina U. made a better comprehension of the aetiology of rosacea fulminans and compiled recent publications on management [18]. Then, in 2015, Haenen CC and her colleagues treated a patient who had rosacea fulminans in pregnancy with erythromycin and was successful [19].

When both the treatment options are given in the literature, and the treatments mentioned in the dermatology books are compiled, early and aggressive treatment is recommended, yet pregnancy poses a therapeutic dilemma; the recognised effective treatments, including retinoids, tetracycline antibiotics, antiandrogenic contraceptives and dapsone are all contraindicated during pregnancy [12].

Isotretinoin has been associated with congenital anomalies; tetracyclines are associated with discolouration of the teeth and impaired bone growth, dapsone may cause neonatal haemolysis [12].

Metronidazole is not recommended before the second trimester and although erythromycin is considered safe it is not always effective because of resistance in some bacteria [12].

Erythromycin administration is usually a safe option in pregnancy, but it has been shown in the literature that erythromycin administration is more effective in cases with comedones and bacterial reoccurrence [12].

In our case, erythromycin was not administered because of the low incidence of comedones, as we have already mentioned, and the other options were directed.

The use of systemic steroids can only be justified if the benefits outweigh the risks of

intrauterine growth retardation, maternal diabetes mellitus and hypertension [12].

For these reasons, we do not consider the use of systemic steroids in our case. Since topical medicines are generally known not to damage the fetus during pregnancy, they have been tried in the treatment of our case.

After taking other options that were not available in pregnancy, we also gave the patient topical fusidic acid and systemic penicillin antibiotics and found that there was a significant improvement.

In conclusion, there is no substantial evidence as to the mechanism by which pregnancy may trigger this conditioner whether the gender of the fetus influences the development of rosacea fulminans, but is generally accepted that hormonal changes in pregnancy play an important role. In pregnancy, the use of topical drying compounds, surgical drainage, and topical and systemic antibiotics and corticosteroids have been reported, but the best practice remains inconclusive.

We suggest a multidisciplinary approach with early diagnosis, shared obstetric and dermatology care and provision of emotional support to the mother. The pathogenesis of rosacea fulminans remains uncertain, but it is obvious that the further basic and clinical research is required to optimise the management of this rare facial dermatosis.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1442-1445.
https://doi.org/10.3889/oamjms.2018.276
eISSN: 1857-9655
Case Report

Schizophrenia as Potential Trigger for Melanoma Development and Progression! The Psycho-Neuro-Endocrine-Oncology (P.N.E.O) Network!

Georgi Tchernev[1,2*], Ilia Lozev[3], Ivanka Temelkova[1], Svetoslav Chernin[4], Irina Yungareva[1]

[1]Medical Institute of Ministry of Interior (MVR), Department of Dermatology, Venereology and Dermatologic Surgery, General Skobelev Nr 79, Sofia 1606, Bulgaria; [2]Onkoderma, Policlinic for Dermatology and Dermatologic Surgery, General Skobelev 26, Sofia 1407, Bulgaria; [3]Medical Institute of the Ministry of Interior, Surgery, Sofia, Bulgaria; [4]Medical Institute of the Ministry of Interior, Common, Vascular and Abdominal Surgery, Sofia, Bulgaria

Citation: Tchernev G, Lozev I, Temelkova I, Chernin S, Yungareva I. Schizophrenia as Potential Trigger for Melanoma Development and Progression! The Psycho-Neuro-Endocrine-Oncology (P.N.E.O) Network!. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1442-1445. https://doi.org/10.3889/oamjms.2018.276

Keywords: Malignant melanoma; Antipsychotics; Levodopa; Advancing

***Correspondence:** Georgi Tchernev. Medical Institute of Ministry of Interior (MVR), Department of Dermatology, Venereology and Dermatologic Surgery General Skobelev Nr 79, Sofia 1606 Bulgaria; Onkoderma-Policlinic for Dermatology and Dermatologic Surgery General Skobelev 26, Sofia 1407, Bulgaria. E-mail: georgi_tchernev@yahoo.de

Received: 26-Jul-2018; **Revised:** 09-Aug-2018; **Accepted:** 10-Aug-2018; **Online first:** 16-Aug-2018

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Skin, nervous tissue, dopamine and melanoma share a common neuroectodermal origin. Hence, processes that modulate nervous tissue formation, patient mental status, motor regulation of individuals, and skin cancerogenesis are inextricably linked. Psycho-neuro-endocrine oncology (or dermato-oncology), i.e. P.N.E.O., is a new model or trend in medicine and science presented for the first time in the world literature by us, that aims to examine the relationship between the mental state, the hormones and the malignant transformation. Schizophrenia and Parkinson's disease are the two main patterns of disease where the main symptoms are related to dopamine levels in the human body. According to our analyses of the available literature, the amount of dopamine is related to the incidence of melanocytic or non-melanocytic cutaneous tumours in patients with central nervous system diseases and those affecting the motor function and coordination. Such patterns of interaction are extremely indicative of the elucidation of the ubiquitous hypothesis or statement: "My illness is on a mental basis, caused by stress ..."

CASE PRESENTATION: We present a 44-year-old patient with untreated schizophrenia for approximately 25 years, associated with advanced acral localised melanoma. Schizophrenia is generally associated with a higher level of dopamine, which is also a key precursor to melanin synthesis. After a careful analysis of all literature on melanoma in patients with 1) treated and untreated schizophrenia, 2) those with untreated and untreated forms of Parkinson's disease, it would be logical to conclude that the high level of dopamine in the described patient groups is a risk factor for the development of melanoma.

CONCLUSIONS: The possible mechanisms for the occurrence of malignant melanoma within the so-called psycho/neuro/endocrine oncology (P.N.E.O.), as well as the effective methods of prevention, are under discussion.

Introduction

Schizophrenia is a mental disorder affecting approximately 1% of the world population [1]. Standard therapy for patients with this disease is given with dopamine antagonists [2]. They are thought to have a protective effect on the development of malignant melanoma in patients with schizophrenia [3].

The statement of the anti-tumour effect of dopamine antagonists is based on the results of various studies which postulate that patients with Parkinson's disease and dopamine agonist therapy have an increased incidence of malignant melanoma [4] [5].

Case report

We present a 44-year old woman with head trauma suffered in childhood (falling from a bridge), soon afterwards diagnosed with paranoid schizophrenia. Patient complaints began approximately 26 years ago. Last, about 2 years ago, she received the antipsychotic drug Olanzapine 5mg

for 3-4 months. The patient is now receiving Biperiden Hydrochloride 2 mg (1-1-0) and Aripiprazole (antipsychotic) 15 mg (0-0-1) therapy. Her therapy is currently discontinued for schizophrenia. The patient reports the presence of a pigmented lesion on the medial surface of the left lower leg for approximately 10 years. About 6 months ago she noticed the occurrence of nodules on the medial surface of her left lower leg, and subsequently, the pigment lesion progressively increased its size (Figure 1a).

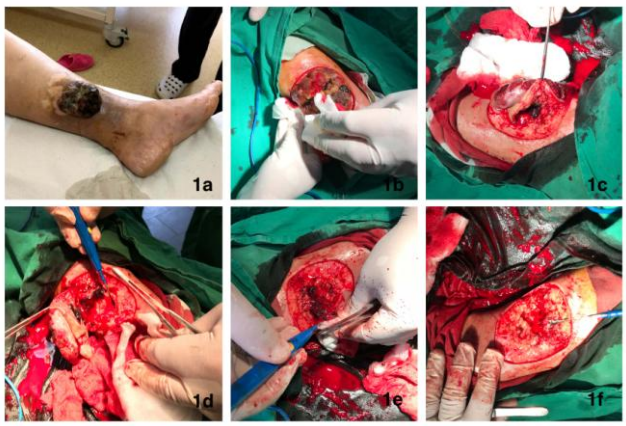

Figure 1: a) Primary cutaneous giant melanoma in a schizophrenic patient; b-f) Surgical excision of the primarius under general anaesthesia. A split skin meh graft was planned after 3 weeks

Subjectively there is a complaint about itching. Approximately 4 months ago, the patient consulted a surgeon for that lesion and was referred for surgical removal of the formation. However, the patient refuses to undergo surgery and is conservatively treated with Povidone-iodine ointment and herbs. A subsequent significant increase in the size of the lesion was observed, which is the reason for hospitalisation. Dermatological examination revealed the presence of an exophytic, tumour-like formation with an erosive surface, in decay, releasing stenching smell, located on the medial surface of the left lower leg (Figure 1a).

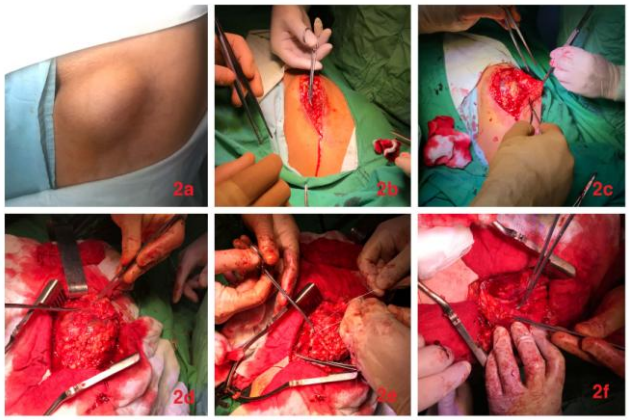

Figure 2: a-f) Inguinal lymph node dissection was carried out

Additionally, the presence of enlarged lymph nodes in the left inguinal region (Figure 2a) was established. Radical removal of the primary tumour formation along with lymph dissection (inguinal and parailliacal) of the enlarged lymph nodes (Figure 1b-1f, 2b-2f, 3a-3f iguinal/4a-4h parailliacal, Figure 5a-5c defect closure after the lymph node dissections44) was carried out.

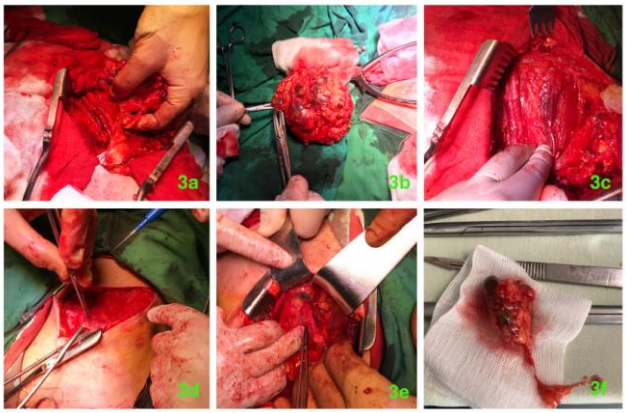

Figure 3: a-c) Arterial and venous vessels were not injured (Fig. 3a); the giant tumorous metastatic formation was completely removed (Fig. 3b, 3c)

Histopathological evidence was for ulcerated nodular type of malignant melanoma, Breslow thickness of 18 mm, Clark IV-V, and 3 metastatic lymph nodes, two of them in the inguinal and 1 of them I the parailliacal area, staged as III D. The patient was redirected to the oncology department and a therapy with Interferon 3 x 3 Mio was planned.

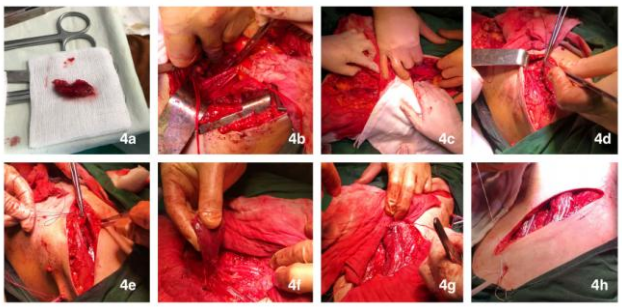

Figure 4: 3d-4h) The parailliacal lymph node dissection was performed parallel with the inguinal one (Fig. 4b). Several pigmented metastatic lymphatic nodes were removed additionally (Figs. 3e, 3f, 4a)

Discussion

Standard care treatment of schizophrenia as a chronic mental illness involves antipsychotic agents [2]. These drugs act as antagonists of the dopamine D2 receptors and serotonin 5-HT2A receptors, respectively, dopamine and serotonin [2]. There is an interesting hypothesis of a possible association between the intake of antipsychotics and the possibility of subsequent melanoma formation [3]. Already in 1982, an antitumor effect of dopamine

antagonists on the development of melanoma was discussed [3]. Melanoma culture research in mice then showed that the antidopaminergic agent (Pimozide) used had an inhibitory effect on mouse melanoma cells [3]. Similarly to other authors, we maintain that schizophrenic patients receiving anti-dopamine therapy show a lower incidence of melanoma [6].

Figure 5: a-c) Defects closure with single cutaneous surgical stitches

Under the effect of sunlight, mutations in melanoma cells occur, thus increasing the risk of melanoma in general [7]. Solar radiation induces mutations in the p53 genome regulator that is capable of inducing a cell block on the one hand (via certain inhibitory proteins such as p21, p27, p16) and, on the other, activating certain pro-apoptotic proteins such as Bax/Bak that in turn, cause cell death in tumor cells [8] [9]. The presence of mutations in p53 deactivates its functions and causes uncontrolled cellular proliferation, lack of possibility of the altered cells being eliminated by the activation of programmed cell death-apoptosis, ultimately resulting in the generation of the corresponding tumour branch [8] [9]. Whether this malignant branch will develop by impaired melanocyte or keratinocyte, at least hypothetically, should depend on the concentration or density of melanocytes localised on the basal level of the epidermis (as well as on the individual sensitivity of each patient that is multifactorial conditional).

When melanin synthesis is enhanced or is enhancing (by external intervention such as dopamine agonist therapy for Parkinson's disease or by internal intervention such as in untreated schizophrenia, for example), then mutations affect melanocytes (which density in the epidermis should have been increased) more often. Melanoma incidence in the two categories of patients described is as shown in other studies [4] [5]. Currently, there are no scientific studies to compare the incidence of melanoma and melanocyte density in patients with Parkinson's disease and schizophrenia both across patients with Parkinson's disease and healthy volunteers.

From an anatomical point of view, albeit somewhat speculatively, the increased number of melanocytes could also be seen as a protective factor in the development of keratinocyte tumours. This is also anatomically determined by the fact that the network of the increased number of melanocytes, as well as the dendrites of the latter, cover the greater part of keratinocyte cells, thus "absorbing" the majority of the negative effect of solar radiation on "their account". The more melanocytes in the basal layer of the epidermis, the higher the risk of melanoma development (when patients are exposed to UV radiation, and possibly have a genetic predisposition).

In schizophrenic patients with increased levels of dopamine, it should be assumed that the number of melanocytes in the basal part of the epidermis is further increased. In the presence of intensive sunbathing in areas exposed to solar radiation, the cumulative risk of melanoma formation should further increase [10].

According to this hypothesis, antipsychotic drugs should reduce the number of melanocytes in the epidermis by reducing dopamine and hence the risk of melanoma formation. Indirectly, the risk of developing epithelial skin tumours such as basal cell and spinocellular carcinomas [11] should also increase. It is believed that the genes regulating skin pigmentation play a key role in the development of melanoma [12]. How, however, these genes involved in melanin synthesis affect the risk of melanoma development is still the subject of further studies [13]. It is believed that *CDKN2A, MC1R, MITF* genes are involved in melanocytes differentiation and development and that mutations in these genes are associated with the formation and progression of malignant melanoma, together with pigmentation [14]. Probably the common biochemical pathway of dopamine and melanin (with a common precursor, i.e. tyrosine) is the starting point that should be considered critically regarding the risk of melanoma formation in patients receiving dopamine preparations (antagonists or agonists) [15].

This is the place to mention an equally discussed topic about the risk of malignant melanoma in patients with Parkinson's disease [16]. The therapy of patients with Parkinson's disease includes dopamine agonists that increase dopamine levels and, hence, the risk of melanoma [4] [5] [17].

It is open and somewhat speculative question whether melanomas in patients with increased dopamine in the body develop on the basis of 1) increased melanocytes in the basal part of the epidermis, which determines completely randomly the possibility of mutations (due to the higher probability of occurrence following intense ultraviolet radiation), or this is due to 2) distorted processes within the melanogenesis itself (due to the presence of a certain product in abundance, which would lead to a possible blocking of some and activation of other pathogenic chains)?

If we base our discussion on the described theory of psycho-neuro-endocrine oncology, it should be inevitably confirmed by studies in patients with Parkinson's disease with elevated levels of melanin precursor dopamine [4]. Available data from a study in Parkinson's patients show a 4.4 to the 7-fold higher risk of melanoma occurrence in these patients [4] [5].

In conclusion, the risk of malignant melanoma development depends on some factors. We believe that in patients receiving dopamine antagonist's therapy, the reduced dopamine level may reduce the potential for melanoma development, and the biomechanisms of this anti-tumour effect are determined by a genetic level. In support of this, there is evidence that dopamine agonists used to treat patients with Parkinson's disease have increased the incidence of melanoma in these patients several times.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1446-1449.
https://doi.org/10.3889/oamjms.2018.330
eISSN: 1857-9655
Case Report

An Intracerebral Penetration of Air Shotgun Pellet in Toddler: A Case without Neurological Sequelae

Wayan Niryana[1], Hendry Irawan[1], I Putu Eka Widyadharma[2*]

[1]Neurosurgery Division, Department of Surgery, Faculty of Medicine, Udayana University, Bali, Indonesia; [2]Department of Neurology, Faculty of Medicine, Udayana University, Bali, Indonesia

Citation: Niryana W, Irawan H, Widyadharma IPE. An Intracerebral Penetration of Air Shotgun Pellet in Toddler: A Case without Neurological Sequelae. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1446-1449. https://doi.org/10.3889/oamjms.2018.330

Keywords: Air shotgun pellet; Intracerebral; Toddler

***Correspondence:** I Putu Eka Widyadharma. Neurosurgery Division, Department of Surgery, Faculty of Medicine, Udayana University, Bali, Indonesia. E-mail: eka.widyadharma@unud.ac.id

Received: 12-May-2018; **Revised:** 27-Jul-2018; **Accepted:** 29-Jul-2018; **Online first:** 17-Aug-2018

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: A non-powder firearm including air shotgun remains a significant source of injury to children. It causes severe damage and can involve the brain, eyes, heart, abdomen, and other body parts.

CASE REPORT: A toddler boy was accidentally shot by an air shotgun at the forehead, and there was no sign of neurological deficit, both before and after surgical removal of the pellet. Herein, we report a case of air shotgun pellet which penetrated a toddler's head from the forehead, all the way up to the occiput. Removal of the pellet was successfully performed without eliciting any neurological sequelae.

CONCLUSION: Air shotgun pellet may potentially cause severe injury to the central nervous system when the head is affected, which can be safely prevented by a prompt but deliberate surgical removal. The study would also like to emphasise the importance of education to reduce gunshot incidence in the pediatric population.

Introduction

Firearms, according to its energy source, can be classified into traditional and non-powder firearms. Traditional firearms generate energy by burning gunpowder to propel a projectile, while non-powder firearms derive its power from compressed air or carbon dioxide. Non-powder firearms such as pellet gun, air rifles, ball-bearing guns, paintball guns, are significant sources of injury and death among children and adolescents. The injury can involve the brain, eyes, head, neck, chest, abdomen, and extremities [1] [2]. Non-powder firearms have a velocity ranging from 80 to 300m/s and can cause injury up to 20-60 feet away. Pellets have several designs such as wad cutter, round nose, sharp-pointed, and hollow point [2].

The head, neck, and eyes are the most frequent area injured by air shotgun. Pellets may enter the skull through eyes, as well as the temporal and occipital bone because they are relatively thin and less resistant compared to other head regions. Air shotgun pellet injury to the head may be either penetrating in which pellet enter the skull but does not exist, or perforating through the skull in which there are inlet and outlet wound injury. The penetrating injury is usually due to a pellet shot with high velocity from a modified air shotgun, fired from a very close distance [3].

Non-powder firearms are dangerous as it can inflict severe injuries and even death to children [1] [2] [4] [5]. Non-powder firearms are widely available in toy stores, department stores, and online stores with the premise that non-powder firearms are considered toys which are relatively safe for children to play with [4]. In contrast, the injury caused by shooting another person or himself can be serious and fatal. It usually occurs by accident, typically boys being the usual victim, potentially due to a lack of adult supervision [5].

In the United States, approximately 3.2 million

non-powder guns are sold each year, and not all areas have the law to regulate the purchase [1] [4]. Non-powder firearms typically have a velocity of 150-1,200 feet per second, compared with 0.22-caliber handguns which generate the velocity of 800 feet per second. Non-powder firearms have the power to penetrate human skin and bone. The risk of death increases if the speed is more than 350 feet per second [1].

We reported a toddler with penetrating air shotgun pellet from the forehead to the occipital bone without outlet wound injury. The case is considered unique and because the patient had a normal neurological function and recovered without any significant complication.

A 2-year-old male toddler with fully conscious, came to the emergency room with his parent after his forehead was wounded by an air shotgun. He vomited twice, had no history of decrease of consciousness, seizure, headache, and extremity weakness. The accident occurred after he and his grandfather were playing with air shotgun and did not know that there was pellet in that air shotgun. The grandfather fired at patient's forehead from a distance less than one meter. Upon admission, his vital signs were stable with blood pressure 100/60 mmHg, pulse rate of 110 beats per minute, respiratory rate 20 breathes per minute, and axillary temperature of 36.7^{0}C. There was an open wound on his left forehead, 5mm in diameter. All other physical examinations were within normal range. The neurological investigation revealed that the patient was fully conscious with Glasgow Coma Scale of E4V5M6, his pupils were round, isochoric, 3mm in diameter, excellent muscle strength on all four extremities, with an absence of meningeal signs or pathological reflex. The skull radiograph found thick round shape material on the right occipital region.

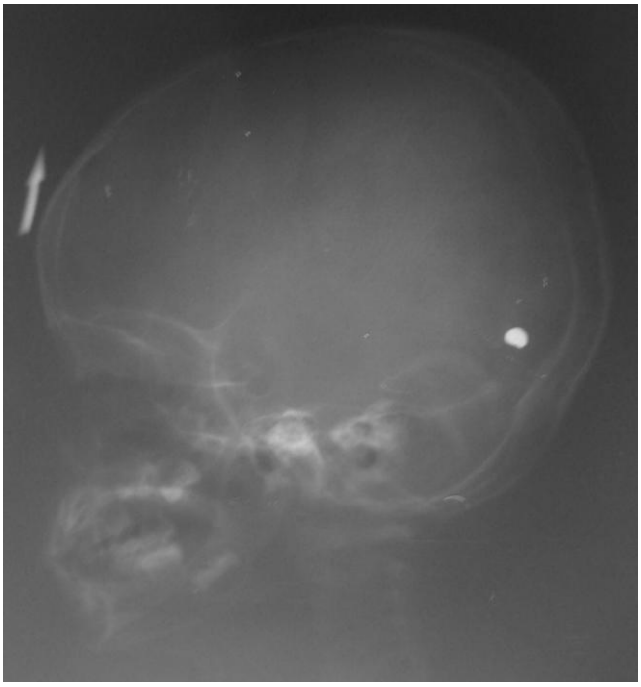

Figure 1: Skull radiograph lateral view; marker (arrow) and the pellet (opaque round shape material)

We predicted that the pellet only lodged in one side of the brain hemisphere and attached to the occipital bone. The head CT scan revealed a foreign object with metal density in the right occipital region which enter from the left frontal area, accompanied with an intracerebral hematoma from the frontal to the occipital areas, by the foreign body pathway. Besides, pneumocephalus was found around the foreign object, as well as intraventricular haemorrhage, brain swelling, and left frontal bone fracture. We then performed a three-dimensional reconstruction of the skull, which revealed an entry wound of 6mm in diameter without any other apparent fractures and pellet nested in internal tubule of the right occipital bone.

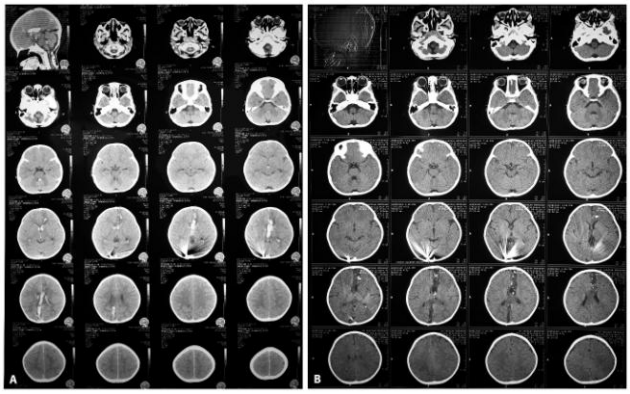

Figure 2: Head computed tomography for the first time came to the emergency room (A) and at the next two weeks after the incident (B)

After two weeks of closed monitoring, the patient was stable, fully conscious with no apparent neurological deficit or signs of infection. We run a head computed tomography, which showed a foreign body in the right occipital region with neither intracerebral hematoma nor intraventricular haemorrhage but presented with few fragments along its pathway.

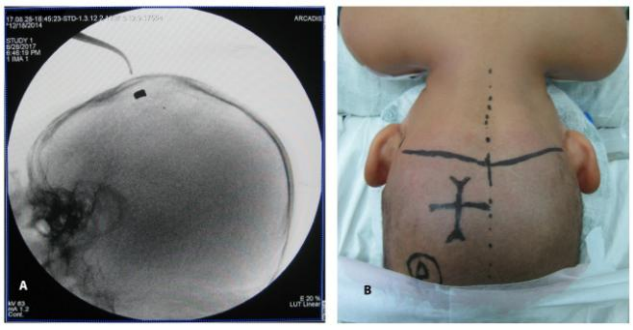

Figure 3: The C-arm to detect the position of the pellet (A) and draw site marking on the right occipital region (B)

We then performed a surgical procedure to remove the corresponding foreign body guided by mobile C-arm. The patient was in a prone position with head down. We used C-arm to detect pellet position and draw site marking for incision. We did a

single borehole in the middle of the marked zone and evaluated the corresponding area using C-arm, by which we discovered the position of the pellet was right below that hole. We later removed the pellet and its fragments with subsequent C-arm imaging to confirm that there was no remaining unextracted shrapnel. The pellet was 6mm in diameter, with round shape.

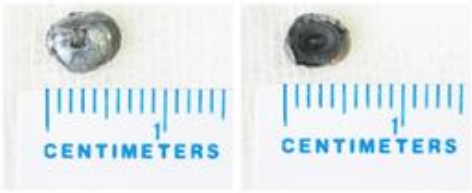

Figure 4: The pellet

The patient was stable after surgery. The wound was fully healed, and there was no sign of neurological sequelae or symptoms of cerebral infection. He was discharged 7 days after the surgery. To date, he was completely functional and independent for doing daily activities adjusted for his age.

Discussion

According to O'Neill et al., the majority of pediatric injury involved children between 12 and 17 years old (58.6%). Based on that data, 17 of 29 patients (56.8%) had a serious injury, 9 patients (31%) required surgery, 6 patients (20.7%) had significant morbidity, and 2 patients (6.9%) was death. The injuries were common to intracranial, eye, head, and neck (65.6%). Unfortunately, 2 of 3 patients who had intracranial injury was dead [1].

Similarly, Veenstra et al also found that eye was the most commonly affected area due to non-powder firearm (63%), followed by head (12%), neck (10%), abdomen (5%), lower extremity (5%), upper extremity (3%), and chest (2%) [2].

A cohort study from 2009 to 2014 discovered that there were 43 cases of pediatric injury caused by non-powder firearms, among which 84% was male with median age of 11 years old. The most common mechanism of injury was unintentional 84%. The other was assault 12%, suicide 2%, and unknown 2%. This study showed median injury severity score of non-powder firearms was 10, of which 14% was requiring surgery [4].

A similar 10-year period cohort study from 2003 to 2013 also found that 57 children had a non-powder gun injury (pellet, ball-bearing, or paintball gun). There was 77% ball-bearing gun injuries, 23% pellet gun injuries, and 3% paintball gun injuries. The mean age was 11 years old; with 23% injury involved children age 10-13 years old and 19% of age between 14-17 years old. Boys were injured more often than girls (i.e., 84%). The non-powder gun injury was caused by accident (68%) and violence (32%) [2].

The most frequent entry sites for intracranial projection were thin bones like orbital, temporal, and occipital bones. Intracranial pellet projection was found in temporal (44.6%), parietal (23.2%), occipital (10.7%), frontal (7.1%), and the rest were multiple sites. The most common intracranial haemorrhage was intracerebral hematoma 51.7% [3].

Based on Zidan et al., study, the mortality of intracranially-lodged air gun pellet among adults and children was 10.3%. The morbidity comprises superficial wound infection, cerebrospinal fluid leakage, meningitis, brain abscess, hemiparesis, seizure, and dysphasia [3]. The primary factor necessitating immediate surgery was fear of intracranial abscess, of which commonly occurred. Multiple factors are leading to intracranial infection such as skull base fracture, cerebrospinal fluid leakage, extensive brain damage, delayed operation, and retained intracranial foreign body [3].

Even with appropriate sterilisation procedure and administration of antibiotics, brain infection may be rendered ineluctable. For instance, Zidan et al. used the prophylactic antibiotic with 3rd generation cephalosporin and metronidazole, yet there was still 5.1% of subjects developed brain abscess. Other preventive measures should also be advocated. For instance, the use of seizure prophylaxis. In that study, only 5% developed seizure after given antiepileptic drug, which one patient had retained pellet intracranial and the other not [3].

According to the Center for Disease Control and Prevention, factors affecting health are socioeconomic, laws and regulations, protective interventions, clinical interventions, and education [4]. Our case was an unintentional injury to air shotgun which can be prevented if the family members were well-educated and not assumed air gun as a toy for children. Fortunately, the injury was not fatal, and the patient did not get a neurological deficiency. It is thus critical to educate people who possess non-powder firearms on its safety, and proper education about using non-powder firearms is essential to reduce preventable injury in children.

Further continuous observation is needed as any sequelae may be developed in the future. No one can ensure this patient will always sequelae free. According to the pellet location and the wound tract, the left frontal and right occipital region were involved, the patient can develop any cognitive and visual impairment. The psychological aspect also important, like this 2 years old boy underwent such serious head surgery. The most common psychological impacts for children who had undergone surgery and anaesthesia before 4 years of age are low listening comprehension

and performance IQ [6]. So, language abilities and cognition are needed to be evaluated in the next following years.

Intuitively speaking, such prevention would require integrated cooperation between government, health personnel, and other significant stakeholders, such as child protection and social services. A tighter and strict law should be imposed for regulation of non-powder firearms. Hard punishment should also be applied to those who inadvertently harm others using this type of gun. The mass media may also be useful to educate people and raise awareness of air shotgun and its potential injuries.

Conflict of Interest Disclosure

The authors declare no conflict of interest or any financial support. We had consent letter to the patient's family to publish this case.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1450-1453.
https://doi.org/10.3889/oamjms.2018.316
eISSN: 1857-9655
Case Report

The outcome of Pregnancy with Fetal Primitive Neuroectodermal Tumor

Vesna Livrinova[1*], Igor Petrov[2], Adela Stefanija[1], Jasminka Josheva[3], Viktorija Jovanovska[1], Igor Samardziski[1], Selim Komina[4], Rubens Jovanovic[4], Ana Daneva-Markova[1], Slagjana Simeonova-Krstevska[1], Irena Todorovska[1], Ajla Shabani[1]

[1]University Clinic for Obstetrics and Gynecology, Skopje, Republic of Macedonia; [2]University Clinic for Neurology, Skopje, Republic of Macedonia; [3]Private Hospital Acibadem Sistina, Skopje, Republic of Macedonia; [4]Institute for Pathology, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia

Citation: Livrinova V, Petrov I, Stefanija A, Josheva J, Jovanovska V, Samardziski I, Komina S, Jovanovic R, Daneva-Markova A, Simeonova-Krstevska S, Todorovska I, Shabani A. The outcome of Pregnancy with Fetal Primitive Neuroectodermal Tumor. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1450-1453. https://doi.org/10.3889/oamjms.2018.316

Keywords: Fetus; Brain tumour; Outcome

***Correspondence:** Vesna Livrinova. University Clinic for Obstetrics and Gynecology, Skopje, Republic of Macedonia. E-mail: livrinovii@yahoo.com

Received: 15-May-2018; **Revised:** 22-Jul-2018; **Accepted:** 27-Jul-2018; **Online first:** 17-Aug-2018

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Fetal intracranial tumours are very rare. The overall incidence is 0.34 per one thousand live birth newborns. According to the new classification of central nervous system tumour (2016), a primitive neuroectodermal tumour of (PNETs) is an embryonal tumour group; these are tumours with high malignancy and belong to group IV (WHO). In our case, we will present a case of PNETs in 28 gestation week old fetus, diagnosed antenatally and confirmed postnatally.

CASE REPORT: We present the third pregnancy in 29 years old patient, with two previous term deliveries of healthy newborn. She came to University clinic at 27+3 gestational week for fetal hydrocephalus. After an ultrasound and MRI scan, possibilities were explained to the parents. During the medico-ethical counselling, explain to the parents the need for operation and the possibility of postoperative adjuvant therapy, quality of life with potential future disabilities. They choose to terminate the pregnancy. Postmortem the diagnosis was PNETs. Summary of analysis: peripheral neuroectodermal tumour with ganglion and neuronal differentiation

CONCLUSION: Antenatal management depends on the gestational week in the time of diagnosis and the decision of parents. If the lesion is before viability fetus, it should be offered termination of pregnancy. Another important factor is the mode of delivery, because of increased intracranial pressure although this aggressive combined modality of treatment, recurrence is often. Tree year of survival is between 53% and 73% when the adjuvant radiotherapy is included. For that, they should be diagnosed as soon as possible before achieving fetal viability. Only 18% of those tumours presenting in the first year of life are diagnosed before or at delivery.

Introduction

Fetal intracranial tumours are very rare. The overall incidence is 0.34 per one thousand live birth newborns. It is very difficult to determinate the origin of tumour mass during the pregnancy due to its big dimension and supratentorial location. In older children, the brain tumours are often infratentorial. According to the new classification of central nervous system tumour (2016), a primitive neuroectodermal tumour of (PNETs) is an embryonal tumour group; these are tumours with high malignancy and belong to group IV (WHO) [1]. Primitive Neuroectodermal Brain Tumor together with Medulloblastoma and Atypical teratoid/ a rhabdoid tumour, belong to a subgroup of Embryonal tumours which originate from neuroepithelial tissue which consist 29.9% of all a brain tumour. Embryonal tumours consist of 6.62%. PNETs account for less than 5 % of embryonic CNS tumours. Most occur during childhood, with 80 % diagnosed before age 10 years, and 25 % present within the first two years. An analysis of PNETs in adults suggests that these tumours are molecularly different from the more common childhood PNETs. Only 18% of those tumours presenting in the first year of life are diagnosed before or at delivery.

In our case, we will present a case of PNETs in 28 gestation week old fetus, diagnosed antenatally and confirmed postnatally.

Case report

This was the third pregnancy in 29 years old patient, with two previous spontaneous term deliveries of healthy newborn. She came to University Clinic for Obstetrics and Gynecology in Skopje at 27+3 gestational week, after the suggestion of her ordinate gynaecologist because of his suspicion for fetal hydrocephalus. The patient had previous 3 ultrasound examinations. The ultrasound was performed, and the diagnosis by the US was: Tu cerebri. Hydrocephalus internet. Agenesis corporis callosi. Cardiopatia (VSD). Ultrasound exam: fetal biometric measurements shown 28 g.w. Fetal head: in right brain hemisphere, supratentorial located there is heterogonous texture tumour-like mass with a dimension of 70x66mm, with whole dilated right ventricle with a dimension of the posterior horn of 26 mm and shift of cerebral falx on the left (Fig. 1).

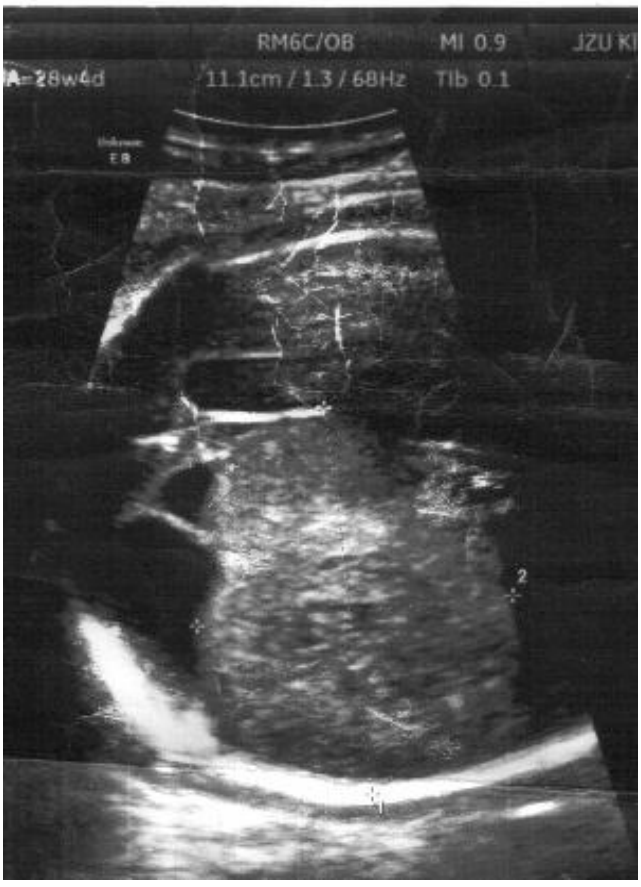

Figure 1: Ultrasound 2-D image of the fetal head

Additional imaging technique was performed such as MRI of the fetus. The report from MRI: single pregnancy in cephalic presentation. Fetal cranium: posterior cranial fossa with normal appearance. On the right side partly solid, a partly cystic tumour mass which has a heterosexual appearance in T2 pulse sequence with hypo signal solid part and the cystic peripheral part which is extended in frontoparietal direction. On T2 pulse sequence this lesion is from isosignal till hyposignal with existing linear peripheral hyper signal toward parietal, suggesting necrosis and haemorrhage.

The lesion has an extensive mass effect, resulting in dilatation of the right ventricle more evident in occipital horn with a diameter of 29 mm with reduction of brain tissue. The left hemisphere is with regular gyration. Also, there is digenesis of the colossal body. According to the above description and appearance, the lesion suggests PNET s with differential diagnosis glioblastoma (Fig. 2).

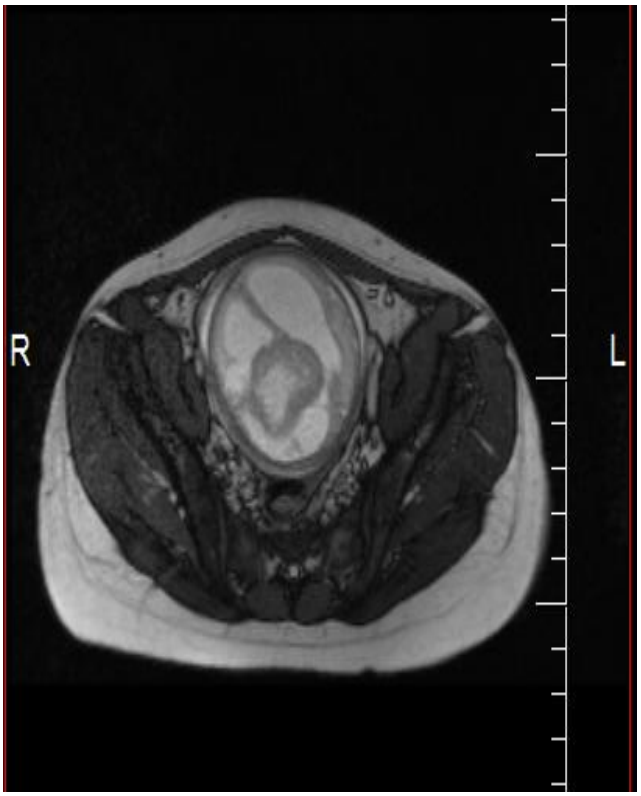

Figure 2: MRI of the fetal head

During the medico-ethical counselling, we performed testing for aneuploidies and explain to the future parents about the need for operation and possibility for postoperative adjuvant therapy, quality of life with potential future disabilities according to the data of literature.

They decide to terminate the pregnancy with intrauterine feticide. The dead male newborn weighted 1700 g and 42 centimetres long. The newborn was evaluated by pathologists. Diagnosis was: Primitive neuroectodermal tumour lobe parietalis cerebri lateris dextri. Hydrocephalus internus. Encephalomalacia cerebri precipice lobi parietal-occipitalis bilateral.

Haemorrhagia subarachnoidal parietooccipital bilateral (Fig. 3, 4).

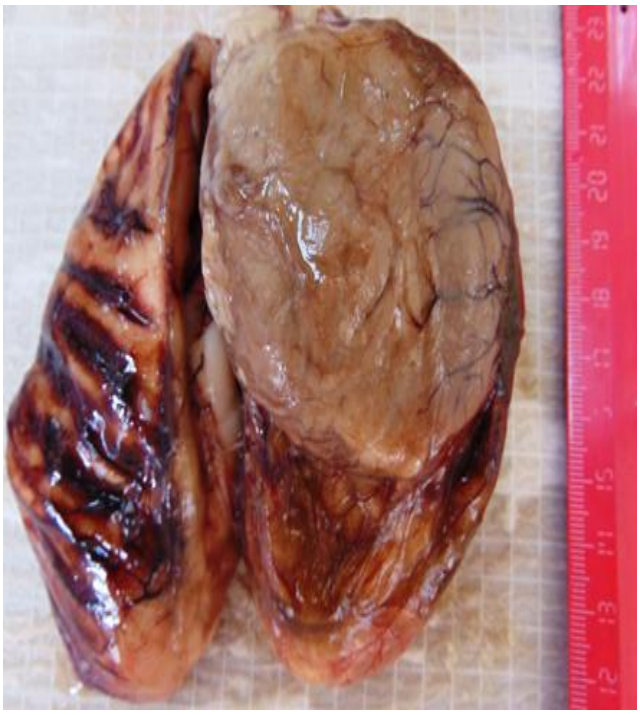

Figure 3: Macroscopic appearance of the lesion

Photomicrograph of a tumour demonstrating Homer-Wright rosette formations, composed of moderately differentiated, round to oval cells with moderately abundant eosinophilic to the amphophilic cytoplasm and hyperchromatic nuclei, surrounding central core of neurofibrillary material, (haematoxylin-eosin, original magnification x 400) (Fig. 4).

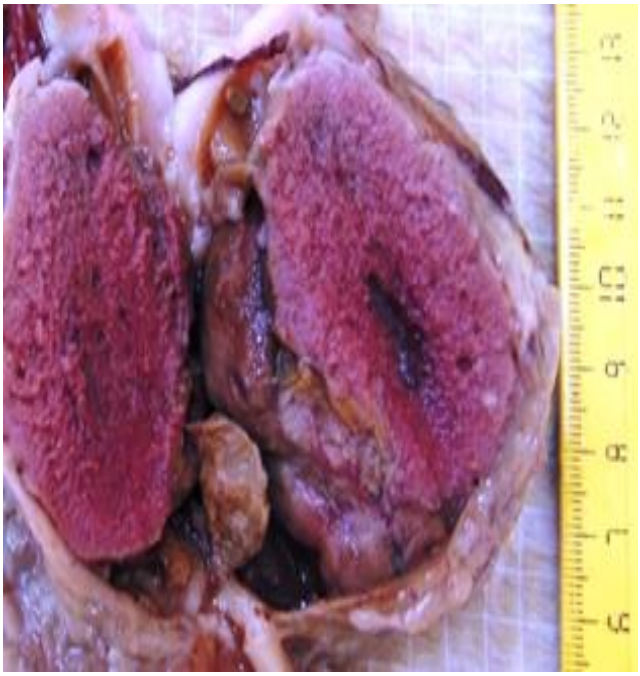

Figure 4: Macroscopic changes of lesion

Additionally, immune hystochemical staining of tumor lesson shown this profile: Vimentin(+), S100(+), CD34(-), GFAP(+), Actin(-/+), Desmin(-), CD99(-), EMA(-), CKWS(-), Chromogranin(-), NSE(+), WT1(+), Synaptophysin: positive single cells with their dendrits, Ki 67 proliferative index: 2-3%. Summary of analysis: peripheral neuroectodermal tumor with ganglional and neuronal differentiation.

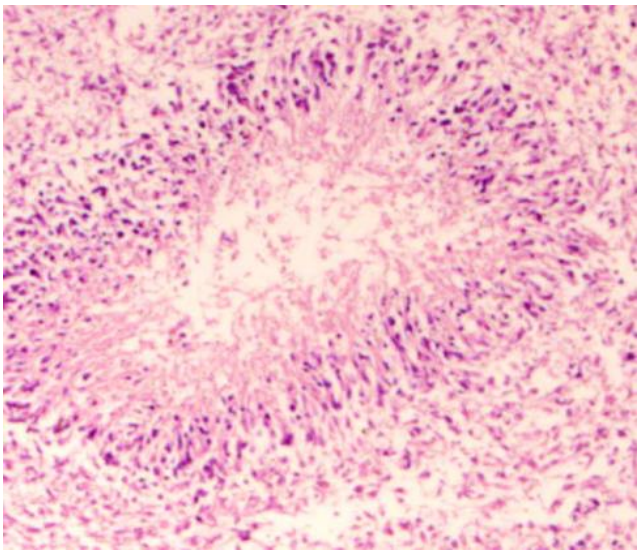

Figure 5: Microscopic picture of lesion

Discussion

PNETs of CNS are low differentiated (WHO-gr.IV) [1], rapid growth, neuroepithelial tumours which originated from the germinative matrix of the primitive neural tube. They have a potential to differentiate in more different cell lines. Tumours with clear neuronal differentiation are classified as neuroblastoma of CNS and others with mixed differentiation of neuronal and glial cells, clasifficated as CNS ganglioneuroblastomas. Some PNETs could be clasifficated of their tissue origin-retinoblastoma, pineoblastoma, but the most of them originate from cerebral hemisphere tissue (suprasellar parts), and for that, they were classicised as supratentorial PNETs. Primitive neuroectodermal tumours constitute less than 5% of embryonal CNS tumours. Lot of them are with clinical manifestations in childhood, 80% before 10 years ago, one quarter are present in the first two years of life. The tumours that occurred in adult life are molecularly different from PNETs in childhood [2].

On MRI scans there are heterogenous formations with hypodense regions correlating with hemosiderin deposition or calcifications; T1 hyperdense regions correspondents with haemorrhage and T2 bright region reflected cystic

components. The relative absence of peritumor oedema is persisted [3].

Antenatal management depends on the gestational week in the time of diagnosis and the decision of parents. If the lesion is before viability fetus, it should be offered termination of pregnancy. Because these tumours have rapid growth potential, often they are diagnosed in advanced gestational age [4]. Another important factor is mode of delivery in the cases when the parent will decide to deliver such babies. Because of increased intracranial pressure due to hydrocephalus and intracranial lesion. With an aim to minimise the additional increase of intracranial pressure, the cesarean section should be offered, before being of uterine contraction [5].

The management includes aggressive surgical resection followed with radiotherapy [6] [7] [8]. Although this aggressive combined modality of treatment, recurrence are often. Three year of survival is between 53% and 73% when the adjuvant radiotherapy is included [9]. The children under three years and with pineal PNETs have a worse prognosis [10].

For that, they should be diagnosed as soon as possible before achieving fetal viability. Only 18% of those tumours presenting in the first year of life are diagnosed before or at delivery [11].

In conclusion, fetal tumours are usually different in their histological characteristics, anatomical distributions, pathophysiology and biological behaviour, compare with the same tumour in children.

Routine antenatal ultrasound exams lead to increase the rate of detection of fetal tumours, especially MRI could be useful in differential diagnosis in some cases. Very important is the skills of obstetrician and ultrasonographer and their experience. This could be very important in the course of changing decisions of parent and management of these pregnancies and postnatally. PNETs are low differentiated malignant tumours of the central nervous system with unfavourable prognosis. Although these are very rare tumours, the importance is their prognosis.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1454-1457.
https://doi.org/10.3889/oamjms.2018.282
eISSN: 1857-9655
Case Report

Renal Arteries Embolization in Unresectable Clear Cell Renal Carcinoma: First Time Experience at Haji Adam Malik Hospital

Noor Riza Perdana[1*], Elvita Rahmi Daulay[2], Fauriski Febrian Prapiska[3]

[1]Department of Urology, Cipto Mangunkusumo National Hospital, University of Indonesia, Jakarta, Indonesia; [2]Department of Radiology, Haji Adam Malik Hospital, University of Sumatera Utara, Medan, Indonesia; [3]Urology Division, Department of Surgery, Haji Adam Malik Hospital, University of Sumatera Utara, Medan, Indonesia

Citation: Perdana NR, Dulay ER, Febrian Prapiska F. Renal Arteries Embolization in Unresectable Clear Cell Renal Carcinoma: First Time Experience at Haji Adam Malik Hospital. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1454-1457. https://doi.org/10.3889/oamjms.2018.282

Keywords: renal arterial embolisation; renal tumour; case report; Indonesia

***Correspondence:** Noor Riza Perdana. General Hospital Haji Adam Malik, Sumatera Utara, Jakarta, Indonesia. E-mail: rizaperdana13@gmail.com

Received: 27-Apr-2018; **Revised:** 15-Jun-2018; **Accepted:** 20-Jun-2018; **Online first:** 19-Aug-2018

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

OBJECTIVE: To report a case of renal arterial embolisation (RAE) in unresectable renal tumour before nephrectomy.

CASE REPORT: On presentation, the clinical features of this patient, including medical history, signs and symptoms, imaging examinations were recorded. After diagnosis and initial treatment, the result and histopathological examination were performed and discussed. We performed RAE in the unresectable renal tumour in the 28-year-old male that was complaining a palpable pain right flank mass and intermittent hematuria that had been observed five months earlier. A month after RAE, the tumour shrinks and become resectable. The parameter used was tumour volume, propulsion and component, with subjective value VAS, hematuria symptom and Quality Of Life Score EORTC-QLQ C30. The next step we performed nephrectomy with histopathology results in Clear Cell Renal Carcinoma (CCRC).

CONCLUSION: RAE is an effective therapeutic and adjuvant tool because it facilitates the dissection of unresectable large renal tumours and tumours with extensive involvement around the renal hilum; it leading to lower overall morbidity. However, the lack of randomised prospective studies is the primary reason that RAE is not used often before surgery.

Introduction

Renal cell carcinoma (RCC) constitutes approximately 90–95% of all kidney neoplasms, and 25–30% of all patients had metastatic disease upon its diagnosis. The incidence rates are high in the Czech Republic, and low in much of Africa and South-east Asia country [1]. In Indonesia, renal cell carcinoma was ranked 18th in both sexes for the overall incidence. It occurs in 1.4-1.8 cases per 100.000 populations [2].

Renal arterial embolisation (RAE) has been proven safe and effective in managing renal cancer for several decades of experience. This procedure was first performed in 1973 by Almgard [3]. Since then, the procedure has developed due to advances in technology and instrumentation. The main indication of RAE is preoperative infarction of renal cancer before nephrectomy. It can also be done as palliation therapy for unresectable renal cancer, symptomatic hematuria, and treatment of angiomyolipomas (AMLs), treatment for vascular malformations, correcting of complications following renal transplantation and treatment for patients who are poor for surgical candidates [4].

In our country, systemic therapy for renal cancer has not been covered by the national health insurance. Therefore, RAE can be a promising treatment option for renal cancer patients. The long-term outcome of RAE, however, remains unknown. In this case report, we described a patient with RCC who underwent RAE as preoperative infarction of renal cancer before nephrectomy.

Case presentation

Previously 28-year-old man was admitted to the hospital with the chief complain of a palpable painful right flank mass. There was a history of intermittent hematuria that had been observed five months earlier. He also had a history of nausea and weight loss. The patient had no family history of a renal tumour. On physical examination, the patient looked pale. There was a mobile palpable mass in the right flank across the midline. The diameter was estimated to be more than 25cm. Urinalysis examination showed microscopic hematuria. We also performed upper lower abdominal CT scan for this patient who showed a huge mass in the right Kidney (Figure 1 and 2).

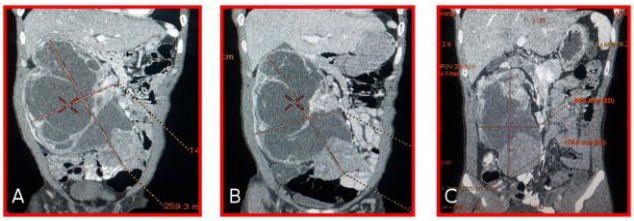

Figure 1: Abdominal CT Scan in coronal plane showed the tumour extent (1A) before RAE was performed, (1B) after 1st RAE performed, (C) after 2nd RAE performed, it shows that a tumour gradually decreased in size according to coronal view

We diagnosed this patient with unresectable Renal Tumor with grading T4BN1M0, with the extension of the lymph node in paracaval and interaortocaval without long distance metastatic, the tumour pressing through the midline and suspected compelling the aorta and inferior vena cava.

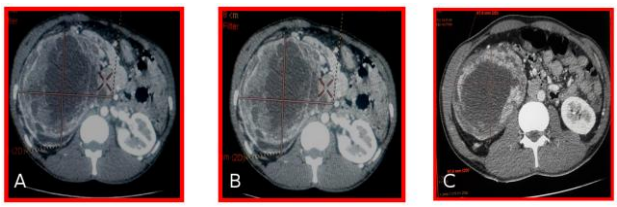

Figure 2: Abdominal CT Scan in axial plane showed the tumour extent (2A) before RAE performed, (2B) after 1st RAE performed, (C) after 2nd RAE performed, it shows that a tumour gradually decreased in size according to axial view

After the patient had signed the written informed consent, the patient was scheduled for the procedure. Under local anaesthesia, we performed arterial vascular access by inserting a vascular sheath (5 Fr-Simmon-shaped catheters) via the right common femoral artery (CFA). An aortography was then performed with a flush catheter which placed slightly superior to the expected origin of the renal arteries. Aortography is beneficial to evaluate renal arteries vascularisation and to determine, if present, the accessory renal arteries. Supplying tumour artery was selectively catheterised and embolize using polyvinyl alcohol (PVA). PVA 300-500 µm were exposed to the target using occlusion balloon catheter delivery technique.

Table 1: Tumor evaluation parameter

No.	Parameter	Before	After RAE 1st	After RAE 2nd
1	Tumor volume	2800 cc	2300cc	1700cc
2	Tumour aggravation	Across the midline, compelling the aorta and vena cava	Across the midline Compelling the aorta and vena cava	Not crossing the midline and compelling the aorta and vena cava
3	Tumor component	Cystic HU: 0-45	Necrotic (+) HU: 0-191	Necrotic (+) HU: 0-300
4	VAS	6-8	2-3	0
5	Hematuria	(+)	(-)	(-)
6	Quality of Life score measured by EORTC-QLQ C30	100	86	70

Note: HU = Hounsfield unit; VAS = Visual Analogue Score; EORTC-QLQ = European Organization for Research and Treatment of Cancer Quality of Life Questionnaire.

After the procedure was completed, the patient again undergoing aortography to evaluate the outcome of the procedure. In this patient, we performed RAE two times with further evaluation. Table 1, Figure 3 and Figure 4 show the tumour response after the first and second procedure. The parameter used was tumour volume, tumour aggravation and tumour component from the abdominal CT Scan, visual analogue score (VAS), the presence of hematuria and the patient quality of life measured by EORTC-QLQ C30.

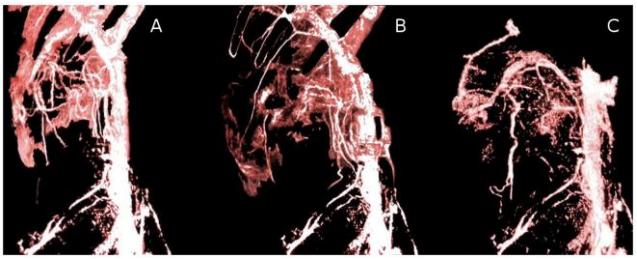

Figure 3: Abdominal CT Scan with vascular description in the 3D plane shows the vascular profile (3A) before RAE was performed, (3B) after 1st RAE, main renal artery was successfully blocked but remaining artery in inferior mesenteric and extrarenal collateral vascular supply cannot be blocked by RAE, (3C) after 2nd RAE was performed, it shows that tumor vascular supply in mesenteric inferior and collateral artery had minimized

After a month of evaluation, we performed nephrectomy with chevron incision in this patient. During the procedure, we found only small adhesion in anterior and inferior with the ileum and no adhesion in solid organ (Fig. 5). The total blood loss is 500 cc. No intraoperative transfusion is needed. The surgical specimen was then delivered to the pathology department. Histological analysis revealed a Clear Cell Renal Carcinoma (CRCC) (Fig. 6).

Discussion

CRCC is the most common and aggressive

RCC subtype with the highest rates of local invasion-metastasis and mortality. It constitutes 70–80% of all renal cancers, these tumours are commonly yellow when they are bivalve and are highly vascular on microscopic examination. The type of CRCC can be a clear cell, granular cell or mixed type. In general, patients with clear cell RCC have a worse prognosis compared with papillary or chromophobe RCC, even after stratification for stage and grade [5] [6].

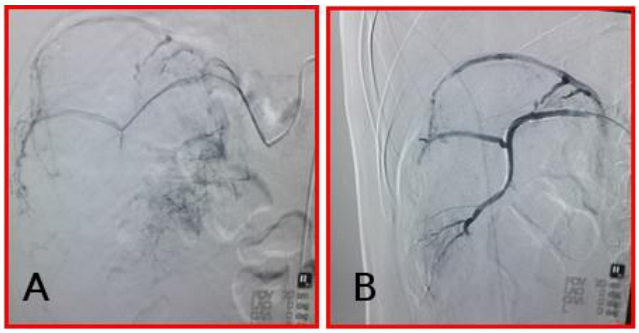

Figure 4: Fluoroscopy result shows (4A) first embolisation, (4B) second embolisation

RAE was first popularised by Almgard et al. In the 1970s, Almgard et al. performed occlusion treatment in 19 patients with three different diagnoses. Eleven cases were a large metastasising tumour, four cases were a large tumour without metastases, and the other four cases were troublesome haematuria. The method used was the same method that had been tested on animals two years earlier [3].

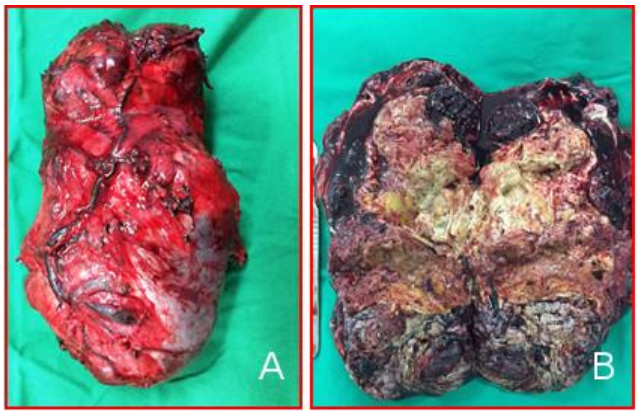

Figure 5: Tumor description after nephrectomy (5A) tumor with size 25cm x15 cm x 6 cm, (5B) tumor with midline dissection

At the first time, RAE was primarily used to treat unresectable symptomatic renal cancer. Currently, the indications for RAE have considerably expanded including renal trauma, renal tumours, iatrogenic complications, and medical renal disease [7] [8]. The advantages of RAE in the preoperative setting include a decrease in perioperative blood loss, the creation of a tissue plane of oedema, facilitating dissection, and reduction in tumour bulk including the extent of vascular thrombus [4].

Before performing the procedure, it is important to have sufficient knowledge of anatomy and its variations of renal vascularisation. Normally, a single renal artery arises from each left and right inferior side of the abdominal aorta at the level of the L1–L2 interspace. The main renal arteries branch into anterior and posterior divisions then continue as segmental, lobar, interlobar, and arcuate arteries. There are anatomical variations in more than 30% people, like the early division of the main renal arteries or extrarenal arteries further subdivided into accessory (hilar) or aberrant (polar) entry into the kidney [9].

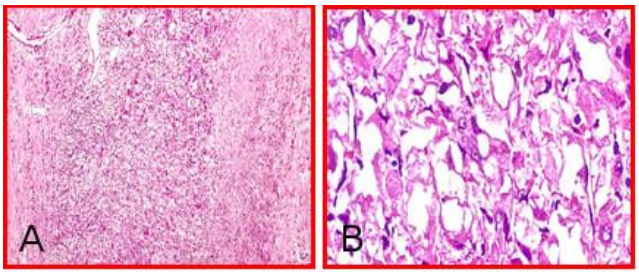

Figure 6: a) cRCC Histopathology is shown in a microscope with 20 x larger; b) The Blue Arrow Showed cRCC Histopathology in 40 x larger with clear arrow showed clear cytoplasmic with nuclei with irregular contour with Fuhrman Classification grade III

The common femoral artery (CFA) had been increasingly used as the preferred access site for renal arterial embolisation. The vascular access is generally performed using the 18 or 19-gauge puncture needles with the modified Seldinger puncture technique. In some cases where the access cannot be done through the CFA, the axillary or brachial arteries can be used as an alternative. The 5-French sheath used mostly to minimise the risk of access site bleeding. For selective embolisation of the renal arteries, some catheter can be used, including an RC-2 shaped catheter, SOS-shaped catheter, Cobra or Simmons-shaped catheter. There are some conditions that require special consideration for the sake of patient safety including atherosclerosis, abdominal aortic aneurysm, narrowing iliac artery, renal artery stenosis, or mass effect of a retroperitoneal tumour. In this patient, there are no such conditions [10] [11].

Renal tumours are typically hypervascular and often with extrarenal arterial involvement. Therefore, the alternative agent that can reach the small vessel and capillary bed occlusion is needed. In the literature, for kidney cancer cases, there is no specific agent recommended to have a better effectivity than others. In our institution, we use polyvinyl alcohol as embolant agent [12] [13]. Foam forms of PVA were first used in the 1970's [14]. The small amount of PVA for tumour embolisation may result in significant tissue ischemia. PVA is delivered through the catheter in suspension form. PVA causes direct mechanical obstruction and induces a foreign body type reaction with the permeation of the particles by granulation tissue. Over time this reaction

subsides, and months to years later, the vessel may recanalise. Although PVA is considered to be a permanent agent, it will recanalise over time [15].

Although this procedure is less invasive than open surgery, RAE has its share of complications, related to the procedure and the underlying pathology. The most common complication is a postembolization syndrome that affects over 90% of patients. It is defined as fever, mild flank pain, nausea, vomiting, paralytic ileus, and leucocytosis for one until three days after the procedure. Supportive treatment is often enough to resolve the symptoms. The other complications such as infection and coil migration are rare [16].

There are some limitations to this case report. The patient needs to be evaluated for a longer period. 1-year-follow-up is recommended to investigate any side effect of the procedure, relapse, or another progression. More subjects undergoing RAE should be investigated to obtain more significant outcomes.

In conclusion, RAE is an effective therapeutic and adjuvant tool because it facilitates the dissection of unresectable large renal tumour and tumour with extensive involvement in the renal hilum. It leads to lower overall morbidity and can also be a neoadjuvant treatment before radical nephrectomy. However, the lack of randomised prospective studies is the primary reason why RAE is still rarely used as premedication before surgery.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1458-1461.
https://doi.org/10.3889/oamjms.2018.320
eISSN: 1857-9655
Case Report

Hydroxyurea Associated Cutaneous Lesions: A Case Report

Viktor Simeonovski[1*], Hristina Breshkovska[2], Silvija Duma[3], Ivana Dohcheva-Karajovanov[3], Katerina Damevska[3], Suzana Nikolovska[3]

[1]Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia; [2]University Clinic for Plastic and Reconstructive Surgery, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia; [3]University Clinic for Dermatology, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia

Citation: Simeonovski V, Breshkovska H, Duma S, Dohcheva-Karajovanov I, Damevska K, Nikolovska S. Hydroxyurea Associated Cutaneous Lesions: A Case Report. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1458-1461. https://doi.org/10.3889/oamjms.2018.320

Keywords: Hydroxyurea therapy; Cutaneous side effects; Leg ulcer; Basal cell carcinoma

***Correspondence:** Viktor Simeonovski. Faculty of Medicine, Ss. "Cyril and Methodius" University of Skopje, Skopje, Republic of Macedonia. E-mail: viktor93@gmail.com

Received: 13-Jun-2018; **Revised:** 26-Jul-2018; **Accepted:** 27-Jul-2018; **Online first:** 19-Aug-2018

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Hydroxyurea (HU) is an antimetabolite agent that interferes with the S-phase of cellular replication and inhibits DNA synthesis, with little or no effect on RNA or protein synthesis. It is used in the treatment of many myeloproliferative disorders (MD) and is particularly a first line treatment drug for intermediate to high-risk essential thrombocythemia. Although safe and very well tolerated by the patients suffering from MD, there have been numerous reports of a broad palette of cutaneous side effects associated with prolonged intake of the medication. These may include classical symptoms such as xerosis, diffuse hyperpigmentation, brown-nail discolouration, stomatitis and scaling of the face, hands, and feet or more serious side effects such as actinic keratosis lesions, leg ulcers and multiple skin carcinomas.

CASE REPORT: We report a case of a 52-year-old man, on long-term therapy with HU for essential thrombocytosis, with several concurrent skin lesions. Despite the perennial use of HU, the cutaneous changes were neglected. The local dermatological examination revealed oval perimalleolar ulcer on the right leg, with dimensions 6 x 4 cm, clearly demarcated from the surroundings with regular margins, periulcerous erythema, with very deep and highly fibrinous bed of the ulcer, positive for bacterial infection. The ulcer was treated with topical wound therapy with alginate and parenteral antibiotics. The extended dermatological screening also showed two nummular lesions in the right brachial region, presenting as erythematous papules with sharp margins from the surrounding skin, gritty desquamation and dotted hyperpigmentations inside the lesion. Further dermoscopy and biopsy investigations confirmed a diagnosis of basal cell carcinoma. Nasal actinic keratosis was also noted. The patient was advised for discontinuing or substituting the HU therapy.

CONCLUSION: We present this case to draw attention to the various cutaneous side effects that occur with perennial HU use and suggest an obligatory reference to a dermatological consult.

Introduction

Hydroxyurea (HU), a hydroxylated molecule of urea, is an antimetabolite drug that interferes with the synthesis of DNA, with little or no effect on RNA or protein synthesis [1][2]. It is used in the treatment of many myeloproliferative disorders (MD) such as chronic myeloid leukaemia, polycythemia vera or management of other diseases like sickle cell anaemia or thalassemia [3]. It is particularly a drug of choice as a first-line treatment for intermediate to high-risk essential thrombocythemia [4]. Although safe and very well tolerated by the patients suffering from MD with primary thrombocytosis [5], there have been numerous reports of cutaneous side-effects associated with prolonged intake of the medication [6]. These include major skin changes as actinic keratosis lesions, leg ulcers and multiple skin carcinomas, [6][7] with leg ulcers affecting as many as 9% of HU-treated patients [8].

The objective of our report is to raise awareness of the possible cutaneous lesions induced by long-term use of hydroxyurea.

Case report

A 52 years old Caucasian male patient, affected by essential thrombocytosis on perennial therapy of more than 10 years with hydroxyurea, presented to the Department of Dermatology with painful leg ulceration.

The patient has experienced two myocardial infarctions in the past (2001/2013) which was both treated with angioplasty through stent placement. He also had an episode of bleeding gastric erosion. Moreover, the patient suffers from hypertension. The chronic therapy that the patient receives is as follows: Furosemide, Carvedilol, Acetylsalicylic acid and Lisinopril. After being diagnosed with essential thrombocytosis, the patient is on a perennial therapy with hydroxyurea (1.5 g/day).

Eight months before the examination, the patient reports an oval skin defect in the perimalleolar region of the right leg, which he does not associate with a mechanical injury. He was never referred to a regular check-up. Owing to the neglected nature of the problem, the lesion progressed with ulceration and became very painful. The patient treated the lesion himself, with dough, sugar and alcohol-unsuccessfully, after which he presented himself to the Department of Dermatology. The local dermatological examination revealed oval perimalleolar ulcer on the right leg, with dimensions 6 x 4 cm, clearly demarcated from the surroundings with regular margins and definite limits, periulcerous erythema and hyperalgesia over the lesion, all the way down to the foot. The bed of the ulcer appeared very deep, involving cutaneous and subcutaneous tissues, highly fibrinous and showed signs of bacterial infection. The microbiological smear was performed and came back positive with a *Staphylococcus aureus*, *Streptococcus β haemolyticus* and *Enterococcus* contamination. The patient was treated with parenteral antibiotics by antibiogram (Clindamycin), as well as topical wound therapy with alginate. Doppler sonography and photoplethysmography were also commenced, by which vascular etiology of the ulcer was excluded. A consultation with a hematologist was advised right away for discontinuing or substituting the HU therapy. A consultation with a hematologist was advised right away for discontinuing HU therapy and substituting it with recommended second-line therapy with pegylated IFN-α. Treatment with barotherapy was introduced and shaving and meshed graft transplantation planned after cleaning the bed ulcer.

The patient was then examined for other cutaneous side effects. The dermatological screening revealed two nummular lesions in the right brachial region, presenting as erythematous papules with sharp margins from the surrounding skin, gritty desquamation and dotted hyperpigmentations inside the lesions.

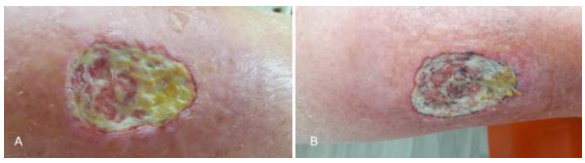

Figure 1: a), b) Oval perimalleolar ulcer on the right leg, with dimensions 6 x 4 cm, clearly demarcated from the surroundings with regular margins and definite limits, periulcerous erythema. The bed of the ulcer appears very deep and highly fibrinous. The a and b figures are respectively time framed to cover 5 months, from initial examination (Fig. 1a) to last control (Fig. 1b). We see no significant change in the course of treatment

When asked, the patient reported that he detected them several years ago and recorded discreet bleeding with mechanical trauma and crust formation over time, but the changes were also neglected, and he did not report them to a doctor before. A dermoscopy was initiated, which supported the initial suspicion of a cancer lesion.

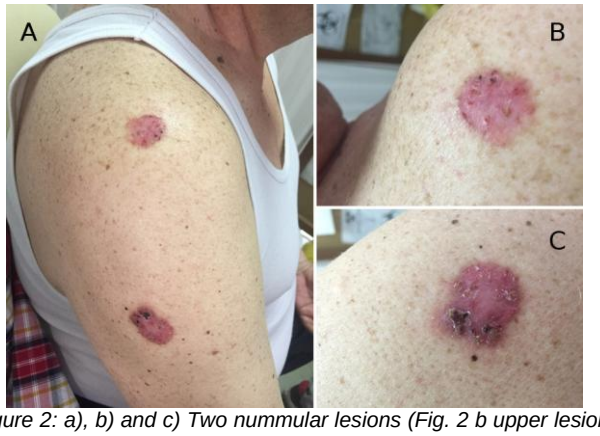

Figure 2: a), b) and c) Two nummular lesions (Fig. 2 b upper lesion, Fig. 2 c lower lesion) in the right brachial region, presenting as erythematous papules with sharp margins from the surrounding skin, gritty desquamation and dotted hyperpigmentations inside the lesion

Per protocol, biopsy followed, and the histopathological findings sustained basal cell carcinoma (BCC). The patient was advised for surgical treatment of the lesions.

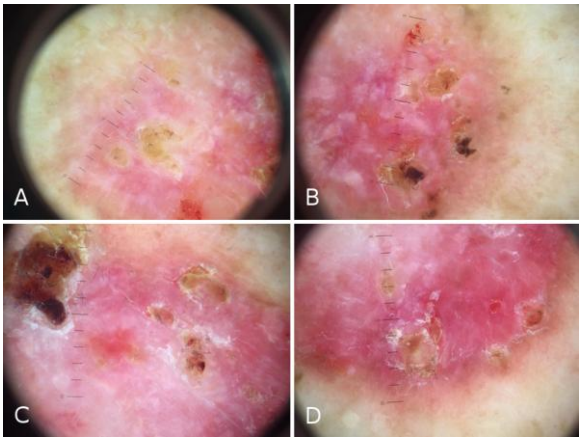

Figure 3: a), b) (upper lesion), c), d) (lower lesion) Dermoscopic findings of cancer like lesions: shiny white to red areas, short fine telangiectasias, leaf-like areas and small surface erosions

Furthermore, an actinic keratosis lesion in the nasal region was to be noted, which was dermoscopically verified. The treatment of choice was cryotherapy, with a successful outcome.

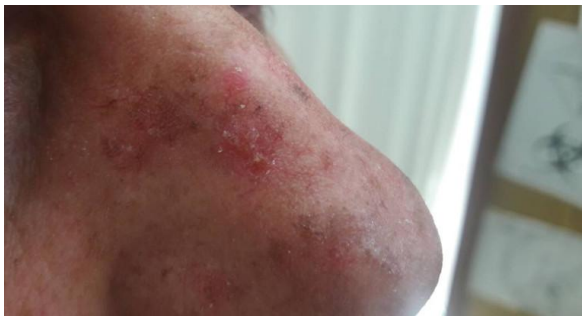

Figure 4: Nasal actinic keratosis lesion

Discussion

Essential thrombocythemia is a myeloproliferative disorder, characterised by stem cell-derived clonal myeloproliferative. The therapeutic approach is directly influenced by the risk grade of the disease, which is determined by the extensity of thrombocytosis, history and advanced age [3]. Thus, our patient is classified as an intermediate to high-risk thrombocytosis.

As presented by Tefferi et al., (2018) the current treatment algorithm for intermediate and high-risk ET is hydroxyurea as a first line drug of choice and a low dose of Aspirin [4], guidelines, which were correspondingly followed for the disorder management in our case.

HU is an antimetabolite agent, which acts upon the S-phase of cellular replication and inhibits DNA synthesis by barring the ribonucleotide reductase but does not affect RNA synthesis [1] [2]. As expected by an antineoplastic medication, Guillot et al., (2004) illustrate a broad palette of skin-related side effects associated with the long-term utilisation of the drug [7]. These may include classical symptoms such as xerosis, diffuse hyperpigmentation, brown-nail discoloration, stomatitis, erythema, and scaling of the face, hands, and feet, but three cutaneous lesions, in particular, are more specific to this drug: leg ulcers, actinic keratosis and increased frequency of skin carcinomas [2] [7] [9]. As in numerous other cases, our patient underwent cutaneous changes which are believed to be closely related to the long-term use of 1.5 g daily dose of HU. Most strikingly observant is the leg ulcer.

Stahl and Silber (1985) were the first one to report an HU-induced leg ulcer in 1985 [10]. Quattrone et al., (2013) appraised a pathophysiologic theory of three pathogenic factors in the formation of HU-induced skin ulcers- minor external traumas, direct HU toxicity on basal cells and hypoxia due to HU-induced macrocytosis [9]. Multiple publications show similarities to our case regarding the proposed aetiology of the leg ulcer. Consistently, dosage and duration of HU administration, as well as the location of the ulcer and the progression of the lesion: long-term use of HU (> 5 years), (1 or 1.5 mg per day), very painful perimalleolar ulcers, with no prior trauma [5] [11] [12] [13] [14]. Notwithstanding the standard wound therapy, treatment of choice in HU-induced leg ulcers is discontinuation of the drug or substitution therapy [8]. In contrast, there were few cases, where only standard debridement therapy, without discontinuation of HU, was enough to achieve successful partial or complete remission of the ulcers [6] [11] [13]. Correspondingly to some publications [6] [13] [14], our case showed refraction to standard conservative therapy. Hence a consultation with a haematologist for discontinuation and substituting it with recommended second-line therapy with pegylated IFN-α was commenced [4].

We also report of two carcinoma-like lesions, which were thoroughly examined according to protocol. As the physical examination and dermoscopy gave ambiguous findings, a biopsy was indicated. The result was a histopathological image of BCC. The significance of reporting these lesions is by other publications of similar lesions, with similar dermatological status, but histopathologically proven SCC [6]. Consequently, we advise an in-depth examination of all cancer-like lesions, due to the broad spectrum of different possible pathohistological findings.

The actinic keratosis lesion is reported within this paper, not only in association with HU [6] but because of its pre-malignant nature and high rate of malignisation, so a detailed follow-up is always advised.

In conclusion, our case study highlights the importance of understanding the cutaneous side effects of long-term HU use. Haematologists and patients should be drawn to attention to cutaneous lesions induced by prolonged HU intake. If there are skin changes already present, substitute therapy should always be advised if possible. Dermatological consult ought to be mandatory for all patients on perennial HU use.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1462-1467.
https://doi.org/10.3889/oamjms.2018.295
eISSN: 1857-9655
Dental Science

Frontal Sinus Obliteration Utilizing Autogenous Abdominal Fat Graft

Abla Eledeissi[1*], Mamdouh Ahmed[2], Emad Helmy[2]

[1]Oral and Maxillofacial Surgery Department, Nasser Institute Hospital, Cairo, Egypt; [2]Oral and Maxillofacial Surgery Department, Faculty of Oral and Dental Medicine, Cairo University, Cairo, Egypt

Citation: Eledeissi A, Ahmed M, Helmy E. Frontal Sinus Obliteration Utilizing Autogenous Abdominal Fat Graft. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1462-1467. https://doi.org/10.3889/oamjms.2018.295

Keywords: Frontal sinus fractures; Frontal sinus obliteration; Abdominal fat graft

***Correspondence:** Abla Eledeissi. Oral and Maxillofacial Surgery department, Nasser Institute Hospital, Cairo, Egypt. E-mail: ablaedc@hotmail.com

Received: 20-Apr-2018; **Revised:** 03-Jul-2018; **Accepted:** 05-Jul-2018; **Online first:** 21-Jul-2018

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Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: Frontal sinus fractures have always been unique because of the controversy surrounding their ideal treatment protocol and the fatal complications that could follow if the wrong treatment opts.

AIM: The purpose of this study was to assess clinically and radiographically frontal sinus obliteration technique utilising autogenous abdominal fat graft.

PATIENTS AND METHODS: This study was carried out on 20 patients having anterior table fracture of their frontal sinuses indicated for sinus obliteration. All sinuses were obliterated using autogenous abdominal fat graft. Post-operatively, patients were clinically evaluated for any signs or symptoms of intracranial infections, wound dehiscence, sinus affections, or aesthetic deformity. Computerized tomography (CT) radiographic evaluations were carried out immediately and 12 months postoperatively to evaluate any uneventful healing of the graft.

RESULTS: Clinical follow-up showed no cerebrospinal fluid leak, no postoperative infection or wound dehiscence in 18 cases. There were two cases however that showed infection. Radiographic follow-up revealed uneventful healing of the abdominal fat grafts with no abnormality detected in the sinus cavity throughout the whole postoperative period.

CONCLUSION: Autogenous abdominal fat graft appears to be a successful obliteration material in the frontal sinus cavity and is beneficial in fractures of the anterior table.

Introduction

Frontal sinus fractures represent a challenge and are relatively infrequent. The incidence of frontal sinus fracture ranges from 2% to 15% of all facial fractures [1] [2]. Restoring esthetics and function and preventing complications to the frontal sinus and other critical related structures are the major objectives intended in the treatment of frontal sinus injuries. Reported complications included chronic sinusitis, meningitis, brain abscess and mucocele formation [3] [4] [5] [6].

One of the most commonly accepted management algorithms is that proposed by Rohrich and Hollier in 1992 [7] Injury of the nasofrontal duct and CSF leak were the key determinants in deciding the treatment plan in this algorithm. Rohrich and Hollier suggested that patients with frontal sinus fractures were treated in four different ways based on the type of injury: 1 no surgical intervention, when the fractures were non comminuted, nondisplaced, not accompanied by cerebrospinal fluid leaks, and not involving the nasofrontal duct; 2 open reduction and internal fixation of the anterior table with sinus preservation in patients with fractures not involving the nasofrontal duct; 3 open reduction and internal fixation of the anterior table with sinus obliteration in fractures involving the nasofrontal duct; and 4 cranialization, when the posterior table had to be removed during exploration of intracranial lesions [3].

It is well agreed in all treatment protocols proposed in the literature that violation of the

nasofrontal duct requires sinus obliteration. Obliteration consists of eliminating the frontal sinus cavity while maintaining the anterior and posterior tables [8].

Various materials both autogenous and alloplastic have been advocated in the literature with varying degrees of success for the obliteration of the frontal sinus and the nasofrontal ducts. These include adipose tissue, bone, temporalis fascia, gelfoam pericranium, bio-glass, oxidised cellulose and others [8] [9]. Although each graft material has its merits and perils, autogenous grafts are favoured over allogeneic materials because of their generous clinical history and positive long-term treatment results [10].

Bergara and Itoiz [11] endorsed the use of autogenous fat for obliterating the frontal sinus. They showed that viable implanted adipose tissue along with the meticulous removal of the sinus lining mucosa typically prevented regrowth of the mucoperiosteum. After Bergara's proposition, autogenous fat has been approved as a reliable material for obliterating the frontal sinus and had been widely used.

The purpose of our study was to evaluate the abdominal fat graft and assess its complication rates when used as an obliteration material in the frontal sinus obliteration technique.

Materials and Methods

The present study was conducted on 20 adult patients admitted to the Cranio-Maxillofacial Surgery Department, Nasser Institute hospital and faculty of Oral and Dental Medicine, Cairo University. The criteria for patient selection included fractures indicated for duct obstruction and frontal sinus obliteration. These included anterior table frontal sinus fractures associated with injury to the nasofrontal duct with no or little involvement of the posterior table (not requiring cranialization).

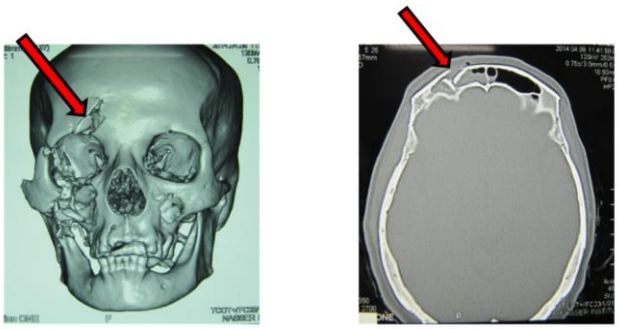

Figure 1: a) Preoperative 3D CT showing a right side anterior table fracture of the frontal sinus with comminuted right ZMC and orbital floor Fracture; b) An axial cut CT showing the unilateral displaced anterior table fracture of the right frontal sinus cavity

General rules of emergency care were applied to all patients presented to the emergency department. Frontal sinus fractures were assessed as part of general head injury evaluation. Neurological and ophthalmological consultations were performed to rule out associated injuries.

Computed tomography (CT) was done for all patients with coronal, axial, sagittal and 3-dimensional reconstructions preoperatively. Based on radiographic findings, the degree of injury to the anterior and posterior tables as well as the nasofrontal duct was determined (Figure 1a, b).

All surgical procedures were performed under general anaesthesia. Exposure of the sinus was done via a bi-coronal incision or through an existing laceration (Figure 2).

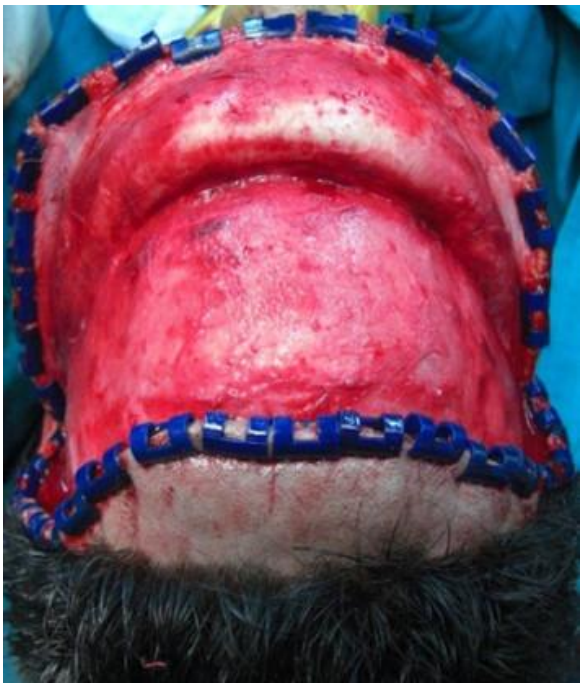

Figure 2: Coronal incision and dissection in the subgaleal plane from one superior temporal line to the other and flap reflection to approach the fractured frontal sinus

The fractured anterior table fragments were removed and debrided of any mucosal linings with rotary drill under copious irrigation and stored in normal saline. The frontal sinus cavity was then addressed where debridement of the sinus membrane was performed using curette then a bur for curettage of the bony cavity of the sinus together with the removal of the inner cortex of the sinus wall to remove any invaginations of the sinus membrane from the foramina of Breschet (Figure 3).

The nasofrontal ducts were finally obstructed using either pericranium, temporalis muscle, or fascia together with bone chips.

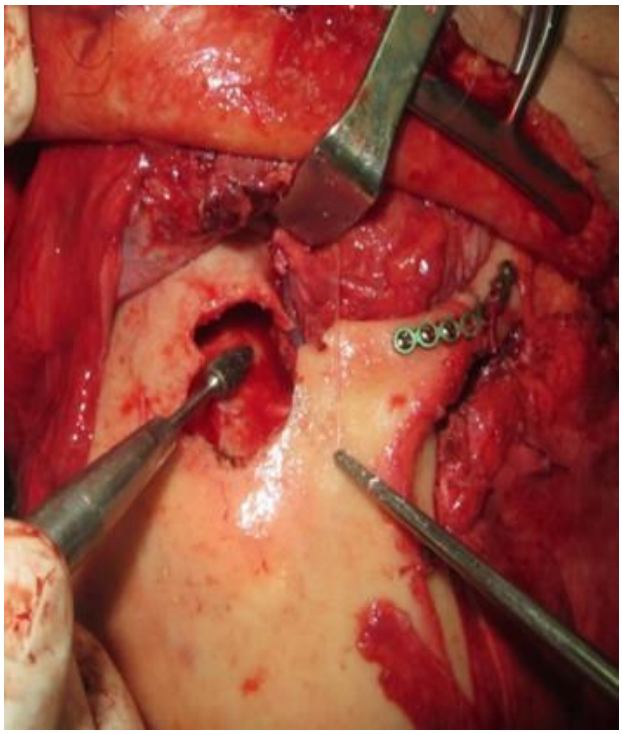

Figure 3: After anterior table removal, the frontal sinus membrane and the nasofrontal duct epithelium were removed with rotary bur

Harvesting the abdominal fat graft was performed using a 4 cm transverse, midline or side incision below the umbilicus (Figure 4a). An appropriate amount of fat was harvested and the wound closed by subcutaneous and skin sutures. The harvested fat was then used to obliterate the sinus (Figure 4b).

Finally, the anterior table was reconstructed using a 1.0 or 1.5 mm plating system or a titanium mesh depending on the degree of comminution (Figure 5). The bi-coronal incision or the forehead laceration was closed in layers, and a pressure dressing was placed for 48 hours. A drain was placed whenever a bi-coronal incision was used.

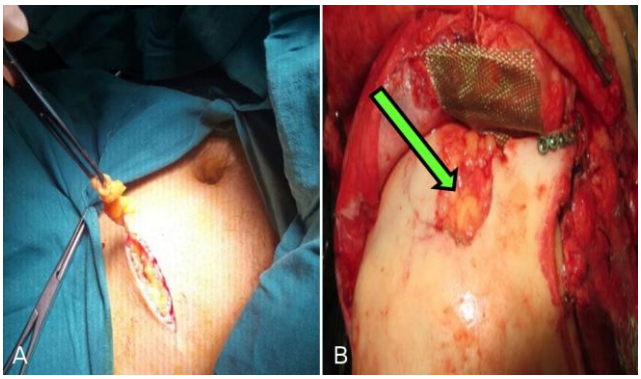

Figure 4: a) Harvesting of the ab¬dominal fat graft using a 4cm transverse side incision below the umbilicus; b) Frontal sinus right side cavity obliteration with abdominal fat graft

Immediately post-operatively, patients were given antibiotics (Amoxicillin/Clavulanate Potassium) 1.2 gm, every 12 hours for 5 days, steroids (Dexamethasone) 8 mg, 4 times daily then gradually withdrawn in the following days according to a standard protocol, and methylprednisolone acetate 80 mg intramuscular injection once with the last dose of dexamethasone.

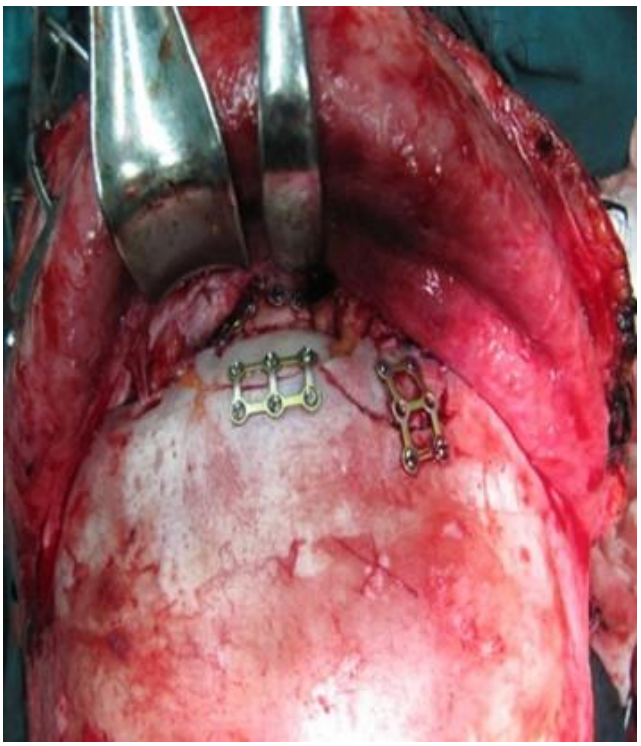

Figure 5: Reduction of anterior table fragments of the fractured frontal sinus and rigid fixation with plates and screws

Analgesics (Diclofenac sodium) were also given twice daily for a week. Antihistaminics (Loratadine) twice daily for five days following the surgery, and nasal decongestants (Oxymetazoline), nasal drops 3 times daily were also prescribed.

Patients were discharged 3 days after their surgery, during this time at their stay at the hospital, they were seen every day and clinically evaluated (days 1 to 3). They were then seen after 1 week from the day of discharge (week 1) then 1 week afterwards (week 2) followed by a visit after 6 months and finally after 12 months.

The postoperative clinical evaluation was carried out to evaluate the following: intracranial Infections (brain abscess or meningitis), Wound Infection, osteomyelitis, wound dehiscence, aesthetic deformity and sinus affections (sinusitis, mucocele, pyomucocele). Radiographic evaluation was done using computerised tomography (CT) scanning immediately and at 12 months postoperatively. Cases were considered radiographically successful when sinuses were free of pathology, and the anterior table was correctly reduced.

Results

This study included 20 adult patients (18 males and 2 females) with the mean age of 29.5 years (19-50). The mechanism of injury was road traffic accident in 18 patients, and two patient's injury was a result of a fall from height.

In the current study, isolated anterior table fracture of the sinus was found in half of the patients (10 patients) while combined anterior and posterior fractures were found in the other half (10 patients). Eighteen frontal sinuses were approached through a coronal incision, and two approached through an existing laceration.

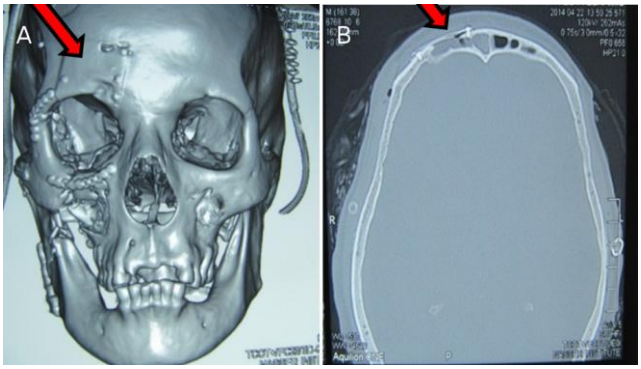

Figure 6: a) 3D CT showing titanium mesh reconstruction of the anterior table of frontal sinus and reduction and fixation of the right ZMC with plates and screws; b) Immediate Postoperative Axial CT, showing a reduced anterior table of the frontal sinus and fat in the sinus cavity

Clinical follow-up showed no signs of infection or wound dehiscence in 18 cases while two cases were infected at 1 and 2 weeks postoperatively with oedema and pus oozing through the wound. Those cases showed no signs of infection pre-operatively. In both cases, the sinus was approached through an existing laceration. The treatment consisted of incision and drainage, irrigation and antibiotic treatment with an uneventful course afterwards. No CSF leak or mucoceles were observed during the 1 year follow-up period in any of the twenty cases. Radiographically, all the abdominal fat grafts underwent uneventful healing, and no abnormality was detected in the sinus cavity throughout the whole postoperative period (Figure 6a, b). One year follow-up showed no evidence of complications, bone occupying lesions or infection (Figure 7).

Discussion

Frontal sinus trauma is a heated topic because there are considerable controversy and dispute in the literature regarding what defines the "appropriate" management. Recommended treatment principles are aiming at restoring the preoperative frontal contour and facial aesthetics, isolating the brain and preventing any CSF leak, and preventing early and avoiding delayed postoperative complications from the central nervous system [12].

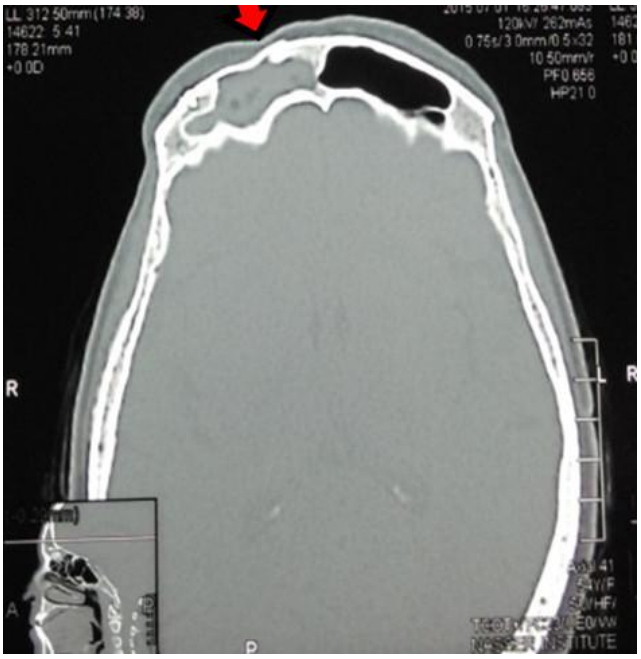

Figure 7: One year postoperative axial CT showing no evidence of complications, bone occupying lesions or infection, good maintenance of fat graft volume and complete frontal sinus obliteration after repair of the right frontal sinus cavity, and good drainage of the non-operated left side sinus cavity

In the past, physical examination in combination with plain films were the key tools in diagnosing frontal sinus fractures; these tools were inaccurate in predicting the degree of frontal bone or intracranial injury. Earlier, many prominent authors made treatment decisions based on these tools alone and most likely operated on patients more often than was needed. Nowadays, CT scan images present an accurate, 3-dimensional image by which the clinician can assess the need for and extent of operative intervention. It is now widely agreed upon that CT evaluation is mandatory in all patients with clinical findings suspicious for frontal sinus injury [13].

Computed tomography (CT) imaging was used in evaluating the fractures in our study. The choice between the surgical or nonsurgical treatment of frontal sinus fractures is crucial. This choice is dependent on some factors which subsequently influence the risk of complications. These factors include fracture type, the presence of comminution, cerebrospinal fluid leakage, the extent of posterior table involvement, neurological status of the patient, preference of the treating physician and the nasofrontal outflow tract (NFOT) injury. CT is an essential tool in evaluating several of these factors [3] [4].

Regarding the NFOT, diagnostic criteria have been recently described in an attempt to identify specific findings on CT imaging that carry a high suspicion for injury and obstruction of the NFOT. These criteria include fracture of the frontal sinus floor, fracture of the medial aspect of the anterior table (anterior ethmoid cells), and frank bony outflow tract obstruction. These criteria were relied upon in our study to diagnose the NFOT injury [3] [4].

Fracture repair depends on good exposure and complete identification of the fractured components. Reports from the literature show that exposure via a standard coronal incision is favoured over using an existing laceration. Exposure via existing lacerations is usually an inadequate approach and should be reserved for only small fractures of the anterior table. The bicoronal approach, on the other hand, seems to have a lower recurrence rate and better aesthetic results. Moreover, complications such as numbness, frontal branch (cranial nerve VII) weakness, dural damage with cerebrospinal fluid leakage, and damage of the orbital contents or intracranial structures, are not so common and have seldom been reported [14] [15].

It is well agreed among most authors that a understanding of the sinus drainage apparatus is the key element to successful management of frontal sinus fractures. The nasofrontal duct is the structure responsible for drainage of the frontal sinus. It is more vulnerable to injury because of its posteromedial position in the sinus causing it to be injured in as many as one-third of patients presenting with frontal sinus trauma. Clinical and experimental evidences suggest that obstruction of the nasofrontal duct is a significant predisposing factor in the development of complications such as mucocele or mucopyocele formation and that this risk is life-long. The seriousness of the NFD compromise in frontal sinus fractures was shown through the work of Zonis et al., [16] who noted that all 4 cases of untreated fractures with NFD injury developed suppurative sequelae.

Consequences following NFD injury can be fatal. When frontal sinus drainage is impaired, and mucus is retained, a mucocele may develop and act as an expanding tumour causing erosion of the bony walls of the frontal sinus, orbits, and skull base. An anaerobic environment may subsequently develop, causing frontal sinusitis that may lead to osteomyelitis, meningitis, or brain abscess. These grave sequelae led to the recommendation that fractures resulting in NFD obstruction should generally be treated in the manner as to create a “safe sinus” by complete sinus membrane removal and obliteration of the sinus cavity [17].

In our study, it was decided that every displaced anterior table fracture (isolated or combined with a non-displaced posterior table fracture not requiring cranialization) defined as bony displacement more than or equal to the width of the outer table associated with NFD fracture was an indication for Frontal sinus obliteration. This is by the conclusion of Heller et al., [17].

There is a myriad of methods and biomaterials available for frontal sinus obliteration. These include obliteration by spontaneous regeneration, autogenous grafts such as bone, fat, and muscle, and alloplasts such as hydroxyapatite bone cement, methyl methacrylate, calcium phosphate bone cement, and glass ionomer. Although advocates for each of these techniques or materials exist, autogenous abdominal fat is the most well-studied and has the longest track record of success [2] [18].

The technique of obliteration with freshly removed abdominal fat dates back to Bergara [19] and Tato et al., [20]. Goodale and Montgomery [21] however established the technique of frontal sinus obliteration with fat obliteration as the standard approach for managing the difficult cases. Their results showed no cases of infection or recurrence and a lack of osteogenesis on plain x-ray films 5 years after surgery.

The work of Tato et al., [20] further endorsed the technique of fat obliteration formerly described. They reported a failure rate of only 3% and stated that adipose tissue survives in the sinus and also that an unobliterated sinus cavity completely cleansed of its lining membrane may obliterate spontaneously with fibrous tissue.

Fat obliteration was further validated by the study of Montgomery and Pierce [22] with only one failure among 61 cases. Calcaterra and Strahan [23] again reported successful obliteration with fat with only one case requiring reoperation and no recurrences. Another study by Sessions et al., [24], showed a 3.7% rate of postoperative infection and no postoperative mucocele.

The most comprehensive series and follow-up were that of Hardy and Montgomery [25]. Two hundred eight (208) patients had their frontal sinuses obliterated with abdominal fat. 4% of cases had to be revised, and the overall complication rate was 18%: there were abdominal wound complications in 5.2%, acute postoperative infections with necrosis of the implanted fat in 3%, and recurrent chronic sinusitis in 3% of patients. No mucoceles were reported throughout the length of the study. In the series, 93% of the patients had no significant symptoms, 6% had persistent pain, and 1% had persistent neuralgia. Our results confirm the high success rate of frontal sinus fat obliteration very comparable to other previous studies [19].

In conclusion, our treatment results suggest that the frontal sinus obliteration utilising autogenous abdominal fat is a highly effective method in the management of fractures of the anterior table. Short term complications were uncommon in our study.

However, the effectiveness of fat obliteration for frontal sinus treatment on the long term cannot be predicted from our study, and further studies are recommended with longer follow-up periods. Moreover, the sample in our study is small, and further studies should be considered with a greater sample.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1468-1474.
https://doi.org/10.3889/oamjms.2018.291
eISSN: 1857-9655
Dental Science

Effect of Laser Therapy on the Osseointegration of Immediately Loaded Dental Implants in Patients under Vitamin C, Omega-3 and Calcium Therapy

Faten Fawzy Mikhail[1*], Mouchira El-Din[2], Tarek Ibrahim[3], Khaled Zekry[2], Amany Nemat[1], Sherine Nasry[1]

[1]Department of Surgery and Oral Medicine, National Research Centre, Cairo, Egypt; [2]Periodontology and Oral Radiology, Department of Oral Medicine, Faculty of Oral and Dental Medicine, Cairo University, Cairo, Egypt; [3]Department of Medical Laser Application, National Institute of Laser Enhanced Sciences, Cairo University, Cairo, Egypt

Citation: Mikhail FF, El-Din M, Ibrahim T, Zekry K, Nemat A, Nasry S. Effect of Laser Therapy on the Osseointegration of Immediately Loaded Dental Implants in Patients under Vitamin C, Omega-3 and Calcium Therapy. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1468-1474. https://doi.org/10.3889/oamjms.2018.291

Keywords: Calcium; Immediately loaded implant; Laser therapy; Omega-3; Osseointegration; Vitamin C

***Correspondence:** Faten Fawzy Mikhail. Department of Surgery and Oral Medicine, National Research Centre, Cairo, Egypt. E-mail: liliradi782008@yahoo.com

Received: 23-May-2018; **Revised:** 24-Jun-2018; **Accepted:** 03-Jul-2018; **Online first:** 15-Aug-2018

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: The use of laser therapy in the biostimulation of bone repair has been growing steadily.

AIM: This study aimed to evaluate the radio-densitometric effect of low-intensity laser therapy on the osseointegration of immediately loaded dental implants in patients under vitamin C, omega-3 and calcium therapy.

PATIENTS AND METHODS: A single implant was placed in the mandibular first molar region of twenty patients which were equally divided into two groups. In the non-laser group, the healing phase was left to progress spontaneously without any intervention, while in the laser group it was augmented with low-level laser therapy of wavelength 904 nm in contact mode, continuous wave, 20 mW output power and exposure time 30 sec with a dose 4.7 J/cm^2. Patients in both groups were given vitamin C, calcium and omega-3 starting one month preoperatively. Postoperative digital panoramas were taken immediately after surgery, 1.5 months and 6 months postoperatively. Changes in bone density along the bone-implant interface at the mesial, distal and apical sides were assessed using the Digora software.

RESULTS: Independent student t-test was used to compare means of variables between the laser and the non-laser group while repeated measures ANOVA was used to compare bone densities at different times for the same group. Significant increased differences were observed at the mesial, distal and apical sides surrounding the implants of both groups per time. However, the rate of increase was significantly higher in the laser group. The mean difference at the mesial side after 6 months was 21.99 ± 5.48 in the laser group and 14.21 ± 4.95 in the non-laser group, while it read 21.74 ± 3.56 in the laser group and 10.78 ± 3.90 in non-laser group at the distal side and was 18.90 ± 5.91 in the laser group and 10.39 ± 3.49 in non-laser group at the apical side. Significance was recorded at P = 0.004, P = 0.0001, and 0.001 at the mesial, distal and apical sides respectively.

CONCLUSION: The low-intensity laser irradiation significantly promoted bone healing and speeded up the osseointegration process emphasising the laser's biostimulatory effect.

Introduction

The success of the endosseous dental implants depends mainly on the successful osseointegration of the implant with bone, and many attempts have tried to enhance this process, one of which was the use of low-level laser therapy (LLLT) [1].

The effect of LLLT on bone regeneration has become a focus of recent research, as it improves vascularisation, enhances collagen synthesis and concerning the bone, it modulates inflammation, accelerates cell proliferation and enhances healing [2] [3] [4]. In several studies, it was demonstrated that LLLT stimulates stem cells of the bone and accelerates its repair process [5] [6].

Recently, immediate implant loading has become more common and is better accepted by many patients as it negates the need for second surgery where provisionalization is simplified by the

immediate loading of the implant after surgery [7] [8].

Vitamin C is an essential water-soluble vitamin for humans, as it is a powerful reducing agent and is important for proper wound healing as it leads to fibroblast differentiation and collagen synthesis [9, 10]. Furthermore, vitamin C has immune-modulating functions influencing the susceptibility of a host to infectious disease, playing a role in bone formation due to hydroxylation of proline and lysine, and protecting the tissue from harmful free radicals [11] [12].

Fish oils are rich sources of the omega-3 polyunsaturated fatty acids (PUFA) eicosapentaenoic (EPA) acid and dexahexaenoic acid (DHA) that are distributed in almost all body cells, thus impacting cell functions, cell communication, production of various biomolecules and antioxidant activities [13]. Furthermore, decreased bone resorption or enhanced bone formation may be a consequence of the dual bone-sparing effect of omega-3 [14].

Calcium (Ca) is one of the most important minerals for bone health. The size of calcium reserve was affected by dietary calcium through the mobilisation necessary to maintain a normal blood calcium level. However, it never impairs those cellular functions. The current suggested daily intake of calcium is 1200 mg for adults [15]. It has been established that the osseointegration of dental implants was improved by the local delivery of calcium, in the form of hydroxyapatite [16]. A beneficial interaction between calcium and omega 3 FAs is plausible based on work done mainly in animal and *in vitro* models suggesting up-regulation of duodenal calcium absorption and decreased calcium excretion with the treatment of omega 3 FAs [17] [18].

The present study attempted to evaluate the radio-densitometric effect of LLLT on the osseointegration of immediately loaded dental implants in patients under vitamin C, omega-3 and calcium therapy.

Subjects and Methods

Sample size calculation was determined to detect an expected difference between laser and non-laser group of bone density changes from baseline to 6 months about 7 ± 3.2 [19]. Using power 95% and 5% significance level, 7 patients were required in each group. Recruitment of 10 participants per group was done to account for possible losses. Sample size calculation was achieved using PS: Power and Sample Size Calculation software Version 3.1.2 (Vanderbilt University, Nashville, Tennessee, USA).

The present study was conducted on twenty patients with age ranging from 30-40 years old, who were selected from the outpatient clinic of the National Research Centre, Cairo, Egypt in the period between 2017 and 2018. The study has been carried out by The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. All patients signed an informed consent before enrollment, and the Ethical Committee of the National Research Centre approved the protocol. Inclusion criteria were patients with good oral hygiene having a missing tooth in the mandibular first molar region with the adjacent teeth free from peri-apical pathology, and sufficient bone volume in the receptor site to accommodate for implant length and diameter. Patients who were smokers, alcoholic or drug abusers, suffering from bruxism, having a history of jaw irradiation or exhibited signs and symptoms of any systemic diseases that could influence the outcome of the therapy, were excluded.

A complete medical and dental history together with a preoperative panoramic X-ray was taken for each patient. A detailed oral and general examinations and thorough scaling and root planing were done for all selected patients. Patients were instructed to use Hexitol (Chlorhexidine HCL, The Arab Drug Company, A.R.E) mouthwash twice daily and take Augmentin (Amoxicillin 875 mg and clavulanic acid 125 mg, GlaxoSmithKline, A.R.E.) 1 gm tablet one hour preoperatively. All patients were instructed to take 500 mg tablet of vitamin C (C-Retard 500 mg, Hikma pharma S.A.E., 6th of October City - Egypt) once daily, 500 mg tablet of calcium (Bone-Cal, Amoun Pharmaceutical Co. S.A.E El-Obour City, Cairo, Egypt.) twice daily and 1000 mg tablet of omega 3 (Omega-300, The Arab Co. For Gelatin and Pharmaceutical Products for MONTANA PHARMACEUTICAL) once daily starting one month preoperatively.

Endosseous root form dental implants (Dentium, made in Korea) were used in the present study. All implants used were of length ranged from 10-12 mm, and diameter ranged from 4-4.5 mm. One implant was placed in each patient. Patients were divided into two groups, in the non-laser group; the healing phase was left to progress spontaneously without any intervention, while in the laser group healing phase was augmented with LLLT.

A gingival incision was performed through interdental papillae of the teeth on both sides of the edentulous area and connected by a crestal incision deep into the alveolar bone. The flap was then elevated buccally and slightly lingual, and a trephine bur was used to penetrate the alveolar crest.

Drilling was accompanied with copious saline irrigation, and enlarged sequentially by a series of gradually increasing drills, to a dimension just smaller than the implant diameter. The implant was then inserted in the bone by hand driven screw tightened with a ratchet wrench. Before wound closure, a temporary abutment, adjusted to the desired height,

was secured onto the implant.

The surgical wound was irrigated with sterile saline then the flap was repositioned back and sutured. A temporary crown was made, trimmed, smoothened, polished and temporarily cemented to the secured abutment. The temporary crown was adjusted to be out of occlusion with the opposing maxillary teeth. All immediately loaded implants had implant insertion torque of 35 N-cm.

After removing the temporary crowns and freeing the abutments, the final abutment was screwed, and the prosthetic part (porcelain-fused-to-metal) was fabricated (five months postoperatively).

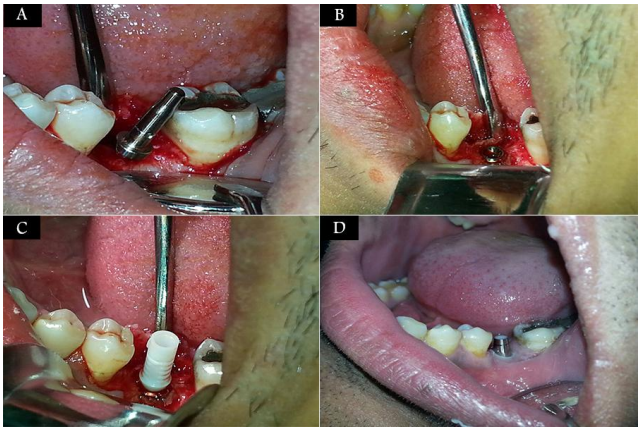

Figure 1: A) A photograph showing flap elevation and the parallel pin inside the implant bed; B) A photograph showing complete seating of the implant at the alveolar crest; C) A photograph showing seating the temporary abutment into the implant; D) A photograph showing screwing the final abutment into the implant

A 904 nm Gallium-Arsenide diode laser device (OPTODAN, Saratovskaja provinces, Saratov, Russia) was used in the present study. Implant site in the laser group was irradiated using contact mode, continuous wave, 20 mW output power, spot diameter 4mm and exposure time 30 sec [20] with a dose 4.7 J/cm^2 [21].

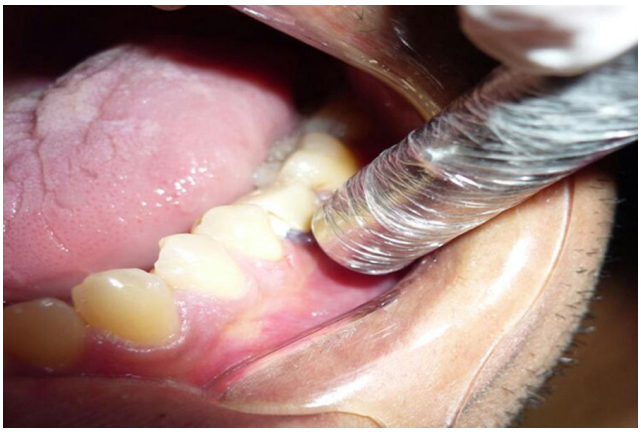

Figure 2: A photograph showing laser application at the implant site

The laser probe was directed towards the implant site, gently touching the tissues, and moving in a continuous slow circular motion to assure full exposure of the target surface to the laser beam. The patients were subjected to 9 sessions during the first week postoperatively (on 2nd, 4th and 6th days), three sessions per day with (1-hour) rest period in-between each session.

Baseline digital panoramic radiographs were taken postoperatively in the same day of surgery, 1.5 months postoperatively and the final digital panoramic radiographs were taken 6 months postoperatively.

Radio-densitometric evaluations were done using the Digora software system, around the mesial, distal and apical surfaces of the implant in both groups. The bone density was measured using grayscale value.

The peri-implant densitometric measurements were performed as follows: Three lines were drawn mesial, distal and apical to the implant. The first line extended mesially from the first thread of the implant to the apex of the implant passing just tangential to the threads, the second line extended distally from the first thread of the implant to the apex of the implant passing just tangential to the threads and the third line extended apically from the mesial aspect of the implant to the distal aspect of the implant.

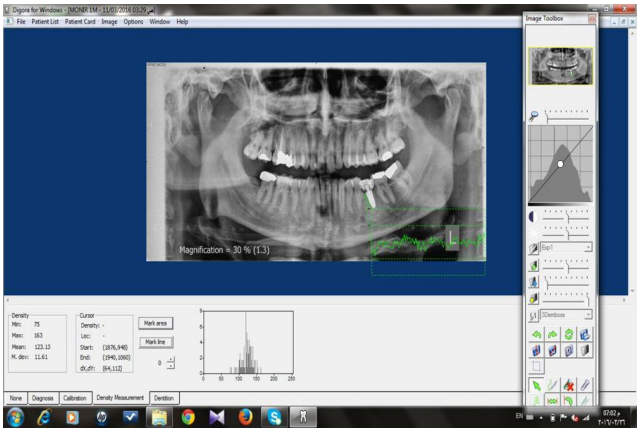

Figure 3: Measurement of bone density using Digora software

Numerical data were presented as mean and standard deviation (SD) values. Data were explored for normality using the Kolmogorov-Smirnov test of normality, variables were found to be normally distributed. Independent student t-test was used to compare means of variables between the laser and the non-laser group. Repeated measures ANOVA was used to compare bone densities at different times for the same group. Least significant difference (LSD) test was used as a post-hoc test to detect the follow-up time responsible for significance. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM SPSS software 18.0, Chicago, IL, USA [22].

Results

As regards the changes of the mean bone density per time, the calculation of the repeated measures ANOVA revealed statistically significant values with time. This increase was seen in all three zones and both laser and non-laser groups.

Table 1: Repeated measures ANOVA comparing mean bone densities at different times for the non-laser group

	Time			
	Immediate	1.5 months	6 months	P-value
Bone densities(mesial)	131.24 ± 10.99[a]	138.18 ± 10.26[b]	145.45 ± 11.44[c]	0.0001*
Bone densities (distal)	127.08 ± 18.60[a]	132.06 ± 19.57[b]	137.86 ± 18.81[c]	0.0001*
Bone densities (apical)	129.01 ± 18.09 [a]	134.67 ± 17.91[b]	139.40 ± 18.83[c]	0.0001*

*Statistically significant difference, p-value ≤0.05. [a, b & c] Different small letters indicate significant differences between the two follow-up times.

Immediate post-operatively, the mean and standard deviation values of bone density in the laser group were 131.85 ± 13.95 and were 131.24 ± 10.99 in the non-laser group. While after 1.5 months, the mean and standard deviation values of bone density in the laser group were 143.45 ± 16.68 and were 138.18 ± 10.26 in the non-laser group. Moreover, after 6 months the mean and standard deviation values of bone density were 153.84 ± 16.41 in the laser group and 145.45 ± 11.44 in the non-laser group.

Table 2: Repeated measures ANOVA comparing bone densities at different times for the laser group

	Time			
	Immediate	1.5 months	6 months	P-value
Bone densities(mesial)	131.85 ± 13.95[a]	143.45 ± 16.68[b]	153.84 ± 16.41[c]	0.0001*
Bone densities (distal)	129.02 ± 9.29[a]	139.53 ± 11.43[b]	150.76 ± 9.86[c]	0.0001*
Bone densities (apical)	130.19 ± 6.58[a]	142.76 ± 5.96[b]	149.09 ± 5.27[c]	0.0001*

*Statistically significant difference, p-value ≤0.05. [a, b & c] Different small letters indicate significant differences between the two follow-up times.

Immediate post-operatively the mean and standard deviation values of bone density in the laser group were 129.02 ± 9.29 and were 127.08 ± 18.60 in the non-laser group. While after 1.5 months, the mean and standard deviation values of bone density in the laser group were 139.53 ± 11.43 and 132.06 ± 19.57 in the non-laser group. Moreover, after 6 months the mean and standard deviation values of bone were 150.76 ± 9.86 in the laser group and 137.86 ± 18.81 in the non-laser group.

Immediate post-operatively the mean and standard deviation values of bone density in the laser group were 130.19 ± 6.58 and were 129.01 ± 18.09 in the non-laser group. After 1.5 months, the mean and standard deviation values of bone density in the laser group were 142.76 ± 5.96 and were 134.67 ± 17.91 in the non-laser group. Moreover, after 6 months the mean and standard deviation values of bone 149.09 ± 5.27 in the laser group and were 139.40 ± 18.83 in the non-laser group.

Results of Independent student t-test comparing the mean differences of both groups revealed statistically significant differences at the three zones during all the follow-up periods (being higher in the laser group).

There were significant differences in the mean bone density values at the mesial, distal and apical sides when the baseline mean bone density values were compared to values of the first follow-up (after 1.5 months), where at the mesial side the mean difference was 11.59 ± 4.87 in the laser group and was 6.94 ± 3.99 in the non-laser group, while it read 10.51 ± 4.33 in the laser group and 4.98 ± 4.67 in non-laser group at the distal side, and it was 12.57 ± 6.23 in the laser group and was 5.66 ± 2.87 in non-laser group at the apical side. Significance was recorded at P = 0.031, 0.013, and 0.007 at the mesial, distal and apical sides respectively.

Table 3: Comparison of the mean difference in bone density between times at different sites between the 2 groups (change by time in bone densities)

Time change		Group		
		Laser Mean ± SD	Non-laser Mean ± SD	p-value
Immediate-1.5 months	Mesial	11.59 ± 4.87	6.94 ± 3.99	0.031*
	Distal	10.51 ± 4.33	4.98 ± 4.67	0.013*
	Apical	12.57 ± 6.23	5.66 ± 2.87	0.007*
Immediate-6 months	Mesial	21.99 ± 5.48	14.21 ± 4.95	0.004*
	Distal	21.74 ± 3.56	10.78 ± 3.90	0.0001*
	Apical	18.90 ± 5.91	10.39 ± 3.49	0.001*
1.5 months-6 months	Mesial	10.39 ± 1.86	7.27 ± 2.49	0.005*
	Distal	11.23 ± 4.37	5.80 ± 4.34	0.012*
	Apical	6.33 ± 1.67	4.74 ± 1.58	0.042*

*Statistically significant difference, P-value ≤ 0.05.

There were significant differences in the mean bone density values at the mesial, distal and apical sides when the first follow-up (after 1.5 months) mean bone density values were compared to the values of the second follow-up (after 6 months), where at the mesial side the mean difference was 10.39 ± 1.86 in the laser group and was 7.27 ± 2.49 in the non-laser group, while it read 11.23 ± 4.37 in the laser group and 5.80 ± 4.34 in non-laser group at the distal side and was 6.33 ± 1.67 in the laser group and 4.74 ± 1.58 in non-laser group at the apical side. Significance was recorded at P = 0.005, 0.012, and 0.042 at the mesial, distal and apical sides respectively.

There were significant differences in the mean bone density values at the mesial , distal and apical sides when the baseline mean bone density values were compared to the values of the second follow-up (after 6 months), where at the mesial side the mean difference was 21.99 ± 5.48 in the laser group and 14.21 ± 4.95 in the non-laser group, while it read 21.74 ± 3.56 in the laser group and 10.78 ± 3.90 in non-laser group at the distal side and was 18.90 ± 5.91 in the laser group and 10.39±3.49 in non-laser group at the apical side. Significance was recorded at P = 0.004, P = 0.0001, and 0.001 at the mesial, distal and apical sides respectively.

Discussion

The replacement of missing teeth using implants over classic prosthetic solutions has gained wide attraction because of superior functional and aesthetic acceptance and the fact that implants stimulate the alveolar bone and induce an increased density in response to functional loading [23].

Increased failure rates with implants placed in type IV bone have been reported. The mandibular first molar region that was used in the present study as a standard site for implant insertion is known to have a relatively lower bone quality and a higher failure rate compared to the anterior region. Its bone type is type IV and offers little cortex and minimal internal strength [24][25]. On the other hand, when immediate and early implant loading regimes are applied, higher failure rates seem to be present, as the early occlusal loading during healing may affect the potential of the newly formed bone to repair the zone of damaged bone at the implant-bone interface [26].

Because the present study aimed to evaluate the effect of low-level laser on osseointegration and to decrease the failure possibilities of immediately loaded implants in bone type IV, both laser and non-laser groups were given omega-3, vitamin C and calcium.

In the current study, low-intensity gallium arsenide laser with wavelength 904nm was used as a regenerative approach to enhance osseointegration and increase the density of bone surrounding the implants. Although many researchers investigated the effect of LLLT in bone tissue in various branches of medicine and dentistry, with wavelengths varying from 670 to 1,064 nm, there are few studies on the use of 904 nm laser on bone tissue. The frequently used lasers are 670, 690, 780, 830, and 1,064 nm [27] [28].

In previous studies, the authors recommended the application of the 904 nm infrared laser on bone tissue. The wavelength 904 nm, which is emitted in the near-infrared region, had a low absorption coefficient and hence, better penetration potential into the tissue, thus raising the resistance and improving bone mineralisation [29] [30].

Gallium arsenide laser of wavelength 904 nm in a continuous mode with adjusted power of 0.02 W and a 30 second exposure time in nine sessions on three alternate days starting from the second postoperative day was used in the present study which was in accordance to a previous study observing the strongest bio modulatory effects at exposure time ranging from 30 to 120 seconds [20].

The energy density used in the current study was 4.7 J/cm^2 based on previous research where the authors evaluated the action of laser therapy (830 nm) on the repair of bone defects in rat models histologically. The results concluded a more enhanced repair in the irradiated group with the improved bone formation and collagen fibres around the graft inside the cavity from the 15th day after surgery [21].

It was reported that laser irradiation of bone stimulates the proliferation of fibroblastic, osteoblastic and mesenchymal cells in their early phase. Immediately after injury, the bone repair process starts in the vascularized regions in tissue anoxia and is accelerated by the stimulatory effect of laser on bone matrix [29]. It was demonstrated that the duration of the positive effect of LLLT is not longer than 1 week postoperatively, which is in agreement with the results of the present study [31].

The three bony zones surrounding the implants revealed a statistically significant cumulative effect in bone density of both groups per time (Table 1 and 2). This effect is assumed to be as a result of the cumulative effect of the drugs that started one month preoperatively, providing a circulating reservoir of micronutrients and minerals essential for the bone integrity and health and this was in accordance to previous studies that established the benefits of these drugs on the bone around implants [16].

Moreover, this was in line with a study performed on white New Zealand rabbits using implants coated with eicosapentaenoic acid that was shown to enhance osteoconduction and anchorage of the implant to the surrounding bone [14]. Also, it was in line with a study performed by Park et al., 2007, where the results concluded that Ca and vitamin D supplementation promoted bone healing around dental implants [32].

Although both laser and non-laser groups revealed a statistically significant increase in mean bone density of the three zones during all the follow-up periods, the rate of increase was significantly higher in the laser group where this increase started earlier and was sustained in the three zones in the laser group when compared to the slower and more delayed increase in bone density in the non-laser group (Table 3). This effect might be due to laser provided angiogenesis, improved vascularisation and perfusion that facilitated the presence of high levels of such micronutrients and minerals in the wounded area, with a subsequent increase in mineral deposition and bone density during a relatively short period.

These findings are in line with a previous study who reported that bone formation and maturation around the implants were enhanced by the use of low-level laser [33]. Furthermore, it was concluded that cellular proliferation, bone nodule formation and alkaline phosphatase (ALP) activity were improved by the application of LLLT [34] [35]. LLLT was proven to enhance the functional attachment of titanium implants to bone and improves bone healing and mineralisation [36] [37] [38].

In conclusion, the low-intensity laser

irradiation significantly promoted bone healing and speeded up the osseointegration process surrounding immediately loaded titanium implants emphasising the laser's bio stimulatory effect.

Acknowledgements

The study was done for partial fulfilment of the requirements of PhD and was partly funded by the National Research Centre. All authors have made a substantive contribution to this study, and all have reviewed the final paper before its submission.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1475-1479.
https://doi.org/10.3889/oamjms.2018.293
eISSN: 1857-9655
Dental Science

In Vivo Comparative Evaluation of Periapical Healing in Response to a Calcium Silicate and Calcium Hydroxide Based Endodontic Sealers

Dalia Y. Zaki, Mohamed H. Zaazou, Maram E. Khallaf, Tamer M. Hamdy*

Restorative and Dental Materials Department, National Research Centre (NRC), El Bohouth St., 12622 Dokki, Giza, Egypt

Citation: Zaki DY, Zaazou MH, Khallaf ME, Hamdy TM. In Vivo Comparative Evaluation of Periapical Healing in Response to a Calcium Silicate and Calcium Hydroxide Based Endodontic Sealers. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1475-1479. https://doi.org/10.3889/oamjms.2018.293

Keywords: Bioceramic sealer; Calcium hydroxide; Root canal sealer

***Correspondence:** Tamer M Hamdy. Restorative and Dental Materials Department, National Research Centre (NRC), El Bohouth St., 12622 Dokki, Giza, Egypt. E-mail: dr_tamer_hamdy@yahoo.com

Received: 07-May-2018; **Revised:** 25-Jun-2018; **Accepted:** 03-Jul-2018; **Online first:** 15-Aug-2018

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: The composition of the root canal filling materials together with the apical limit of the root canal obturation affect the complete periapical healing after root canal therapy.

AIM: This study was performed to evaluate and compare the periapical healing in response to calcium-silicate (iRoot SP) and calcium-hydroxide (Apexit) based-sealers.

MATERIAL AND METHODS: Seventy-two upper premolars root canals of six dogs were used. The teeth were randomly assigned to four groups: Group one: roots were obturated using gutta-percha and Apexit-sealer; Group two: roots were obturated using gutta-percha&iRoot SP-sealer; Group three: the teeth were left open without obturation; Group four: where healthy teeth were used as a negative control. Teeth were evaluated after one, two and three months. The newly formed mineralised apical tissue and the periapical inflammatory infiltrate of the obtained photomicrographs were evaluated, and scorings were statistically-analysed.

RESULTS: The mean percentage of the periapical inflammatory infiltrates and mineralisation scoring after one, two and three months evaluation period were not significantly different among the four groups ($P > 0.05$).

CONCLUSIONS: Regardless of the sealer used, iRoot SP and Apexit promote healing of periapical tissues. IRoot SP sealer showed early insignificant more partial and almost full healing after two and three months.

Introduction

The main objective of endodontic therapy is to eliminate microorganisms, their products, and sub-products [1] [2]. Complete periapical healing after root canal therapy may be influenced by the apical limit of the root canal obturation and the composition of the filling material [3]. Root canal sealers used for the treatment of teeth with periapical lesions should be biologically compatible and allow for periapical healing due to their close contact with living periapical tissues over a long period [4]. Their biocompatibility may be responsible for completing the histologic repair of periapical tissues after root canal treatment, without triggering any adverse reactions, including, inflammation, carcinogenicity, toxicity or allergy [5].

Sealers currently being used in clinical practice include resin-based, zinc oxide-eugenol-based, glass ionomer-based, calcium hydroxide-based, and silicone-based endodontic sealers [6] [7]. iRoot SP (Innovative BioCreamix Inc, Vancouver, Canada) is a ready-to-use injectable white hydraulic cement paste developed for permanent root canal filling and sealing applications.

According to the manufacturer, iRoot SP is an aluminium-free, calcium silicate and resin-based material similar in composition to white MTA and is hydrophilic that utilises the water inherent in the dentinal tubules to drive the hydration reaction. The iRoot SP chemical composition includes zirconium oxide, calcium silicates, calcium phosphate, calcium hydroxide, filler, and thickening agents. It has both excellent physical properties and antimicrobial activity [8]. The calcium silicates content in the powder hydrate to produce a calcium silicate hydrate gel C-H-C and calcium hydroxide CH. The calcium hydroxide reacts with the phosphate ions in the dentinal fluid and

precipitates hydroxyapatite and water [9].

On the other hand; according to the manufacturer, Apexit (Ivoclar Vivadent, Schaan, Lichtenstein) is a two-component (base and activator) calcium hydroxide-based sealer. The base is formed mainly of calcium hydroxide/calcium oxide, hydrated collophonium, fillers and other auxiliary materials. The activator contains disalicylate, bismuth hydroxide/bismuth carbonate, fillers and other auxiliary materials. The material sets by complex formation. Apexit promotes hard tissue formation but tends to dissolve over time and may thus compromise the endodontic seal [10].

The aim of the present study was to in vivo to evaluate and compare the periapical healing in response to a calcium silicate based sealers (iRoot SP) and a calcium hydroxide-based sealer (Apexit).

Materials and Methods

All animal procedures in this study were performed according to the protocols reviewed and approved by the Ethical Committee of National Research Centre, Giza, Egypt in compliance with the applicable ethical guidelines and regulations of the international guiding principles for biomedical research involving animals.

Seventy-two root canals from the upper premolars of six dogs (ages 12-18 months and weight 8-15 kg) were used during this study. The animals were anaesthetized intravenously with sodium thiopental (30 mg/kg body weight, Thiopental eipico, Eipico, Egypt). Standardized periapical radiographs were taken for later comparison with that obtained after root canal treatment. The dental arch was isolated using a rubber dam and 2% chlorhexidine gluconate was used as an antiseptic agent. Access cavity was prepared, and pulp was removed.

The apical cementum layer characteristic of dogs' teeth was then perforated with the sequential use of size #15 to #30 K-files, thus creating standardised apical openings [11]. Root canals were left exposed to the oral cavity for 7 days to allow microbial contamination in the experimental groups. Access openings were then sealed using zinc oxide-eugenol-based temporary filling (Coltosol-Ident). After 45 days, the development of apical periodontitis was radiographically confirmed.

The root canals were instrumented to the working length up to a size #60 K-file. A size #30 K-file was taken to the total root length to ensure apical patency. After final irrigation with saline solution, the root canals were dried and then filled with 14.3% buffered EDTA, pH 7.4 (Meta BioMed Co., Chungbuk, Korea), for 3 minutes then rinsed using saline solution and dried.

The teeth were then randomly assigned to four groups as follows: group 1: (n = 24); roots were obturated using gutta-percha and Apexit sealer with lateral condensation technique; group 2: (n = 24) roots were obturated using gutta-percha and I-Root SP sealer, Group 3: (n = 12) the teeth were left open without obturation or coronal restoration (+ve control group) and Group 4: (n = 12) where healthy teeth were used as a -ve control samples for comparison with the findings obtained from the experimental groups. Amalgam restorative material was used for coronal restorations for all teeth. The dogs were fed a soft diet for 3 to 5 days after dental procedures.

Dogs were then divided into three groups (n = 2) according to the post evaluation periods (1 month, 2 months and 3 months). At the end of each evaluation period, standardised radiographs were taken to detect the presence or absences of periapical radiolucency then 2 dogs were sacrificed by the use of overdose of sodium thiopental. The mandibles were removed immediately by dissection of the surrounding soft tissues. The experimental teeth with the surrounding bone were sectioned using the electrical surgical saw. Block sections were fixed in 10% formalin and decalcified in EDTA.

Three sections from each block were cut 4-6 µm thick and stained with hematoxylin-eosin. The sections were evaluated by two blind evaluators at different magnifications using a CX21 Olympus microscope (Tokyo-Japan). The following parameters were evaluated and scored as follows: (a) newly formed mineralized apical tissue: absent (0), partial (1), almost complete (2) and complete (3); and (b) periapical inflammatory infiltrate: absent (0), mild (1), moderate (2), and severe (3).

A non-parametric one-way ANOVA (Kruskal–Wallis) test followed by paired group comparisons using Mann–Whitney U tests at a 5% significance level were used to analyse the effect of the two materials on the bone deposition (the mineralisation process) and inflammation together with the effect of time on the inflammation and mineralisation. Statistical analysis was performed with IBM® SPSS® Version 22 for Windows (SPSS Inc., IBM Corporation, NY, USA).

Results

Percentages of periapical inflammatory infiltrate and mineralisation scoring after 1, 2, 3 months evaluation period for the tested groups are listed in Table 1 and 2.

Table 1: Percentages of periapical inflammatory infiltrate scorings after 1, 2 and 3 months evaluation period for the tested groups

		Post-operative evaluation periods			
		1 Month	2 Months	3 Months	
		Scoring %			p-value
-ve control	0	100.0%	100.0%	100.0%	
	1	0.0%	0.0%	0.0%	
	2	0.0%	0.0%	0.0%	1.00 NS
	3	0.0%	0.0%	0.0%	
		a	a	a	
+ve control	0	0.0%	0.0%	0.0%	
	1	0.0%	0.0%	0.0%	
	2	100.0%	0.0%	0.0%	1.00 NS
	3	0.0%	100.0%	100.0%	
		c	c	c	
iRoot SP	0	60.0%	60.0%	60.0%	
	1	40.0%	40.0%	20.0%	
	2	0.0%	0.0%	20.0%	0.459 NS
	3	0.0%	0.0%	0.0%	
		b	b	b	
Apexit	0	20.0%	80.0%	40.0%	
	1	60.0%	20.0%	60.0%	
	2	20.0%	0.0%	0.0%	0.308 NS
	3	0.0%	0.0%	0.0%	
		b	b	b	
p- value		0.011*	0.006*	0.008*	

Mean scoring % with different lower case letters in the same column indicate statistically significant difference. *, significant (P < 0.05); ns, non-significant (p > 0.05); NS = Non-Significant.

Although a statistically significant difference was revealed between the control groups and the iRoot SP and Apexit sealer groups; no statistical differences were found between the two sealers regarding inflammatory response and mineralisation. However, results showed early signs of mild and moderate inflammatory infiltrate in Apexit sealer when compared with iRoot SP. After two and three months; iRoot SP sealers showed insignificant more partial and almost complete mineralisation scorings than Apexit type.

Table 2: Percentages of mineralisation scoring after 1, 2, 3 months evaluation period for the tested groups

		Post-operative evaluation periods			
		1 Month	2 Months	3 Months	
		Scoring %			p-value
-ve control	0	0.0%	0.0%	0.0%	
	1	0.0%	0.0%	0.0%	
	2	0.0%	0.0%	0.0%	1.00 NS
	3	100.0%	100.0%	100.0%	
		a	a	a	
+ve control	0	100.0%	100.0%	100.0%	
	1	0.0%	0.0%	0.0%	
	2	0.0%	0.0%	0.0%	1.00 NS
	3	0.0%	0.0%	0.0%	
		c	c	c	
iRoot SP	0	60.0%	40.0%	40.0%	
	1	40.0%	40.0%	20.0%	
	2	0.0%	20.0%	40.0%	0.545 NS
	3	0.0%	0.0%	0.0%	
		b	b	b	
Apexit	0	40.0%	60.0%	60.0%	
	1	60.0%	40.0%	40.0%	
	2	0.0%	0.0%	0.0%	0.779 NS
	3	0.0%	0.0%	0.0%	
		b	b	b	
p- value		0.003*	0.014*	0.006*	

Mean scoring % with different lower case letters in the same column indicate statistically significant difference. *, significant (P < 0.05); ns, non-significant (P > 0.05); NS = Non-Significant.

Representative photomicrography of iRoot SP, Apexit and the control groups' specimens after 1, 2 and 3 months evaluation periods are shown in Figures 1 and 2. For iRoot SP sealed specimen; one month evaluation period showed healing with connective tissue (C.T.) with no signs of bone formation (Figure 1A); scattered osteocytes appeared after 2 months evaluation period (Figure 1B); while new bone formation foci appeared after 3 months (Figure 1C).

On the other hand, the photomicrographs of Apexit sealed specimen showed moderate inflammatory areas and moderate angiogenesis with some calcified foci after one month evaluation period (Figure 2A). New blood vessels formation with mild mineralisation and mild inflammation appeared after 2 months (Figure 2B); while new bone formation and periodontal ligament regeneration appeared after 3 months (Figure 2C).

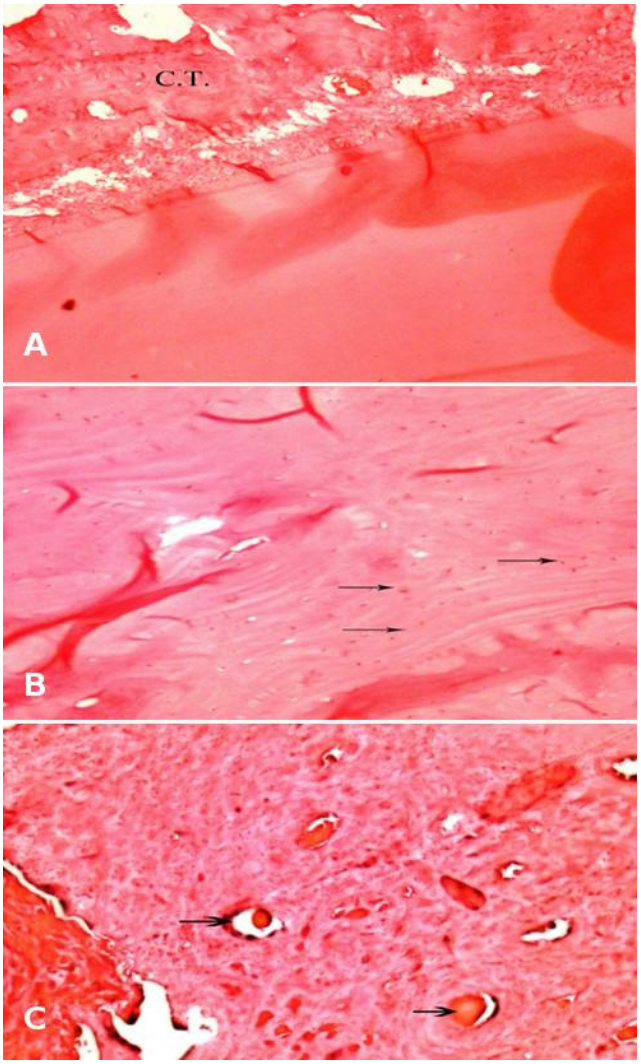

Figure 1: Photomicrograph of representative HE-stained microscopic sections in specimen filled with iRoot SP sealer after: (A) one month, showing healing with connective tissue (C.T.) with no signs of bone formation (x100); (B) two months showing bone formation appeared as scattered osteocytes (arrows) (x100) and (C) three months, showing new bone formation foci with no signs of inflammation (x200)

Discussion

Sealers are responsible for preventing reinfection and filling of irregularities in the prepared canal system. Root canal sealer should support and

accelerate the repair and the regenerative processes of the injured periradicular tissues [12].

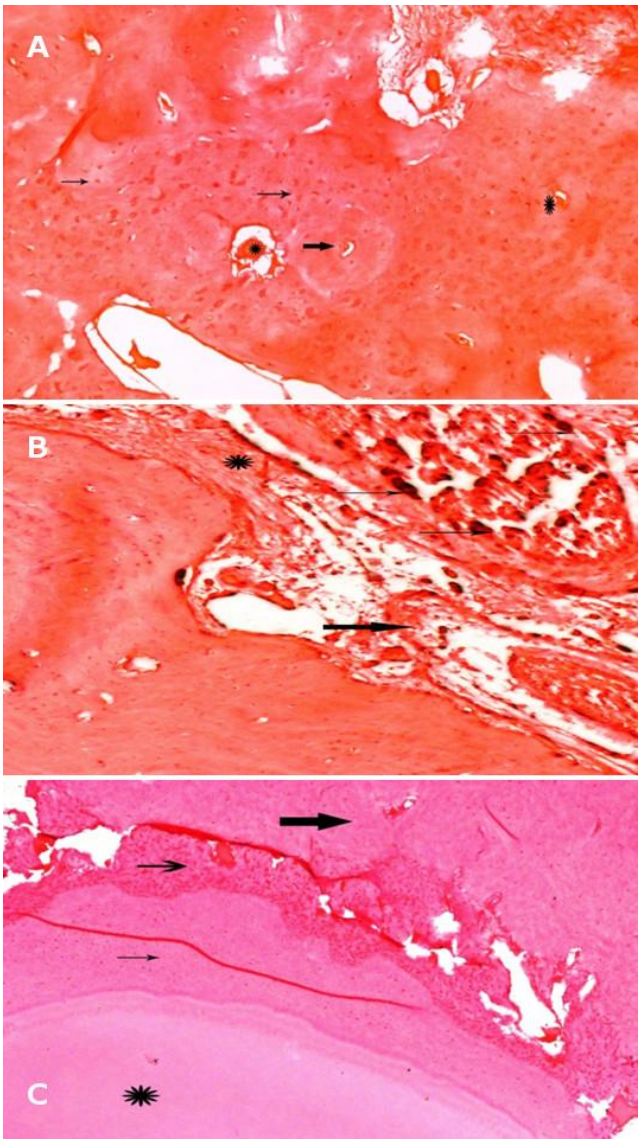

Figure 2: Photomicrograph of representative HE-stained microscopic sections in specimen filled with Apexit sealer after: (A) one month showing moderate inflammatory areas (thin arrows), moderate angiogenesis (thick arrows) with some calcified foci ( ) (x100); (B) two months showing new blood vessels formation with mild mineralization and mild inflammation (*) (x100) and (C) three months showing the root tip (*) with periodontal ligament regeneration (thin arrow) new bone formation (thick arrow) (x100)*

The experimental model of lesion induction used in this study was based on previously established criteria [12]. In this study, the apical cementum barrier of roots was perforated to obtain a patent foramen which is considered as an important step in foraminal debridement [14] [16].

Alkalinity and the ability of a material to release calcium ions were suggested to be responsible for stimulation of repair by deposition of mineralised tissue through the activation of alkaline phosphatase. Additionally, the released calcium ions extracellularly have been reported to induce BMP-2 expression. Calcium ions would also react with the carbonate ions present in the periapical tissue, leading to precipitation of calcite granules, which would trigger the process of deposition of mineralised tissue [17].

Calcium hydroxide-based sealers, are known for their remineralisation effect and antibacterial properties due to the release of hydroxyl ions. Some drawbacks such as poor cohesive strength, greater solubility, marginal leakage and concerns regarding weakening of roots; led to the search for newer calcium silicate based sealers with properties similar to MTA but with lower cost, shorter setting time and proper handling characteristics [18].

iRoot SP has been introduced for use as a bioactive, alkaline, injectable root canal sealer with certain antibacterial properties, of high toxicity when tested in cell culture study on L929 cells but with nontoxic extract [19]. The effect of iRoot SP on the viability of RAW 264.7 macrophages was also tested and proved to be non-toxic [20]. This study was directed to evaluate and compare the periapical healing in response to a calcium silicate based sealers (iRoot SP) with that of a calcium hydroxide-based sealer (Apexit).

It has been suggested that calcium oxide in calcium silicate based sealers reacts with the tissue fluids eventually producing calcium hydroxide. The produced calcium hydroxide would then dissociate into hydroxide and calcium ions causing an increase in the pH of the medium.

The insignificant difference between the tested sealers regarding the presence of inflammatory infiltrate and mineralisation of apical tissue; is probably due to the ability of both materials to release calcium and hydroxyl ions. However early signs of mild and moderate inflammatory infiltrate in case of Apexit sealer was detected. This may indicate the early release of hydroxyl ions; increasing alkalinity and stimulating inflammation in the surrounding tissue. In deeper areas of tissues, calcium hydroxide acts as a mild irritant, stimulating hard tissue formation [17]. Such finding was confirmed by the photomicrographs of Apexit where moderate inflammatory areas, moderate angiogenesis and some calcified foci were detected as early as one month evaluation period. Calcium hydroxide based formulations are known for their initial degenerative response followed by rapid mineralisation and ossification [21] [22].

The early and insignificant more partial and almost complete healing after two and three months revealed by iRoot SP than Apexit type may be the result of the hydrophilicity of the iRoot SP and the presence of nanoparticles providing a homogenous mixture, high solubility, and good flow characteristics. Diffusion of calcium and hydroxyl ions from a material depends on such characteristics. Such finding is in agreement with Chetna Dudeja et al., [23], where

teeth filled with iRoot SP showed higher pH and calcium ion release when compared with a calcium hydroxide based sealer (Ultracal).

Further studies comparing cohesive strength, marginal leakage and roots weakening the effect of both materials are recommended to conclude the clinical efficiency of both materials.

In conclusion, based on the results above, it could be concluded that the investigated sealers promote healing of periapical tissues. IRoot SP sealer showed early insignificant more partial and almost complete healing after two and three months.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1480-1485.
https://doi.org/10.3889/oamjms.2018.304
eISSN: 1857-9655
Dental Science

Children's Social Perception of Peers' Dento-Facial Condition: A Cross-Sectional Study

Sunil Babu Kotha[1*], Ayah AlMenawi[1], Reem Abdullah AlKhalaf[1], Alhanouf Khalid Binhezaim[2], Turki Hamdan AlHarbi[1]

[1]Preventive Dentistry, Riyadh Elm University (REU), Riyadh, Saudi Arabia; [2]Pediatric Dentistry, Armed Forces Hospital, Riyadh, Saudi Arabia

Citation: Kotha SB, AlMenawi A, AlKhalaf RA, Binhezaim AK, AlHarbi TH. Children's Social Perception of Peers' Dento-Facial Condition: A Cross-Sectional Study. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1480-1485. https://doi.org/10.3889/oamjms.2018.304

Keywords: Children perception; Social judgment; Visible dental trauma

***Correspondence:** Sunil Babu Kotha. Preventive dentistry, Riyadh Elm University (REU), Riyadh, Saudi Arabia. E-mail: sunil.babu1606@gmail.com

Received: 23-May-2018; **Revised:** 26-Jul-2018; **Accepted:** 28-Jul-2018; **Online first:** 15-Aug-2018

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

AIMS: This study aimed to compare the participant's perceptions about their peers' dento-facial condition with different incisal appearances (intact, discoloured, fractured, and avulsed incisors).

MATERIALS AND METHODS: A cross-sectional study was conducted among schoolchildren of both primary (8-11 years) and secondary (12-14 years) levels. Each participant was asked to judge photographs with one intact and three digitally modified central incisors giving the appearance of a traumatised tooth. Data on perceptions were collected for each condition using 12 attributes (8 positive and 4 negative; scored on a 4 point Likert scale). The positive, negative and total attribute scores were analysed separately by unpaired Student's t-test. Repeated Measures ANOVA and Bonferroni post hoc analysis was also used.

RESULTS: A group of 587 children participated in the study. The perception for intact and traumatised incisors about demographic factors is well appreciated. Among the gender delineation, girls showed a significant difference in judgment between discoloured and fractured incisors. In comparison to intact incisors, positive and total attributable scores were found to be significantly higher ($P < 0.001$), whereas negative attribute scores were significantly lower ($P < 0.001$) for traumatised incisors. Pairwise comparison showed high significance ($p < 0.001$) between the intact and traumatised incisor conditions.

CONCLUSION: The results demonstrated that visible dental trauma influenced the psychosocial judgment given by children towards their peers. This judgment would, in turn, affect their level of acceptance towards such appearances. Therefore, these conditions ought to be redressed as swiftly as possible.

CLINICAL SIGNIFICANCE: The primary purpose of this study was to highlight the psychosocial perceptions of children in judging their peers, regarding not only attractiveness but also intelligence, friendliness, confidence, outgoing nature, etc.

Introduction

The human face nowadays is drawing attention by playing a very significant role in judging a person for their interpersonal relation [1] [2]. It is safe to assume that the appearance of teeth in judging a person plays a major role. Teeth not only help in functional fulfilment like chewing and speech but also plays a very important role in esthetics regarding attractiveness, which creates a positive impression for the individual who is interacting. Any disturbance in these anterior teeth due to dental trauma affects not only the functional ability but also the self-esteem of the child [3] [4] [5] [6].

During social judgments in face-to-face situations, the eyes and the area of the mouth are most frequently observed [7]. People judged as more enticing, are considered to be more successful and have greater self-esteem than less enticing people [8][9]. This oral appearance, marred by unsightly oral trauma by a child is psychologically influenced by the social judgments made by their peers [10]. Thus, the smile is the most important aspect for a casting better impression. This proposes the difficulty for a child with unappealing teeth to smile [11] [12].

Anterior dental trauma in very young individuals between 2-3 years is mainly due to accidents as their motor coordination is developing, and later during 6-12 years involving the permanent

anterior teeth leading to instability in their functions, aesthetics and most importantly their psychology [13]. The prevalence of dental trauma to permanent incisors is stated to be high worldwide, 15-23% among American teenagers [14], 23-35% among European [15], while in Asian juveniles it was reported to occur in a range of 4-35% [16]. Moreover, the prevalence of dental trauma in Saudi Arabia is reported to be 33% among 5-6 years old boys, 34% among 12-14 years old boys [17], and 31% among 12-15 years old girls [18].

The present paper encompasses the evaluation of children's social perception of their peers' dento-facial condition.

The present study was carried out to compare children's perceptions about their peer's dento-facial condition (visible incisor condition) by using the digitally modified photographs of a single child and to investigate the effect of gender, level of education and nationality on their perception.

The prespecified hypothesis of the present project is to extend previous research by testing the hypothesis that visible dental trauma influences the children in judging their peers and in turn affect their level of acceptance.

Materials and Methods

Riyadh being the capital city of Saudi Arabia has a population from various origins found to be the appropriate site for doing a study on the perception of peers' dento-facial condition.

The participants were divided into two groups.

Group 1: Primary school (8-11 years)

Group 2: Secondary school (12-14 years)

The study sample size of 579 participants was calculated by using a level of precision formula $n = (z_\alpha+z_\beta)^2\ pq/d^2$ (z_α:1.96; z_β:0.82; p:25%; q:75% and d:5%) . Sample size estimation was done a setting power of 80% with a confidence interval of 95%. Simple random sampling was employed for selection of participants. The estimated required sample size of 587 participants was overshot during data collection to further warrant for dropouts owing to incomplete questionnaires.

To obtain the appropriate sample, schools in Riyadh city were divided into three sections: north, south-east and south-west. The process of cluster sampling was used in which each unit is having more than 300 school children were listed according to three sections followed by a table of random allocation used to select 40 schools randomly. Since schools in the north were not as highly populated as, south-east and south-west, the chosen sampling ratio for the schools was 1:2:2; for every school selected from the north, two schools were selected from south-east and south-west. It was planned that approximately 20 (10 each from primary and secondary school) children were examined in each school, giving a predicted sample of 800. Though our estimated size of sample required for our study (as per the equation) was only around 580, we have taken more children (800) expecting some dropouts.

After the Research board, Riyadh Elm University, Riyadh, KSA approved the study, permission was obtained from the Ministry of Education to conduct the survey in the specified schools.

The investigators explained to the children the purpose of the survey and assured them of confidentiality.

Standardized full-face with visible sound teeth colour photographs (A5 portrait) of a primary school and secondary school child each, were taken. Each of these photographs was digitally manipulated using Adobe Photoshop software to include different trauma conditions, i.e. crown fractures, avulsion and non-vital discoloured incisor (Figure 1). A digitally manipulated approach was adopted to prevent the potential confounding effects of a different smile or hairstyle on an overall appearance.

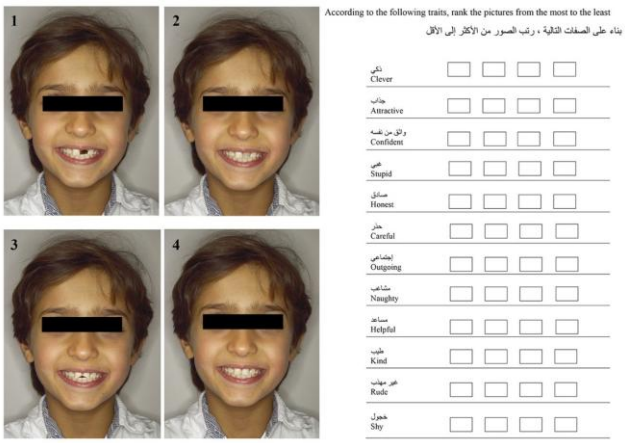

Figure 1: Full face photographs of the different incisal conditions in a single child

Consent was taken from the two volunteers' parents to permit the utilisation of their photographs with digital manipulation for the specific purposes of this study. The specific aim of the study was carefully concealed from the participants during the entire data collecting process.

The survey comprised a two-part self-administered questionnaire. The first section involved demographic data about nationality, age, gender and level of education. The second section included the 4 pictures (intact incisors, fractured, discoloured and avulsed incisor) of the same child and 12 modified descriptors (eight positive and four negative attributes)

included in the validated questionnaire [19] [20].

To avoid an inappropriate scoring by the children who were not well acquainted with English, an English-Arabic version of the questionnaire was formulated. The concept of translation was to obtain an instrument with conceptual equivalence in a different cultural group. The original English questionnaire was translated into the Arabic language by a bilingual native Arabic speaker and after that blindly back-translated by another bilingual native Arabic speaker. Through these rigorous cycles of translation and back translation, it was confirmed that the original meaning of the questionnaire was maintained. A pilot study was carried out using this bilingual instrument with children to ensure equivalence, clarity and comprehension.

Consent was obtained from all participants' parents/guardians one week before the questionnaires were distributed; also, children were given an option not to participate.

The children who participated in this study were schoolchildren of both primary (8-11 years) and secondary (12-14 years) levels who were mentally and physically fit and with the absence of any obvious dental or facial anomalies.

Participants were initially told that the researcher was assessing "the way we look at other people". This mild deception was deemed appropriate to mask the true intention of the research. A brief description of the questionnaire was given to the children to make them understand how to fill out the survey forms. Each participant was asked to judge the personal characteristics of a single subject's photograph with intact and three traumatic conditions (i.e., discolouration, fracture and avulsion) and rank them based on the attributes (8 positives, 4 negative) given in the validated questionnaire. Care was taken not to reveal the fact that how their judgment was going to be assessed later on. Once the questionnaires were distributed among the children, about 15 minutes were allotted to complete them.

Questionnaires with more than 30% of missing data were not considered and the ones with less than 30% missing data, the median value for the individual attribute (from analysis of the whole data set) were replaced for the missing values [19]

The primary outcome was to analyse positive and negative attributes separately and by summing up to examine the aggregate score. The picture, which was ranked one, was given a score of four and the ranking of 4 was given the score of one. Consequently, the positive attribute score (PAS) ranged between 32 (most positive) to 8 (least positive) and the negative attribute score (NAS) ranged between 16 (most negative) to 4 (least negative). The total attribute score (TAS) was also calculated by adding up the scores in which the negative attributes were scored in reverse showing the TAS range from 36 (maximum) to 12 (minimum).

The data acquired was deemed appropriate to carry out the parametric tests since the results were normally distributed. The use of unpaired t-test, repeated measure ANOVA and post hoc analysis using Bonferroni adjustment with the help of the Statistical Software SPSS 24.0 was carried out on the data collected from 587 children. Each subject was a unit of analysis.

A repeated-measures ANOVA placed each on an equal footing in the partitioning of the total variation showing a significant difference between trauma groups ($P < 0.001$). Post hoc analysis using Bonferroni adjustment analysis for pairwise comparisons revealed that children displaying normal intact incisors gained the most favourable ratings compared to the ones with any of the traumatised conditions of the incisors.

Results

Figure 2 summarised the details of expected and the final number of participants in the study. In spite of having the potential number of participants, there was a slight lowering due to various reasons. In primary school children, though the explanation was given on a one-to-one basis, some of them were not able to complete the questionnaire (27.7%). This might have been due to the fatigue involved in correlating and ranking pictures at this primary school level. In contrast to this, the secondary school children had only 9 improper entries.

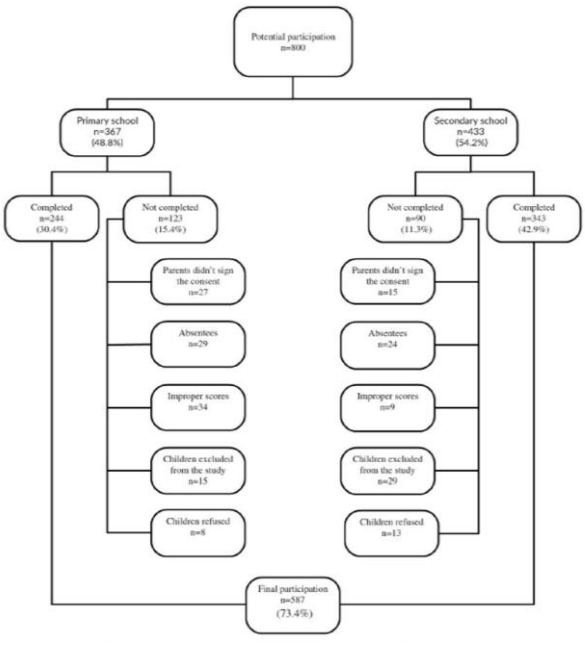

Figure 2: Flowchart outlining the number of participants in the study

Table 1 summarised the distribution and perception of scores about the visible dental condition and the demographic variables. Overall, the demographics of gender, level of education and nationality showed a significant perception difference between intact and traumatised conditions.

Table 1: Distribution and Perceptions scale score variation of incisal condition concerning the demographic factors.

S. No	Demographic Factors	Groups	Total Number (n= 587) with distribution	Dento-Facial Condition					
				Avulsion (1) Mean±SD	Discolouration (2) Mean±SD	Fracture (3) Mean±SD	Intact (4) Mean±SD	p-value[A]	Post hoc[P]
2	Gender	Boys	368 (62.7)	28.16±4.46	29.70±3.17	29.49±3.49	32.57±3.86	<0.001	4>3=2>1
		Girls	219 (37.3)	26.38±4.06	28.8±3.51	30.80±3.13	34.03±4.23	<0.001	4>3>2>1
		p-value[T]		<0.001	<0.001	<0.001	<0.001		
3	School grade	Primary	244 (41.6)	27.97±4.4	28.89±3.6	29.8±3.4	33.0±3.8	<0.001	4>3=2=1
		Secondary	343 (58.4)	27.16±4.3	29.71±3.0	30.1±3.4	32.98±4.2	<0.001	4>3=2>1
		p-value[T]		0.029	0.003	0.29	0.359		
4	Nationality	Arabs	419 (71.4)	27.85±4.45	29.43±3.39	29.99±3.5	32.67±4.02	<0.001	4>3=2>1
		Non-Arabs	168 (28.6)	26.61±4.16	29.19±3.15	29.96±3.1	34.21±3.96	<0.001	4>3=2>1
		p-value[T]		0.002	0.423	0.945	<0.001		

T- Unpaired t-test; A - Repeated Measure ANOVA; P - Bonferroni Post hoc analysis.

Girls showed a significant perception difference between all the 4 groups of the photographs with specific importance between discoloured and fractured teeth (Fig. 3). Primary school children showed no specific inter-differentiation between the 3 trauma conditions. Secondary school children, irrespective of gender or nationality, showed a specific differentiation between avulsed and discoloured incisors with a negative influence towards avulsed teeth and so the same within the nationality (Fig. 3).

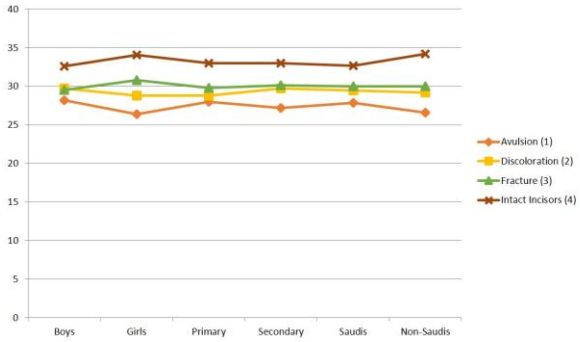

Figure 3: Variation of incisal condition concerning the demographic factors

Table 2 showed that PAS and TAS were significantly varying among the traumatic conditions with intact incisor condition showing significantly higher positive scores compared to others.

Table 2: Perceptions scale positive, negative and total score variation about different clinical situations using Repeated Measures ANOVA with Bonferroni post hoc analysis[*] for pairwise comparisons

Score variation	Situation	Avulsion (1)	Discoloration (2)	Fracture (3)	Intact (4)	df	F	p value[A]	Post hoc[P]
Positive	Mean ± SD	15.45±4.655	19.33±3.693	19.23±3.279	25.95±4.495	3	507.458	<0.0001	1<2=3<4
Negative	Mean ± SD	12.04±2.215	10.03±1.944	10.75±1.966	7.16±2.277	3	425.383	<0.0001	1>3>2>4
Total	Mean ± SD	27.50±4.404	29.36±3.328	29.98±3.421	33.11±4.068	3	164.189	<0.0001	1<2<3<4

A - Repeated Measure ANOVA; P - Bonferroni Post hoc analysis.

Post hoc analysis showed that PAS significantly differed between all groups except fractured and discoloured conditions. NAS was also significantly varying among the traumatic conditions with intact incisor condition by showing significantly lesser negative scores compared to others (Fig. 4).

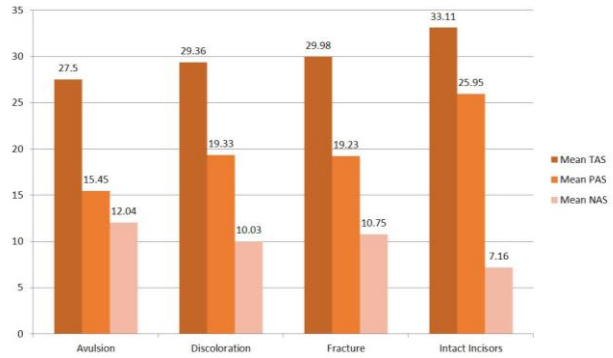

Figure 4: Variation of mean PAS, NAS and TAS perceptions in different clinical situations

Post hoc analysis showed that NAS significantly differ between individual conditions having least score for intact and the highest score for avulsion.

Discussion

Within the acknowledged restrictions of this study, the findings point out that children do make judgments about their peers on the premise of their dental appearance based on the condition of visible anterior teeth. To a certain extent, the outcome of the present study, as predicted, had shown that the negative social judgment to visible incisor defects among children was on par with past studies [19] [20] [21] [22] [23] [24]. The current research incorporated certain methodological changes, wherein defects like discolouration, fracture and avulsion were independently considered for comparison against a control group, unlike previous studies that focused on incorporating a generalised concept of clubbing different types of trauma and comparing them with the normal [20].

The study at hand showed that both primary and secondary school children gave negative judgments towards their peers who had an avulsed tooth, yet had no significantly different judgment for conditions of fractured or discoloured teeth. This could be attributed to the fact that the children were aware that the trauma of a tooth is common at this age and they deliberately chose not to make negative judgments for the same. The avulsion of a tooth, on the other hand, gives an unsightly or unnatural appearance of something missing and is probably why it showed statistical significance. Similar ideas were also proposed by previous studies [20] [21].

It was also seen among the secondary school children in this study that they possessed a more negative judgment towards a discoloured tooth than towards a fractured tooth, which was in concurrence with previous studies [25] [26]. According to Jean Piaget's Cognitive developmental theory [27], some children give negative judgments about visible dental trauma most likely because, during this adolescent age, children are more governed by self-perception. In contradiction, however, Grosofsky et al. [28] stated that tooth shade does not influence the perception of attractiveness.

Surprisingly in the present study, it was found that girls were significantly more negative in their social judgments in comparison with their male counterparts considering any of the traumatic condition; boys gave negative judgments only towards their peers having avulsed tooth. This finding opposes that of previous studies [19] [20] [29], where female adolescents were more positive than males in judging their peers with incisor trauma. However, these results cannot generalise these perceptions, as there are many other factors to be accounted for like the individual's beliefs, values, past dental experience, and their dental attractiveness.

Ethnicity, like in the previous studies [19] [30] [31], showed no significance in judgments towards visual dental trauma related.

It might be debated that photographs provide a judgment without the added parameters of voice, attire, direct view in person, etc., all of which could play an important role in one's perception of a person. On a related matter, certain previous studies comparing the judgment between static 2D images and dynamic video clips showed no significance concluding that these 2D images are suitable for social science research [19] [32] [33].

1. The researchers did not have a verbal discussion with the participants after they finished filling their survey forms as to what reasons governed their responses. This might give a better understanding of a child's perspective while making social judgments.

2. The actual numerical distinction in mean scores of TAS was found to be around 3-4. Larger sample size would have provided a larger numerical distinction, which would change the statistical significance of certain parameters measured.

In conclusion, within the limitations of this study, it was demonstrated that visible dental trauma influenced the psychosocial judgment given by children towards their peers. This judgment would, in turn, affect their level of acceptance towards such appearances. Therefore, these conditions ought to be redressed as swiftly as possible, and it is advisable that parents and caregivers ought to consider these issues genuinely for better social relations and academic performances of their wards. This study provided the quantitative evidence to dental professionals that their peers based on any visible dental trauma negatively judge children. Therefore, it would be prudent and in the best interest of a child's healthy emotional and physical growth, that such visible dental defects are not neglected.

The primary purpose of this study was to highlight the psychosocial perceptions of children in judging their peers, regarding not only attractiveness but also intelligence, friendliness, confidence, outgoing nature, etc. These factors usually make most children conscious about their visible incisal trauma affecting them emotionally and psychologically for their long-term development in life [19] [30]. So greater awareness should be created amongst dental professionals to try and correct small esthetic dental defects by performing non-invasive techniques like simple composite restorations or microabrasions to improve the confidence of the child, rather than by just convincing children to wait till they become older for cosmetic correction. Thus, we must question ourselves regarding clinical, financial and legal concerns while treating children for these visible traumatic incisors for overall wellbeing.

Acknowledgements

The authors thank Dr Jagan Kumar Baskaradoss, Dr Avidapu Rajshekhar, and Dr Mamata Hebbal for helping us in statistics. Special thanks to Dr Rohit Ashok Antony Fernandez in reviewing the manuscript. We also thank principals, teachers and children from all the schools who cooperated for our study.

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https://doi.org/10.3889/oamjms.2018.300
eISSN: 1857-9655
Dental Science

Prescription of Antibiotics for Periodontal Disease among Dentists in the Region of Tirana

Besian Abazi[1*], Joana Mihani[2]

[1]Albanian University, Faculty of Medical Sciences, Department of Dentistry, Tirana, Albania; [2]University of Medicine Tirana, Department of Pharmacy, Tirana, Albania

Citation: Abazi B, Mihani J. Prescription of Antibiotics for Periodontal Disease among Dentists in the Region of Tirana. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1486-1491. https://doi.org/10.3889/oamjms.2018.300

Keywords: Antibiotics; Dental prescriptions; Periodontitis

***Correspondence:** Besian Abazi. Albanian University, Faculty of Medical Sciences, Department of Dentistry, Tirana, Albania. E-mail: Abazi.besian@gmail.com

Received: 10-Apr-2018; **Revised:** 07-Jun-2018; **Accepted:** 04-Jul-2018; **Online first:** 15-Aug-2018

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Periodontal disease has been and will be a challenge for dentists in the entirety of oral pathologies. To date, there is no data regarding the prescription of antibiotics for periodontitis in the district of Tirana.

AIM: Evaluate aspects related to the pattern of prescription of antibiotics among dentists in Tirana region for periodontitis.

METHODS: Prescriptions from dental practitioners were collected from 25 pharmacies, randomly selected. The only prescription containing a diagnosis of periodontitis, with at least one antibiotic given, was included in the study. Data analysis was done with SPSS 20.

RESULTS: Out of 1159 initial prescriptions, only 314 met the selection criteria. The average age of patients was 39.91 ± 15.21 years. Mean duration of therapies was 5.57 ± 1.5 days. The most common form of prescription was one broad-spectrum antibiotic (74.5%), combined antibiotics therapy (22.3%) and narrow-spectrum antibiotic (3.2%). Combined antibiotics involved the use of Metronidazole with Amoxicillin (12.1%) and Metronidazole with Spiramycin (10.2%). Significant differences in the patterns of prescription were identified in relation with patient age and therapy duration ($P < 0.05$). No statistical difference was found in the patient's gender and the typology of the therapy ($P > 0.05$).

CONCLUSIONS: Our study shows prescription characteristics of antibiotics for periodontal disease by dentists in Tirana for the first time. Amoxycillin is the most prescribed antibiotic, followed by amoxicillin with clavulanic acid. We found variation in dosage, frequency and duration for all antibiotics used, and perceptible discrepancies between observed and recommended practice. Guidelines on rational antibiotic use are needed for dental practitioners in Tirana and the Republic of Albania for better management of periodontitis and resistance prevention.

Introduction

Periodontitis is an inflammatory disease which affects the supporting tissues of the tooth. It constitutes one of the most frequent bacterial infection in adults. There are hundreds of bacterial species associated with this disease, thus making it difficult to achieve a successful specific therapy for periodontitis [1]. Among these the most relevant are Aggregatibacter actinomycetemcomitans (*A.a.*), Porphyromonas gingivalis (*P. gingivalis*), Treponema denticola (*T. denticola*), Fusobacterium nucleatum (*F. nucleatum*) and Prevotella intermedia (*P. intermedia*), [2] [3] [4]. *P. gingivalis* is considered as the main cause of chronic periodontitis, though no less important is the A.a., which is recognised as the leading cause of aggressive periodontitis [2] [5] [6]. Difficulties faced by periodontists lie in the fact that the restoration of normality for the periodontal tissues becomes difficult with time. If left untreated, it can progress into an irreversible situation [7]. There are some procedures and protocols aimed to prevent the progression of the lesion, maintain current levels of periodontal tissues and restore periodontal health. To succeed in these procedures, in addition to manual curettage and periodontal surgery, systemic antibiotic therapy is a key factor [8] [9] [10] [11]. Strong

evidence exists to support benefits of manual curettage to remove supra and subgingival plaque. Hence, without the use of antibiotics, this procedure is unable to eradicate pathogenic bacterial species and thus to maintain gingival levels of adhesion [8] [12] [13]. To support such procedures, except monotherapy with antibiotics, dental practitioners use a combination of antibiotics known as a combined therapy or dual antibiotic therapy [14] [15] [16].

To the best of our knowledge, to date, there is no data regarding the prescription of antibiotics for periodontitis in the region of Tirana.

Materials and Methods

This retrospective drug utilisation study was conducted over a period of 3 months, March-June 2016. It involved prescriptions collected from 25 randomly selected pharmacies in the region of Tirana. We collected prescriptions dispensed by dentists and selection criteria included prescriptions: (a) limited and only for periodontitis in patients ≥18 years old; and (b) with at least one antibiotic prescribed. Prescriptions with other systemic drugs or local medications were excluded from the study.

Collected data were analysed using the statistical software SPSS 20 (IBM, USA). Differences between patterns of prescription, patient's age, gender and treatment duration were compared using One-way ANOVA and Chi-square tests. *P*-values < 0.05 were considered statistically significant.

Results

From 1159 collected prescriptions, only 314 met the selection criteria. The gender ratio was 1:1.12 consisting of 148 Males (47.1%) and 166 Females (52.9%). The average age was 39.91 ± 15.21years (18-81). Mean duration of therapies was 5.57 ± 1.5 days (3-10) (Table 1).

Table 1: Age and therapy duration variables parameters

	Age of patients	Duration of therapy
Mean	39.91	5.57
Median	36.00	5.00
Mode	25	5
Standard deviation	15.21	1.50
Minimum	18	3
Maximum	81	10

Three patterns of antibiotic prescription were observed: (a) 74.5% single therapies with a broad-spectrum antibiotic (BSA), (b) 22.3% combined therapies (CT) consisting in a broad-spectrum antibiotic and a narrow-spectrum antibiotic and (c) 3.2% narrow-spectrum antibiotic (NSA) (Table 2).

Table 2: Frequency of type of antibiotic therapies

The pattern of antibiotic prescription	Value	%
Broad spectrum	234	74.5
Broad and narrow spectrum combination	70	22.3
Narrow spectrum	10	3.2

Among 234 BSA prescriptions, Amoxicillin is the most prescribed drug (32,5%), followed by Amoxicillin-Clavulanic Acid (22%) and Spiramycin (12.7%). Instead, Erythromycin (4.1%), Azithromycin (1.6%) and Ciprofloxacin (1.6%) are less prescribed drugs. CT involved the use of Amoxicillin with Metronidazole (12.1%) and Spiramycin with Metronidazole (10.2%). Regarding the use of NSA, only Metronidazole was prescribed (Table 3).

Table 3: Percentage of antibiotic prescription and characteristics of therapy duration

Antibiotic		Therapy duration (days)			
	%	Mean	Maximum	Minimum	Mode
Broad spectrum					
Amoxycillin	32.5	5	7	3	5
Amoxycillin with Clavulanic Acid	22	5	7	3	5
Spiramycin	12.7	5	7	3	5
Erythromycin	4.1	5	6	3	5
Ciprofloxacin	1.6	5	7	4	4
Azithromycin	1.6	5	5	5	5
Mixed spectrum					
Metronidazole+Amoxycillin w/Clavulanic Acid	12.1	7	10	6	7
Metronidazole+Spiramycin	10.2	8	10	5	7
Narrow spectrum					
Metronidazole	3.2	6	7	6	6

One-way ANOVA test indicated the statistically significant difference between the three typologies of antibiotic therapy prescribed, patient's age and treatment duration (Table 4).

Table 4: One Way ANOVA test results for patients age and treatment duration about the type of therapy

	Sum of Squares	df	Mean Square	F	Sig.
Age	3106.280	2	1553.140	6.962	0.001
Treatment duration	399.900	2	199.950	201.206	0.000

Post Hoc test demonstrated that the single BSA therapy and CT do not show significant differences in mean patients age, while NS therapy presented significant difference compared with both therapies (Table 5).

Table 5: Multiple comparisons where the dependent variable is patient age

(I) Antibiotic type	(J) Antibiotic type	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Broad spectrum antibiotic	Combined therapy	1.469	2.035	.751	-3.32	6.26
	Metronidazole	17.912*	4.823	.001	6.55	29.27
Combined therapy	Broad spectrum antibiotic	-1.469	2.035	.751	-6.26	3.32
	Metronidazole	16.443*	5.049	.004	4.55	28.33
Narrow spectrum antibiotic	Broad spectrum antibiotic	-17.912*	4.823	.001	-29.27	-6.55
	Combined therapy	-16.443*	5.049	.004	-28.33	-4.55

Data shows that NSA like Metronidazole tends to be prescribed to younger patients with a mean age of 23 years (Figure 1).

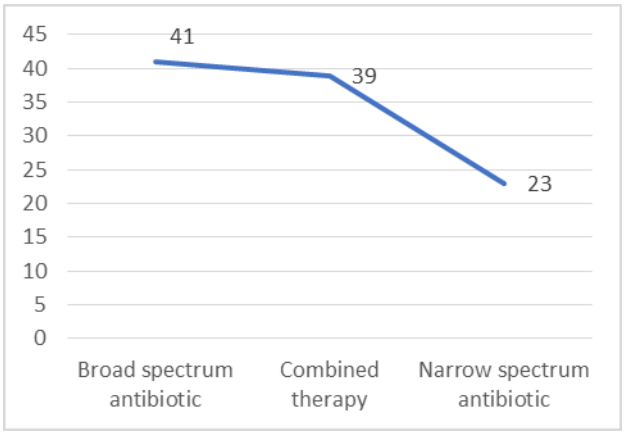

Figure 1: Mean patients age

Furthermore, Post Hoc test showed the statistically significant difference between all three types of antibiotic therapies regarding the duration of therapies (Table 6).

Table 6: Multiple comparisons where the dependent variable is therapy duration

(I) Antibiotic type	(J) Antibiotic type	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Broad spectrum antibiotic	Combined therapy	-2.705*	0.136	0.000	-3.03	-2.39
	Metronidazole	-1.377*	0.322	0.000	-2.14	-0.62
Combined therapy	Broad spectrum antibiotic	2.705*	0.136	0.000	2.39	3.03
	Metronidazole	1.329*	0.337	0.000	.53	2.12
Narrow spectrum antibiotic	Broad spectrum antibiotic	1.377*	0.322	0.000	.62	2.14
	Combined therapy	-1.329*	0.337	0.000	-2.12	-0.53

The single broad-spectrum antibiotic regime has a mean duration of 4.92 days while Metronidazole therapy for 5 days. Dual therapy has the longest regimen, with a mean of 7.63 days (Figure 2).

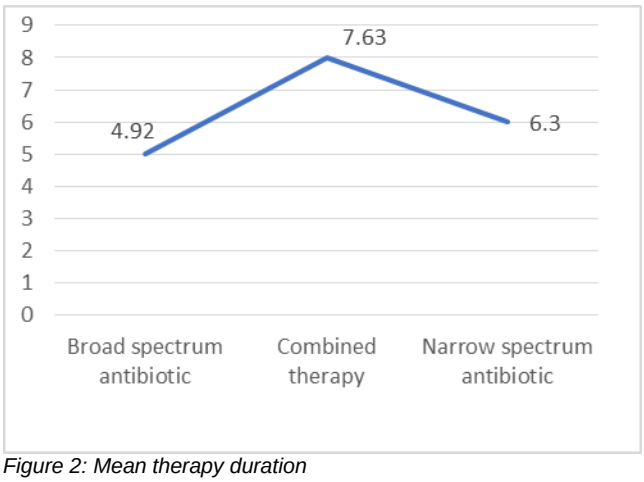

Figure 2: Mean therapy duration

No statistical difference was found in the patient's gender and the typology of the therapy ($P > 0.05$).

Discussion

This study shows that dentists in the Tirana region tend to prescribe BSA in 74.5% of cases. Amoxicillin alone and its combination with Clavulanic acid are prescribed in more than half of cases (54.5%). Amoxicillin is found to be useful in the management of patients with aggressive periodontitis, in both localised and generalised forms [17] [18]. Also, its combination with Clavulanate potassium shows to incline bacterial resistance. Also, Spiramycin is frequently used. In 12.7% of prescription, it is used alone for the management of periodontal disease. Spiramycin has shown promising results in the treatment of advanced forms of periodontitis, and as an adjunct to thorough scaling and root planning, provides a statistically significant improvement in probing depths for up to 24 weeks when compared with scaling and root planing alone. Furthermore, it produces a significant improvement in attachment level [18] [19] [20] [21].

On the other hand, NSA alone, such as Metronidazole remains a non-primary choice for the pharmacological treatment of periodontal disease, with only 3.2% of prescriptions. Although, the literature shows that Metronidazole has a prominent effect on periodontitis, yet there is scepticism among dentists who are reluctant to prescribe it. However, Metronidazole alone it's not the drug of choice for treating A.a. infections. Instead, it's a combination with other antibiotics shows to be effective against these bacteria [22] [23]. Also, it is effective against anaerobes such as *P. gingivalis* and *P. intermedia* [24]. Studies have suggested that Metronidazole combined with Amoxicillin or Amoxicillin–Clavulanate potassium, it may be with great impact in the management of patients with aggressive periodontitis [23] [25] [26] [27] [28]. In our study, Metronidazole was mostly used in combination with Amoxicillin 12.1% and with Spiramycin in 10.2%. Of the cases.

Clinical research suggests that Ciprofloxacin with Metronidazole is a powerful antibiotic combination against mixed infections. At present, Ciprofloxacin is the only antibiotic in periodontal therapy to which all strains of A.a. are susceptible [29] [30] [31] [32]. Metronidazole targets obligate anaerobes, and Ciprofloxacin targets facultative anaerobes. This type of therapy can be of great benefit for the patients since periodontal infections contain a wide diversity of bacteria, and this scenario makes it mandatory to use more than one antibiotic, either serially or in combination [33] [34] [35] [36] [37]. Nevertheless, data shows that dentists in Tirana region do not use this combination.

The duration of the treatment shows to be a problem itself, with a mean of 5.57 ± 1.5 days. Dentists who prescribe only one antibiotic, prefer a 5-days therapy. In contrast, dentists that have chosen a combination therapy have prescribed it for a longer

time, and this can be the case of chronic resistant periodontitis. Only the CT has a mean duration of 7.63 days. None of the prescriptions had a prolonged therapy for more than ten days. Therapies with BSA, have a mean duration of 4.92 days and a therapy with NSA has a mean duration of 6.3 days. This is a short-term therapy which may pose a risk regarding antibiotic resistance rather than a successful treatment for the disease. This, especially in cases of chronic periodontitis where the presence of periodontal pathogens, specifically A.a., is known to endure in tissues after therapy and re-infect the pocket. Thus, the use of systemic antibiotics was thought to be necessary to eliminate pathogenic bacteria from the tissues [27].

Interpretation of the processed data, not only does show that NSA like Metronidazole have a limited number of prescriptions, but it is more used in young adults. Patients with a generalised form of the disease usually appear to be young, and they present generalised attachment loss and poor antibody response. Therefore, these patients often have a fair, poor, or questionable prognosis, and they need an effective systemic antibiotics therapy. This is in contrast with our findings of NSA use in younger adults.

Evidence shows that there is a lack of knowledge for antibiotic prescription patterns and duration of therapy in dentists in the region of Tirana. The increasing resistance problems of recent years are probably related to the over-or misuse of antibiotics. There is a clear need for the development of prescribing guidelines and educational initiatives to encourage the rational and appropriate use of antibiotics in dentistry, especially periodontal problems. These are issues we found to be present also in other countries [38] [39] [40] [41] [42] [43] [44].

While the use of antibiotics in periodontal treatment will probably always be controversial, the position paper of the American Academy of Periodontology contains valuable guidance for their use, and we would recommend their application in everyday practice by dentists. Following exhaustive literature searches, this paper determined that patients with aggressive periodontitis appear to benefit from the adjunctive use of systemic antibiotics during treatment. Systemic antibiotic therapy helps the manual curettage and improves immune response to eliminate subgingival bacteria, which are not affected by manual therapy [12]. The mechanical curettage without the addition of systemic antibiotics would probably be a failure considering the rapid bacterial colonisation of periodontal pockets [36].

Based on WHO latest report, antimicrobial resistance poses a "global health security threat" to public health [45]. Subsequently, to benefit the most from these therapies, we must limit their use and prescribe the right dosage and duration of therapy to prevent further resistance.

In conclusion, our study shows prescription characteristics of antibiotics for periodontal disease by dentists in Tirana for the first time. Most of the dentist in the district of Tirana, for periodontitis, prescribes only one broad-spectrum antibiotic. Amoxicillin was the most preferable, followed by amoxicillin with clavulanic acid. Metronidazole a narrow spectrum antibiotic is prescribed more in young adults, while combined therapies and broad-spectrum antibiotics tend to be prescribed with the increasing of patient's age. We found variations in dosage and frequency for all the antibiotics used, particularly concerning data exists regarding the short duration of therapies prescribed. Perceptible discrepancies were observed between recommendations and practice. Therefore, these observations highlight the need for dentists to improve antibiotic prescribing practices for periodontal problems. Guidelines on rational antibiotic use are needed for dental practitioners in Tirana and in the Republic of Albania for a better management of periodontitis and resistance prevention.

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Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1492-1497.
https://doi.org/10.3889/oamjms.2018.301
eISSN: 1857-9655
Dental Science

Patient Satisfaction with Orthodontic Treatment Received in Public and Private Hospitals in Dammam, Saudi Arabia

Abdulwahab Aljughaiman[1], Ali Alshammari[1], Abdullah Althumairi[2], Abdulaziz Alshammari[3], Naif Almasoud[4], Muhammad Ashraf Nazir[4*]

[1]Dental Hospital, College of Dentistry, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia; [2]Department of Biomedical Dental Sciences, College of Dentistry, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia; [3]College of Dentistry, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia; [4]Department of Preventive Dental Sciences, College of Dentistry, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

Citation: Aljughaiman A, Alshammari A, Althumairi A, Alshammari A, Almasoud N, Nazir MA. Patient Satisfaction with Orthodontic Treatment Received in Public and Private Hospitals in Dammam, Saudi Arabia. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1492-1497. https://doi.org/10.3889/oamjms.2018.301

Keywords: Patient satisfaction; Orthodontic treatment

***Correspondence:** Muhammad Ashraf Nazir. Department of Preventive Dental Sciences, College of Dentistry, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia. E-mail: manazir@iau.edu.sa

Received: 05-May-2018; **Revised:** 05-Jul-2018; **Accepted:** 08-Jul-2018; **Online first:** 19-Aug-2018

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: The demand for orthodontic treatment is on the rise, and there are high patient expectations for improved dentofacial appearance. Patient satisfaction with orthodontic treatment is associated with improving treatment outcomes.

OBJECTIVE: To evaluate patient satisfaction with orthodontic treatment received in public and private hospitals.

MATERIAL AND METHODS: This cross-sectional study was conducted on a calculated sample of patients who received orthodontic treatment in public and private hospitals in Dammam, Saudi Arabia. A validated questionnaire (five-point Likert scale) was used to assess patient satisfaction with orthodontic treatment.

RESULTS: A total of 229 out of 243 patients completed the survey (response rate = 94.2%). The mean age of the participants was 22.69 ± 6.34 years. More females (65.5%) than males (34.5%) participated in the study. The participants gave the highest satisfaction score to the doctor-patient relationship (mean score 4.33). This was followed by dentofacial improvement (mean score 4.23), dental functions (mean score 4.20), and psychosocial improvement (mean score 3.94). The participants provided significantly more positive perspective about doctor-patient relationship in public than private hospitals (P = 0.014). The patients treated in private hospitals were more satisfied with dental functions domain than those who received treatment in public hospitals (P = 0.023). The patients treated by public orthodontists were significantly more satisfied with other domains (situational aspect and residual category) than by the private orthodontists.

CONCLUSION: The doctor-patient relationship was the most important factor in satisfaction with orthodontic treatment. Overall, patients treated in public hospitals were more satisfied with orthodontic treatment than those in private hospitals.

Introduction

Malocclusion is the third most common oral condition, and it negatively impacts individuals' social interaction and their emotional status in addition to affecting dental functions and facial appearance [1] [2] [3]. Research shows high orthodontic treatment needs among adolescents [4]. The evidence is mounting about the impact of orthodontic treatment on the quality of life of individuals, and improvement in appearance, speech, self-esteem, socialising and interpersonal relationships are seen in orthodontically treated patients [5] [6] [7]. High income and education, and awareness about oral health including improved facial esthetics have resulted in an increased demand for orthodontic treatment [8]. A vast majority (69.6%) of teenagers wants orthodontic treatment, and their desire is associated with the severity and different types of malocclusion [9]. Female tend to perceive a greater need for orthodontic treatment than male adolescents [10] [11]. Each year, about 5.75 million Americans and Canadians seek orthodontic treatment, and there was 43.75% increase during the last decade [12].

Many factors such as interpersonal relations between provider and patients, accessibility and convenience of care, quality of services, the competence of provider, and the cost of treatment

determine patient's satisfaction. Patient satisfaction surveys can bring new information about the realities of care provided to them that may not mirror the perceptions of health care providers [13]. Hence, several studies employed different instruments and showed a wide range of patient satisfaction with orthodontic treatment [14] [15] [16] [17].

Satisfaction of patients with orthodontic treatment is associated with gender, age, duration of orthodontic treatment, and an improvement in dentofacial appearance. Also, patient satisfaction is considered crucial for adherence to orthodontic treatment and is related to the stability of orthodontic treatment [18] [19]. The quality of health services provided to patients also affects their satisfaction [20]. On the contrary, Al-Omiri and Abu Alhaija reported no relationship between patient satisfaction and age and gender [14]. A previous study in Saudi Arabia reported that 87.1% of patients were satisfied with orthodontic treatment [21].

Presently, there is an increased demand for orthodontic treatment in addition to high expectations for improved dentofacial appearance. Providing high standards of orthodontic treatment and achieving the highest satisfaction level should be a top priority of practising dentists as these elements are critical to retaining the patients. Most studies reported in the literature involved orthodontic patients visiting teaching dental hospitals/ centres in universities. The level of patient satisfaction with orthodontic treatment could be different if treated in private dental practices or government hospitals/dental centres.

Therefore, this study was conducted to compare the level of patient satisfaction with orthodontic treatment received in private and public hospitals in the Eastern Province of Saudi Arabia. The study was expected to provide valuable information that could be used to achieve the highest level of patient satisfaction with orthodontic treatment and consequently improved treatment outcomes in both the public and private sectors in the country.

Methods

This cross-sectional study was conducted from January 16, 2017, to April 25, 2017, at public and private orthodontic speciality centres or hospitals in Dammam, Dhahran, AlKhobar the Eastern Province of Saudi Arabia. The ethical approval was obtained from the Scientific Research Unit of the College of Dentistry Imam Abdulrahman Bin Faisal University, Dammam (ethical approval #2017008). The researchers contacted the administrators of the speciality centres and hospitals to get their permission to conduct a survey. An estimated sample of 243 patients was calculated based on a 5% margin of error, 95% confidence interval, 80% of response distribution and population size (20,000) [22]. The patients who received orthodontic treatment over one year were eligible to participate in this study. The participants were briefed about the purpose and details of the study. They provide their consent by filling out the questionnaire. The questionnaire was delivered to every patient up to the first 243 patients. The study was conducted in line with the principles of the Helsinki Declaration.

The patients with cleft lip and cleft palate were excluded from the study. A validated questionnaire (five-point Likert scale) was used to assess patient's satisfaction with orthodontic care [15]. However, content and face validation of the questionnaire was conducted by the expert faculty members ensuring readability, comprehensiveness, and clarity of the items in the final questionnaire. This is pivotal because when adapting a questionnaire to a new setting, some of the items may not be conceptually relevant to Saudi culture and ways of life. Moreover, some of the items may not be relevant to certain age groups in the current study. Therefore, some of the items in the original questionnaire were removed. The questionnaire was translated into Arabic and reviewed by a faculty member who was an expert in both English and Arabic languages before it was administered to patients. The questionnaire items are distributed in seven sections which include personal data, the doctor-patient relationship, situational aspects, dento-facial improvement, psychosocial improvement, dental function, and residual category.

The collected data were entered and analysed using SPSS program version 22 (IBM Corp. Armonk, NY, USA). Means and standard deviations were calculated for quantitative variables and frequency distribution for categorical variables. Mean scores of each subscale of the questionnaire were calculated. Mann Whitney U test was used to evaluate the differences in satisfaction levels between the patients treated in private and public hospitals. A p-value ≤ 0.05 was considered significant.

Results

A total of 229 out of 243 patients completed the survey with a response rate of 94.2%. The mean age of the participants was 22.69 ± 6.34 years. Approximately 67.7% (n = 150) of the patients were treated at governmental hospitals and 32.3% (n = 79) at private hospitals. More females (65.5%) than males (34.5%) participated in the study. About 39.3% had insurance coverage, and almost half the sample had a monthly family income of more than 10,000 SAR (1$ U.S. = 3.75 SAR). Minimum duration of orthodontic treatment was one year, and the mean duration was 2.21 years (Table 1).

Table 1: Demographic information of study participants

Characteristics	N	%
Gender		
Male	79	34.5
Female	150	65.5
Nationality		
Saudi	195	85.2
Non-Saudi	34	14.8
Education		
No formal education	4	1.7
Primary education	15	6.6
Secondary education	109	47.6
Bachelor's degree	94	41.0
Master's degree and above	7	3.1
Monthly household income		
Less than SAR 10,000 per month	63	27.5
Between SAR 10,000 to 20,000 per month	69	30.1
More than SAR 20,000 per month	28	12.2
Do not know/not sure	69	30.1
Dental insurance		
Yes	90	39.3
No	139	60.7
Type of hospital		
Public	155	67.7
Private	74	32.3
Age of participants (Mean ± SD)	22.69 ± 6.34 years	
Duration of treatment (Mean ± SD)	2.21 ± 1.21 years	

The comparison of patients' responses to satisfaction regarding orthodontic treatment received in public and private hospitals was carried out. There are six domains in the questionnaire which are displayed in the tables. In the doctor-patient relationship domain, patients gave the highest mean scores to respectfully receiving treatment (mean score 4.63) and liking the orthodontist (mean score 4.62).

Table 2: Patient satisfaction with orthodontic treatment related to the doctor-patient relationship in public and private hospitals

Doctor-patient relationship	Mean score ±SD	Public hospital	Private hospital	p-value
		Mean ± SD	Mean ± SD	
I personally liked the orthodontist(s) who treated me	4.62 ± 0.60	4.68 ± 0.555	4.50 ± 0.687	0.045
Greater efforts should have been made to reduce the pain from braces	3.51 ± 1.31	3.63 ± 1.269	3.24 ± 1.353	0.038
The orthodontist(s) always checked their work carefully	4.51 ± .75	4.54 ± 0.714	4.46 ± 0.831	0.667
The orthodontic care I received could have been better	3.56 ± 1.35	3.66 ± 1.340	3.35 ± 1.359	0.093
The orthodontist(s) was gentle when treating me	4.52 ± 0.72	4.63 ± 0.583	4.30 ± 0.918	0.008
Before treatment began, my orthodontist(s) carefully explained what treatment would be like	4.41 ± 0.82	4.55 ± 0.616	4.12 ± 1.097	0.007
Questions I had about my treatment were answered promptly	4.52 ± 0.68	4.60 ± 0.609	4.34 ± 0.799	0.013
The assistants were gentle when treating me	4.56 ± 0.62	4.61 ± 0.574	4.46 ± 0.70	0.120
The orthodontic staff (assistants and office personnel) treated me with respect	4.53 ± 0.64	4.59 ± 0.556	4.42 ± 0.776	0.190
The orthodontist(s) treated me with respect	4.63 ± 0.58	4.66 ± 0.562	4.55 ± 0.622	0.183
Overall mean score	4.33 ± 0.13	Mean Ranks 124.59	Mean Ranks 94.92	0.014

Patients treated in public hospitals liked orthodontist more than in private hospitals, and the difference was statistically significant (P = 0.045). Similarly, public orthodontists more carefully explained the treatment (P = 0.007) and promptly answered patients' questions (P-value = 0.013) and gently provided treatment (P = 0.008) than private orthodontists. Overall, there was the more positive perspective of the patients about the doctor-patient relationship in public than private hospitals (P = 0.014) (Table 2).

Table 3 shows that the respondents identified cleanliness of treatment areas (mean score 4.47) as the most important item in addition to the timing (mean score 4.33) and appropriateness (mean score 4.31) of appointments in the situational aspects domain. Most items did not differ statistically in both public and private hospitals, however, more satisfaction reported in public than in private hospitals (P-value = 0.025).

Table 3: Patient satisfaction with orthodontic treatment related to situational aspects in public and private hospitals

Situational aspects	Mean score ± SD	Public hospital	Private hospital	p-value
		Mean ± SD	Mean ± SD	
My treatment took about as long as I expected it would	3.83 ±1.001	3.92 ± 0.897	3.65 ± 1.175	0.160
Even though some appointments were short, each was necessary for my treatment to be successful	4.31 ± 0.768	4.29 ± 0.747	4.34 ± 0.816	0.457
Problems that arose during treatment were quickly taken care of	4.24 ± 0.853	4.22 ± 0.832	4.28 ± 0.899	0.346
The treatment area was modern and up to date	4.30 ± 0.777	4.34 ± 0.716	4.20 ± 0.891	0.428
The orthodontist's office was conveniently located	4.25 ± 0.793	4.28 ± 0.761	4.20 ± 0.860	0.645
I was satisfied with the selection of days and times when I could be seen for orthodontic appointments	4.33 ± 0.751	4.39 ± 0.649	4.20 ± 0.921	0.326
Plenty of time was spent with me during each appointment	3.37 ± 1.238	3.31 ± 1.241	3.49 ± 1.230	0.310
I was rarely kept waiting for appointments	3.73 ± 1.167	3.85 ± 1.068	3.49 ± 1.327	0.072
The waiting area was comfortable	4.24 ± .820	4.30 ± 0.751	4.09 ± 0.939	0.152
The treatment area was clean and sanitary	4.47 ± 0.716	4.44 ± 0.739	4.53 ± 0.667	0.426
I had to travel far to reach the orthodontic clinic	2.89 ± 1.478	3.01 ± 1.481	2.64 ± 1.448	0.078
The treatment took much too long	3.45 ± 1.247	3.37 ± 1.274	3.61 ± 1.180	0.194
Overall mean score	3.86 ± 0.129	Mean Ranks 121.78	Mean Ranks 100.80	0.025

The study participants expressed their satisfaction with different items of dentofacial improvement. But no statistically significant differences were reported by patients treated in public and private hospitals (Table 4).

Table 4: Patient satisfaction with orthodontic treatment related to dentofacial improvement in public and private hospitals

Dentofacial improvement	Mean score ± SD	Public hospital	Private hospital	P-value
		Mean ± SD	Mean ± SD	
My teeth are straighter due to orthodontic treatment	4.23 ± 0.812	4.22 ± 0.847	4.24 ± 0.737	0.920
I have a better bite due to orthodontic treatment	4.23 ± 0.822	4.23 ± 0.820	4.22 ± 0.832	0.917
I think I have a more attractive face due to orthodontic treatment	4.18 ± 0.873	4.20 ± 0.871	4.14 ± 0.881	0.578
My appearance has changed exactly as I expected	4.21 ± 0.827	4.25 ± 0.784	4.14 ± 0.911	0.531
My teeth fit very well since I have been treated	4.24 ± 0.762	4.21 ± 0.787	4.32 ± 0.704	0.347
When I look in the mirror, I feel very satisfied with the way my appearance is improved since orthodontic treatment	4.25 ± 0.797	4.24 ± 0.765	4.27 ± 0.865	0.510
I feel very happy because I look so much better since I have been treated	4.33 ± 0.740	4.33 ± 0.713	4.34 ± 0.799	0.720
Overall mean score	4.23 ± 0.016	Mean Ranks 113.57	Mean Ranks 118.00	0.633

The patients reported psychosocial improvement with orthodontic treatment (3.94 ± 0.076). However, no significant differences found in

satisfaction between privately and publically treated patients (Table 5).

Table 5: Patient satisfaction with orthodontic treatment related to psychosocial improvement in public and private hospitals

Psychosocial improvement	Mean score ± SD	Public hospital	Private hospital	p-value
		Mean ± SD	Mean ± SD	
I feel better about myself because of orthodontic treatment	4.42± 0.694	4.39 ± 0.698	4.49 ± 0.687	0.323
I feel more outgoing because of orthodontic treatment	3.83 ± 1.109	3.85 ± 1.052	3.80 ± 1.227	0.939
I feel more confident because of orthodontic treatment	4.04 ± 0.970	4.06 ± 0.884	4.00 ± 1.135	0.758
I feel more popular because of orthodontic treatment	3.76 ± 1.093	3.72 ± 1.042	3.82 ± 1.198	0.298
When I meet people for the first time, they react much more positively to me since I have been treated	3.87 ± 1.060	3.85 ± 0.988	3.92 ± 1.202	0.254
Overall mean score	3.94 ± 0.076	Mean Ranks 112.24	Mean Ranks 120.78	0.360

As it can be seen in Table 6, the respondents found ease in eating (4.21), chewing (4.17) and biting food (4.18) after having orthodontic treatment. Patients treated in private hospitals were more satisfied with dental functions than those who received treatment in the public hospitals (p-value=0.023). They were satisfied with the results of orthodontic treatment (mean score 4.27) and expressed their desire to recommend treatment (mean score 4.34) to other patients as well. Overall, with regards to the residual category, patients treated with public orthodontists were more satisfied than with private orthodontists (Table 6). The highest overall mean score (4.33) was reported for the doctor-patient relationship domain whereas the lowest mean score 3.67 was given to residual category domain.

Table 6: Patient satisfaction with orthodontic treatment related to dental function and residual category in public and private hospitals

Dental function	**Mean score ±SD**	**Public hospital**	**Private hospital**	**p-value**
		Mean ±SD	Mean±SD	
Eating is easier since I have been treated	4.21± 0.898	4.13 ±0.881	4.38±0.917	0.013
Chewing is easier since I have been treated	4.17± 0.904	4.09± .863	4.34±0.969	0.008
I can bite food more easily since I have been treated	4.18± 0.879	4.13± 0.843	4.30±0.947	0.050
I would recommend orthodontic treatment to everyone who has difficulties chewing food	4.25± 0.850	4.17± 0.831	4.41± 0.875	0.014
Overall mean score	4.20± 0.018	Mean Ranks 108.36	Mean Ranks 128.91	0.023
Residual category				
My orthodontic treatment was inconvenient for me	3.26± 1.386	3.46± 1.411	2.85±1.246	0.002
I take better care of my teeth since having braces	4.29± 0.771	4.37± 0 .740	4.12± 0 .810	0.019
I am satisfied with the results of my orthodontic treatment	4.27± 0.776	4.30± 0 .791	4.22± 0.745	0.332
If I had it to do over again, I would still want orthodontic treatment	3.90± 1.054	3.97± 1.053	3.74±1.048	0.086
I would recommend orthodontic treatment to others	4.34± 0.747	4.33± 0.731	4.35± 0.784	0.670
I am dissatisfied with the treatment result	2.79±1.373	2.88±1.391	2.59±1.323	0.146
Generally speaking, I have bad experiences with orthodontic treatment	2.90±1.334	2.96±1.353	2.76±1.291	0.285
Overall mean score	3.67± .177	Mean Ranks 122.54	Mean Ranks 99.20	0.012

Discussion

The study evaluated patient satisfaction with orthodontic treatment and revealed that patients were more satisfied with the orthodontic treatment received in public than in private hospitals. Overall, a high level of patient satisfaction observed in the present study showed that orthodontic treatment was widely accepted as part of health care services in the province. In addition to the prevalence and severity of malocclusion, the gender is an important variable that determines the utilisation of orthodontic services. More female patients were represented in the sample in our study. This is in agreement with the results of a previous study that showed 54% of female compared with 37% of male patients pursued orthodontic treatment [23]. The high rate of orthodontic treatment among female patients was because females give more importance to physical attractiveness than males and they also consider teeth more important for facial appearance than male patients [23]. It was also found that the need for orthodontic treatment was perceived more significantly in female than male subjects [10]. Nevertheless, a previous study observed no association between gender and patient satisfaction with orthodontic treatment [14].

In the present study, the highest mean score (4.33) was given to doctor-patient relationship, and more patient satisfaction was recorded for public than private orthodontists. In the doctor-patient relationship domain, the patients gave the highest mean score to respectfully receiving treatment (4.63) and liking the orthodontist (4.62). The patients treated in public hospitals liked orthodontist more than in private hospitals (P=0.045). Likewise, most of the patients who participated in this study were satisfied with the explanation regarding treatment procedures and prompt answering of their queries in public than private hospitals (P<0.05). Similarly, a previous study by Shahrani et al. found the majority of patients (87.1%) satisfied with the orthodontic treatment and the dentist-patient relationship was an important factor that affected patient satisfaction [21]. Another study by Keles and Bos also demonstrated similar results and showed that the patients gave the highest satisfaction (mean score 4.24) to doctor-patient relationship domain [19]. The reason of high satisfaction with doctor-patient relationship might be explained by the fact that the treatment will be more effective and patient will be more satisfied when the patient-doctor relationship is good. It has been shown that successful orthodontic treatment is significantly related to the positive doctor-patient relationship [24].

The higher satisfaction observed among patients treated by the public than private orthodontists could be because treatment provided in public settings maintained high ethical and quality standards with evidence-based practice [21]. It is also possible that high patient satisfaction in the public

sector might have resulted because of the recent Saudi government's initiatives to improve the quality of services in the public health sector in the country [25]. Moreover, this was supported by a previous study that observed a significant association between patient satisfaction and the quality of health care services in the public hospitals in Saudi Arabia [26]. On the other hand, lower satisfaction with orthodontic treatment in the private hospitals could be related to poor compliance or unrealistic expectations of the patients in the private sector which could have negatively affected their satisfaction [27].

It was reported that patients were highly satisfied with dental functions particularly with eating and chewing and total satisfaction score was significantly correlated with satisfaction with eating and chewing and oral comfort domains [14]. These findings coincide with the results of the present study where patients reported improvement in biting, chewing and eating after orthodontic treatment. Interestingly, patients treated in public hospitals were less satisfied with the dental function category than those who received orthodontic care in private dental centres ($P<0.05$). Satisfaction with the aspects of dental function in private sector could be explained by the fact that healthcare providers in private sectors are more likely to provide a better quality of services due to the competitive environment in the market [28].

In the present study, the patients expressed significantly higher satisfaction with the domains which are related to doctor-patient relationship, appointment systems, and equipment and facilities in a dental office and public hospitals were better than private hospitals in these areas. The studies have shown that difficulty in getting dental appointment including long wait time resulted in patient dissatisfaction [29][30]. Similarly, it was found that patients showed high satisfaction with facilities, latest equipment, and the cleanliness of waiting area and instruments [29].

The study findings can help identify the drivers of patient satisfaction with orthodontic treatment both in private and public hospitals in the Eastern province of Saudi Arabia. The use of a validated questionnaire and face and content validation relevant to culture and age of the participants add strength to the study. However, the study participants conveniently participated in the study which limits the generalizability of its findings to most patient populations in the province. Also, there is a possibility of under and overreporting of patients' responses due to social desirability bias. Nevertheless, the findings of the study can help general dental practitioners and other health care providers to refer the patients for orthodontic treatment. Similarly, the decision-makers in the Ministry of Health can develop policies and programs to improve the quality of services provided in both the private and public sectors.

In conclusion, it was found that patients treated in public hospitals were more satisfied with orthodontic treatment than those in private clinics. There were significant differences in patients' responses about the doctor-patient relationship and dental function domains. The doctor-patient relationship was the most crucial factor in the satisfaction of patients with orthodontic treatment. The orthodontists working in the private sector should improve orthodontic services to ensure the highest patient satisfaction. The Ministry of Health should better monitor the quality of orthodontic services and take appropriate measures so that patients receive the highest standards of orthodontic care in both the private and public sectors. The orthodontists should consider establishing a professional relationship with the patients to achieve the highest level of patient satisfaction and improved orthodontic treatment outcomes.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1498-1502.
https://doi.org/10.3889/oamjms.2018.308
eISSN: 1857-9655
Dental Science - Review

Advantages of CAD/CAM versus Conventional Complete Dentures - A Review

Nadica Mihajlo Janeva[1*], Gordana Kovacevska[1], Saso Elencevski[1], Sanja Panchevska[1], Aneta Mijoska[1], Bisera Lazarevska[2]

[1]Department of Prosthodontics, Faculty of Dentistry, Ss. Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia; [2]University Dental Clinical Centre St. Pantelejmon, Skopje, Republic of Macedonia

Citation: Janeva NM, Kovacevska G, Elencevski S, Panchevska S, Mijoska A, Lazarevska B. Advantages of CAD/CAM versus Conventional Complete Dentures - A Review. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1498-1502. https://doi.org/10.3889/oamjms.2018.308

Keywords: CAD/CAM complete dentures; Digital complete dentures

***Correspondence:** Nadica Mihajlo Janeva. Department of Prosthodontics, Faculty of Dentistry, Ss.Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia. E-mail: njaneva@stomfak.ukim.edu.mk

Received: 07-Jul-2018; **Revised:** 23-Jul-2018; **Accepted:** 27-Jul-2018; **Online first:** 04-Aug-2018

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: The introduction and evolution of CAD/CAM technology into complete dentures fabrication brought high expectations in improving disadvantages associated with conventional methods.

AIM: The purpose of this review was to analyse the existing literature on computer-engineered complete dentures and to determine their advantages over the conventional dentures.

MATERIAL AND METHODS: An electronic search of the English literature from January 1994 to March 2018 was performed in PubMed/MEDLINE, using the following keywords: CAD/CAM complete dentures, computer-engineered complete dentures, complete digital dentures, complete milled dentures, and rapid prototyping dentures.

RESULTS: A total of 179 English language titles were obtained from the database, and 14 were relevant to fulfil the purpose of this review. A review of 7 articles is summarized in 2 tables for presenting a comparison between CAD/CAM and conventional dentures in clinical and laboratory studies.

CONCLUSION: Following the review of articles that discussed the comparison between CAD/CAM and conventional complete dentures in clinical studies, it can be concluded that the main advantages of CAD/CAM dentures are the reduced clinical chair time and the number of visits, digital archiving, significantly higher retention, and more favorable clinical and patient-centered outcomes. As a result of the review of laboratory studies, superior mechanical and physical properties in CAD/CAM dentures were revealed, concerning enhanced accuracy of fit of milled denture bases, less denture tooth movement and increased toughness, ultimate flexural strength, and higher elastic modulus.

Introduction

Due to the increase in population lifespan, the need for dental treatment for edentulous people has become bigger. Despite the advancements in dental treatment possibilities for edentulism, and although implant-assisted complete dentures (CDs) are reported to be more efficient and more preferable option for edentulous patients, conventional CDs remain a choice, due to anatomical, physiological or financial restrictions [1].

Since the conventional method of fabricating CDs was established more than 80 years ago, the continuing goal was to improve all the drawbacks associated with the process of fabrication, and enhancements of the properties of polymethyl methacrylate (PMMA) material. The introduction and evolution of computer-aided technology in the field of CD fabrication is expected to overcome the complications related to conventional CDs and to facilitate the fabrication process.

The first scientific paper on the use of a computer-aided system for designing and fabricating CDs was published by Maeda et al., in 1994, and these first CAD/CAM CDs were made by additive RP technology, from photo-polymerised acrylate material using a 3-D laser lithographic (LL) machine [2]. Since then, because of the complexity of the procedures for fabricating CDs, it took almost 20 years for the emergence of the first commercially available denture systems. Katadiyil et al., present the procedures for the production of digital CDs for the first two CAD/CAM commercial prostheses-AvaDent digital prostheses (Global Dental Science LLC, Scottsdale, Ariz.) and the Dentca CAD/CAM prosthesis system

(Dentca Inc., Los Angeles) [3]. Several researchers contribute to the development of this technology for fabrication of CDs [4] [5] [6] [7].

The process of fabricating CDs with computer-aided technology involves digitisation of the clinical information registered from the patient with light scanning technology and digital designing of CDs on computer software (CAD). The result of designing is virtual dentures in occlusion. This is followed by an automatized process of manufacturing (CAM), which can be additive (rapid prototyping) or subtractive (computerised numerical control milling) process [8]. The subtractive method is a more frequently employed method. The digital record, in the form of an STL (stereolithography) file, is stored in the database.

Beside a lot of advantages in the treatment concept and fabrication process (modified and shortened clinical protocols, digital data archive, automated fabrication of the denture bases), significant improvements in the quality of CAD/CAM CDs is expected from the enhanced physical and mechanical properties of the prepolymerized PMMA pucks (blocks), from which denture bases are milled. Preformed PMMA puck is polymerised by injection, under high temperature and pressure, which prevents shrinkage of the CAD/CAM CDs [9]. The comparison of the processing distortion of traditional techniques and the CAD/CAM fabrication technique, which leads to dimensional changes in the denture base and consequently diminished retention, stability, and support, has been dealt with in few laboratory studies [10] [11] [12].

First clinical studies related to computer-aided CD have led to the publication of a few reviews focused on clinical and patient-centered outcomes, unique applications, and clinical complications of this technology [1] [13]. The purpose of this review is to evaluate data focusing on clinical and material-related advantages of CAD/CAM CDs over the conventional CDs.

Material and methods

An electronic literature search was done through PubMed/MEDLINE database for identifying English articles with keywords "CAD/CAM complete dentures", "complete digital dentures", “computer-engineered complete dentures”, "complete milled dentures", "rapid prototyping dentures" from January 1990 to March 2018. The inclusion criteria for selection were clinical studies, laboratory technical research papers, case reports, and review articles with a comparison between CAD/CAM and conventional processing techniques for CD fabrication, with specified keywords. The criteria for excluding articles were non-English articles, and articles that failed to meet the inclusion criteria.

The search strategy involved three phases: reviewing titles, selecting abstracts of interest and final selection of articles for full-text detailed analysis. During the second phase, the abstracts that fulfil the purpose of this review were selected, and in the third phase, their full-text was analysed.

Results

The electronic search through PubMed resulted in 179 titles in the English language literature, and 14 were relevant to determine the advantages of CAD/CAM-fabricated CDs. Table 1 presents 2 articles chosen for presenting a comparison between digital and conventional CDs in clinical and patient-centered outcomes in clinical studies. Table 2 shows 5 articles from laboratory studies. Another 7 articles were selected and included in this review to contribute to the purpose of this review.

Table 1: Clinical and patient-centred outcomes

Article	Summary	Results
Kattadiyil et al., [14]	This comparative clinical prospective study compared and rated digitally and conventional CDs fabricated by predoctoral students with faculty supervision; each of 15 completely edentulous patients (average age 55 years) received 1 conventional and 1 digital (AvaDent) set of CDs.	Significantly higher average scores were recorded for the digital CDs by the faculty for 14 evaluated and analysed criteria; Significantly higher average patient response scores were recorded for the digital CDs; Students preferred digital CDs compared with conventional; The conventional fabrication process required significantly more clinical time.
Al Helal et al., [18]	This clinical study compared retention values of milled and conventionally processed denture bases; 20 CAD/CAM (AvaDent) and 20 conventional (heat-polymerised) processed maxillary denture bases were fabricated for individuals with completely edentulous maxillary arches; for measuring the retention, with custom-designed testing device, each denture base was vertically pulled by using an advanced digital force gauge 3 times at 10-minute intervals.	Significantly increased retention values were observed for the milled denture bases (an increase of 19,91 N).

Discussion

Encouraging advances in computer-aided in the field of CD fabrication have increased the interest and the number of publications in the last few years. 14 articles were selected to accomplish the purpose of this review, to evaluate the data only focusing on the advantages of CDs fabricated with CAD/CAM technology in comparison to conventional CDs. However, long-term clinical outcome studies are also necessary to be performed.

One of the first advantages that are reported from utilising CAD/CAM technology for the fabrication of CDs is the reduced number of appointments and simplified laboratory work in comparison to the conventional protocol [14].

Table 2: Laboratory findings

Article	Summary	Results
Goodacre et al., [10]	Comparison of the denture base adaptation of conventional (pack and press, pour, injection) and CAD/CAM techniques for fabricating CDs; 40 duplicated gypsum casts were created, and laser scanned; 10 specimens for each of the 4 techniques had been fabricated, hydrated for 24 h, and the intaglio surface laser scanned; using surface matching software, the scan file of each denture was superimposed on the scan file of the corresponding cast; measurements were made at 60 locations, providing evaluation of fit discrepancies at 5 areas; accuracy and reproducibility were assessed.	The CAD/CAM technique showed the best combination of accuracy and reproducibility;
Srinivasan et al., [11]	Comparison of trueness of CAD/CAM milled CDs with injection-molding and conventionally (flask-pack-press) manufactured CRDPs; 33 CDs were fabricated by three different technique, using a single master reference model and incubated in artificial saliva for 21 days; scanning of the intaglio surface was performed 7 days after processing, and again after 21 days in artificial saliva; the corresponding surfaces of the reference model and the 3D images of the dentures were super-imposed using a 3D-software; 5 specific regions of interest were defined and compared.	At baseline, there was no difference in the trueness of the total intaglio surfaces between the groups; after 21 days in artificial saliva, only CDs showed significant trueness; improved trueness for all techniques in most regions of interest.
Goodacre et al., [19]	Comparison of the denture tooth movement of pack-press, fluid resin, injection, CAD/CAM bonded, and CAD/CAM monolithic techniques; 50 dentures (10 for each technique); preprocessing and postprocessing scan files of the cameo surface of each denture were superimposed using surface-matching software; measurements were made at 64 locations, and tooth movement in a buccal, lingual, mesio-distal, and occlusal direction were evaluated.	The CAD/CAM monolithic technique was the most accurate and the most reproducible technique.
Steimassl et al., [20]	Comparison of methacrylate monomer release between CAD/CAM dentures (4 different CAD/CAM denture systems: Baltic Denture Systems, Vita VIONIC, Weiland Digital Dentures, Whole You Nexteeth) and conventional (heat-polymerised) dentures; denture weight, volume, and density were determined; after 7 days of water storage, the monomer release was measured by high-performance liquid chromatography.	No significant difference in released monomer between CAD/CAM and the conventional dentures.
Srinivasan et al., [21]	An in vitro evaluation and comparison of biocompatibility, mechanical properties, and surface roughness of a pre-polymerized PMMA resin for CAD/CAM CDs and a traditional heat-polymerized PMMA resin; Biocompatibility was assessed with cultivation of two types of cells (human primary osteoblasts and embryological mouse fibroblasts) on the substrate separately; mechanical properties were tested with the nanoindentation test, three-point bending test, and surface roughness test.	The tested CAD/CAM and heat-polymerised resins were equally biocompatible; CAD/CAM resins demonstrated improved mechanical properties (higher elastic modulus, ultimate strength, and toughness); higher roughness of the CAD/CAM resin specimens.

Kattadiyil et al., in their study compared the use and effectiveness of CAD/CAM technology in digital CDs fabrication (AvaDent digital CD protocol) with those of the conventional method. Apart from the reduced number of visits to patients for the digital protocol (2 visits), they also emphasise the significantly reduced clinical treatment time for digital fabrication process (approximately 3.5 hours less compared to the conventional protocol). Besides that, clinical evaluation by faculty in this study determined significantly higher retention, fit, and stability in digital CDs; significantly higher average patient response scores were recorded for the digital CDs regarding comfort, retention, masticatory efficiency, and efficiency of the technique. In the clinical retrospective study of Saponaro et al., the average number of visits for the digital protocol was 2.39 (some patients required a third visit) [15] [16]. Still, not every CAD/CAM denture system is intended for a 2-appointment protocol. In the pilot study of Schwindling and Stober, digital CD fabrication with the Weiland Dental system was intended for 4 clinical visits (including a trial placement visit), but a mean of 5.4 visits was needed to complete the fabrication process [17]. The improved retention of digital CDs could be explained by the improved fit and due to the absence of polymerisation shrinkage and the unique method of milling the digital dentures from a prepolymerized block of acrylic resin [14]. In the clinical study of AlHelal et al., the retention values of maxillary conventional heat-polymerised denture bases were compared with digitally milled denture bases, after 24 h storage in water before the testing appointment [18]. The clinical result showed a significant increase in retention for the digital CDs compared with the conventional group. This result indicated that the hydration might not have sufficiently compensated for the polymerisation shrinkage. AlHelal suggested that possible explanation for this could be the increased density of prepolymerized acrylic resin block, which offers higher dimensional stability and is not necessarily influenced by hydration.

A great additional advantage for the digital technique is emphasised, and that is the electronic archiving of all clinical data from the patient, together with the design of the manufactured prostheses, which enables making spare or new prostheses, in case of breaking or losing them, without clinical appointments.

Few laboratory studies have compared the processing distortion of traditional techniques and the CAD/CAM fabrication technique [10] [11] [12]. The results of Goodacre et al., laboratory study indicate that the CAD/CAM processing technique offers a desirable balance of minimal fabrication distortion and better adaptation. They found that CAD/CAM fabrication process is the most accurate and reproducible denture fabrication technique in comparison with traditional techniques [10]. Although Srinivasan et al., in their comparative study have concluded that the trueness of the intaglio surface of all three investigated techniques seems to remain within a clinically acceptable range, they reported that CAD/CAM group showed the strongest compression (with the exception of the tuberosities), especially in the vestibular flange area [11]. The compression in the area of the vestibular flange (tighter inner seal) might be related to improved retention in the findings of the clinical studies. They noted that an edentulous

ridge was chosen without pronounced undercuts and with a shallow palate, and if the palate or the tuberosities been steeper, the shrinkage during heat polymerisation would have probably increased the misfit of the intaglio surface. The results of the in vitro study of Steinmassl et al., designed to evaluate the denture fit in a clinically relevant setting, have supported the findings in Goodacre study [12]. The total number of examined, currently available, CAD/CAM dentures has had a significantly higher precision of denture base fit than the conventional dentures.

The least denture tooth movement during processing with the least denture base distortion at CAD/CAM milled dentures were the combined results of both laboratory studies od Goodacre et al., [10][19]. Accordingly, the CAD/CAM technique would be considered the best processing technique in comparison with traditional techniques. Results of their study for denture tooth movement demonstrated that techniques requiring compression during processing (pack-and-press, injection) showed increased positive occlusal tooth movement compared with techniques not involving compression (fluid resin, CAD-CAM bonded, CAD-CAM monolithic), meaning that it would cause an increase in the patient's OVD. Clinical relevance of these findings might be less laboratory and clinical post-insertion adjustments [16].

Although CAD/CAM dentures are assumed to have a lower methacrylate monomer release than conventionally fabricated dentures, the results from the laboratory study of Steinmassl et al., have not proved that hypothesis [20]. None of the four evaluated different CAD/CAM dentures have released significantly less monomer than conventional (heat-polymerised) dentures. As one of the conclusive explanation for no significant differences, they point out on bonding agents (methacrylate-based) used for fixation of the denture teeth to the milled sockets in CAD/CAM dentures, as a source of methacrylate monomer release.

The claims for superior mechanical properties of the pre-polymerized PMMA pucks were substantiated in the laboratory study of Srinivasan et al., [21]. The increased toughness, ultimate strength, and higher elastic modulus may provide clinical benefits, both for patients and clinicians, regarding designing the denture base with lower minimal thickness, without a common occurrence of denture fractures. However, these claims need to be verified in a clinical study.

In conclusion, based on the published literature, this review has drawn some advantages of the CAD/CAM dentures compared to conventionally fabricated dentures. The main advantages reported in the selected clinical studies were the reduced clinical chair time and number of visits, digital archiving, significantly higher retention, and more favourable clinical and patient-centered outcomes of CAD/CAM dentures. The laboratory studies revealed superior mechanical and physical properties in CAD/CAM dentures, regarding the enhanced accuracy of fit of milled denture bases, less denture tooth movement and increased toughness, ultimate strength, and higher elastic modulus. Long-term clinical research and additional material-related aspects are warranted to reach definitive conclusions.

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https://doi.org/10.3889/oamjms.2018.265
eISSN: 1857-9655
Public Health

Health Promotion Toolkit: An Approach for Empowering Families Caring For Children with Developmental Disabilities in Tabuk

Azza Abdalla Ghoneim*

Tabuk University, Tabuk, Saudi Arabia; Menoufia University, Al Minufya, Egypt

Citation: Ghoneim AA. Health Promotion Toolkit: An Approach for Empowering Families Caring For Children with Developmental Disabilities in Tabuk. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1503-1511. https://doi.org/10.3889/oamjms.2018.265

Keywords: Developmental disabilities; Health promotion; empowerment

***Correspondence:** Azza Abdalla Ghoneim. Tabuk University, Tabuk, Saudi Arabia; Menoufia University, Al Minufya, Egypt. E-mail: a_ghoneim@ut.edu.sa

Received: 29-Mar-2018; **Revised:** 24-May-2018; **Accepted:** 25-May-2018; **Online first:** 05-Aug-2018

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

AIM: The study aimed to determine the effects of the health promotion toolkit on empowering families caring for children with developmental disability. It hypothesised that health promotion toolkit would effectively improve families' empowerment and alleviate parental stress.

METHODS: The research design was quasi-experimental. A convenience sample of 30 children with DD and their families enrolled at Shoaa ElAmal Center in Umluj participated. Tools were Health Promotion Assessment Sheet, Family Empowerment Scale, and the Parent Stress Index.

RESULTS: The results documented significant lower levels of parental stress and higher levels of family empowerment among mothers at posttest than pretest. A significant negative correlation between family empowerment and parental stress was reported.

CONCLUSION: Health promotion toolkit had a positive effect on empowering families as well as lowering parental stress. Recommendation Health promotion toolkit should be integrated as a monitoring method of health care needs of health promotional activities for children with developmental disabilities.

Introduction

Developmental disability is a major health concern. It includes a wide range of impairments that begin before the age of 18 years. These impairments include physical, intellectual and behavioural domains of development that may affect day-to-day functioning [1]. An American study showed that about one in six or about 15% of children have at least one type of developmental disabilities. They include physical disabilities, sensory-related disabilities, communication disabilities and intellectual disabilities [2].

Intellectual disability is a complex phenomenon refers to the mental ability and self-care skills that are below the expected level of an individual's age [3]. It results in significant deficits at an intellectual level in addition to adaptive skills [4] [5].

Approximately, 10% of the population in developed countries and 12% of the population in developing countries are disabled [6]. In Saudi Arabia, The prevalence MR is 8.9 per 1000 children [7]. Another study reported that mental retardation was the most common neurological disorder among Saudi children with a prevalence rate of 26.3 per 10000. The researchers stressed the priority for health care planning for those children [8]. As they are at high risk for adverse health conditions such as epilepsy, neurological, gastrointestinal and behavioural disorders [9]. Generally, they have poor or fair health status [10]. However, children who received early intervention, adequate education, appropriate supports and sustained care generally have improved life outcomes. In fact, they can live independently with family support [11].

Pediatric nurses are vital in providing support to families caring for a child with intellectual disabilities. To build the necessary supportive and collaborative relationships with those families, nurses should

understand the concept of empowerment and its' process [12]. Empowerment concept is widely used in health sciences particularly nursing [13].

Family empowerment is upheld as a creative strategy to provide high quality of care for chronic childhood illnesses and intellectual disabilities across a family-centred care approach [14] [15]. This approach of care delves into providing the effective family strategies that adequate care and support intellectually disable children [16].

In other words, educating parents about their child's condition, teaching them the needed new skills, offering and providing them with the support services they need is known as empowerment strategies [17]. Hence, empowerment strategies possess the essence of health promotion that is about "enabling individuals to take control over their health and its determinants that improve their health. Thereby, they will be able to live an active and productive life [18]. Additionally, health promotion maintenance is a method utilised to maintain and enhance the existed levels of health through the implementation of effective programs, services and policies [19].

It includes health teaching, decision-making, supportive activities (screening, self-care skills, advocating for environmental change, positive health behaviours, and choices) and supportive policies in work and community settings. Therefore, health promotion categorised into three activities: prevention, protection and health education [20].

As the goals of nursing care for developmentally disabled children with are to promote their optimal social, physical, cognitive and adaptive development [21], pediatric rehabilitation nurses must be a part of the interdisciplinary team that can establish an effective management plan. They can help to maximise children's potential by advocating, health teaching, promoting and coordinating health promotion practices as supportive care intervention [22].

Although the prevalence of developmental disabilities particularly, intellectual in Saudi Arabia is similar to that reported in other countries [7] [8], limited studies are conducted. Also, there are various obstacles when researching disability field in Saudi Arabia [23].

Moreover, the cost of preventive efforts is significantly lesser than the management of expected complication; thus, cost-effectiveness favours the prevention approach [24]. The earlier the health/rehabilitative care is delivered, the more the chance of reducing the effect of disability and its' expected complication, and the more quality of life of the person [25].

Therefore, the current study could be of great help for the development of comprehensive strategies for improvement of the quality of life for families that have one or more child with intellectual disability. For these reasons, this study was conducted to determine the effectiveness of health promotion toolkit on empowering families caring for developmentally disabled children.

Research question

1. What are the effects of a health promotion toolkit on empowering families caring for children with developmental disabilities?

This study aimed to determine the effectiveness of a health promotion toolkit for children with developmental disabilities on the family empowerment.

Research Objectives

1. Identify basic health-care needs of children with developmental disabilities.

2. Set a health promotion toolkit for promoting the health of children with developmental disabilities.

3. Examine parental stress among families caring for children with developmental disabilities.

Hypothesis

1. Health promotion toolkit would effectively improve families' empowerment.

2. Utilization of the health promotion toolkit would alleviate parental stress.

Methodology

The current study utilised a quasi-experimental design.

The study carried out at Shoaa El-Aml day care centre for disabled children in Umluj city at Tabuk region KSA. It is a non-profit organisation licensed by the Ministry of work.

The researcher calculated the sample size by the formula: $n = [(Z\alpha)^2*(S)^2]\div d^2$ at a confidence level of 0.95 and test power 80%. Where n is the sample size, Zα is the level of confidence, S is the standard deviation and d is the desired precision. The estimated sample size was 138 participants, however; the convenient number was 30 children and their families. They were included based on inclusion criteria (a) children who had a diagnosis of developmental disabilities, (b) age ranges between 3-18 years and (c) IQ ranges 50-70. The researcher

excluded children who had chronic renal failure and those who enrolled in a health education program during the last 3 months.

The Research Ethics committee at the Deanship of the academic research at Tabuk University granted ethical approval for this research. The researcher clarified the objectives, importance, and safety of the study to participants. Therefore parents were voluntary participate and assured confidentiality of their data.

The researcher utilised three tools for data collection, they include:

1. **Tool one:** Health promotion Assessment sheet. It is a structured interview questionnaire designed by the researcher to assess the current health status of children and mother's knowledge about developmental disabilities. It consisted of four parts.

a) Part one included data about children characteristic such as gender, age, weight, height, parental age, education and occupation and family number.

b) Part two included data regarding medical history, immunisation, health problems and dental problems.

c) Part three included self-care skills, social problems, behavioural problems, physical activity and eating habits and safety.

d) Part four contained data regarding mothers' perception of their children health and their knowledge about developmental disabilities.

Scoring system: - Scores for the evaluated items (health problems, medical history, dental problems, behavioral problems, social problems and safety) are absent (3), to some degree (2) and present (1); - Scores for self-care skills are independent (3), partially dependent (2) and completely dependent (1); - Scores for mothers' knowledge are complete (3), incomplete (2) and wrong (1).

2. **Tool two**: Family Empowerment Scale (FES) that developed by Koren [26]. The scale consisted of three subscales that related to family, child's services and parents' involvement in the community. The items of the scale ranged from 1 (never) to 5 (very often). Scores above 30 on the family and child's services sub-scales and above 25 on the parents' involvement in the community sub-scale indicated empowered families. Total scores on FES were 170 points. Scores above 85 indicate significant familial empowerment.

3. Tool Three: "Parent Stress Index-Short Form" developed by Abidin [27]. It is a 36-item self-reported questionnaire developed to measure parental stress. It composed of three domains that are parent-distress (PD); parent-child dysfunctional interaction (PCDI); and difficult child (DC). The items of the scale range from 1 (strongly disagree) to 5 (strongly agree). Scores above 33 on the PD and DC sub-scales and above 27 on the PCDI sub-scale are considered clinically elevated. Total score of the scale was 180 points. Scores above 90 indicate significant high level of parental stress**.**

Validity and reliability

1. Once the researcher adopted or designed the tools for data collection, she tested its content validity. Three experts in pediatric nursing and a paediatrician ascertain their relevance and completeness.

2. Reliability of the tools was determined to assess the extent to which items were related to each other. The reliability of Health Promotion Assessment tool was r = 0.69 by Cronbach's test. Koren [32] documented that Family Empowerment Scale has adequate internal consistency for each of the three subscales (Family: α= 0.88; Service System: α= 0.87; Community/Political: α= 0.88). The test-retest reliability was also found to be adequate for the three subscales (Family: *r* = 0.83; Service System: *r* = 0.77; Community/Political: *r* = 0.85). Abidin [33] confirmed the reliability of the parent stress index by Test-retest reliability coefficients that was 0.84 and by Cronbach's test was (r = 0.68).

Data collection and procedure

1. **Written permission:** The researcher attained an authorisation from Shoaa El-Aml Day Care Center to conduct the study after explaining the purpose of the study and methods of data collection.

2. **Pilot study:** A pilot study carried out on 10 mothers to assure clarity, consistency and feasibility of the tools. The researcher did not modify the tools.

3. ***Procedure:*** Data collection process is starting from March 2017 to September 2017 and contained three phases:

i. **Phase I (assessment phase**): children and parents assessment were performed to obtain baseline data regarding children and parents' characteristics, parents' knowledge about developmental disabilities, parent's perception of children overall health, children self-care skills, health problems and/or symptoms, behavioral problems, dental health, children safety, parents' stress level and empowerment level. Referral to Umliuj hospital was done for five children.

ii. **Planning Phase:** the researcher designed health promotion toolkit. It consisted of educational and training sessions for mothers which developed based on the identified areas of weakness in mothers' knowledge and health practices; it included the number of sessions, content and

methods of teaching. The objectives for health promotion toolkit were set as follows.

General objective

1. To provide mothers with knowledge, skills and positive attitudes toward caring for children with developmental disabilities.

Specific objectives

By the end of the educational sessions, mothers would be able:

1. To list the causes and types of developmental disability.

2. To identify how to manage different stressors adaptively.

3. To explain how to manage different behavioural problems.

4. To determine the methods used for managing enuresis.

5. To utilise the healthy eating pyramid for planning healthy meals.

6. To follow the steps of prevention of infectious diseases.

7. To perform first aids skills for children.

8. To apply safety measures for children.

9. To show a positive attitude toward the importance of periodic checkup for children.

10. To show a positive attitude toward the importance of dental care and hygiene.

11. To show a positive attitude toward the importance of physical activity.

iii. **Implementation Phase:** The health promotion toolkit implemented in the training unit at Shoaa ElAmal Day Care Center. The program implemented for five days with three educational and training sessions per day. Each session lasted from 30 to 45 minutes. Each session contained from 10-20 mothers. The researcher utilised think-pair-share, workshop, discussion, storytelling, debate, roleplaying, and demonstration teaching strategies. Also, PowerPoint presentation, videos, first aids' manikin, brochures, colouring books for children and dental care equipment teaching aids. These sessions held based on the four steps of family empowerment model which were:

a) Knowledge increase

During this stage, the researcher meant to increase mothers' knowledge. Mothers were assigned to four groups in which five mothers/group. The researcher discussed the following topic: concept of developmental disabilities, causes, types, diseases associated with or caused by developmental disabilities, prevention of infectious diseases, periodic checkup schedule for children, dental health and care, healthy eating, physical activity, children safety, first aids skills, management of behavioral problems, enuresis management and stress management.

b) Improvement of self-efficacy

Once the researcher completed the content of the training sessions, she asked mothers to demonstrate the learned practical skills. Their participation and achievement increase their self-efficacy and encourage them to learn more.

c) Increase self-esteem

At this phase, the researcher asked mothers who achieved the trained skills correctly to train another mother who could not demonstrate the skill. Therefore, they actively participated in the education process that improves their self-esteem.

d) Process evaluation

By the end of each session, the researcher got oral feedback from the mothers by asking questions and allowed free comments. Also, demonstration of selected skills.

iv. Evaluation Phase

Mothers' knowledge, stress and level of empowerment were evaluated after completion of the sessions.

Data Analysis

i. The collected data were coded for entry and analysis. The researcher utilised IBM Statistical Package for Social Science (SPSS) statistical package version 19 for data analysis and Excel program for graphic design.

ii. Data analysed by the mean (X), standard deviation (SD), one-way ANOVA, frequency distribution, chi-square ($\chi 2$) test. However, if the expected value of any cell in the table was less than 5, the researcher utilised the Fisher Exact test. The P-value < 0.05 was the set level of significance for all statistical tests.

Results

Table 1 shows the distribution of parents according to their characteristics. As indicated in the table, the mean and standard deviation of mothers' age was 39.33 ± 7.67 and 44.93 ± 7.99 for fathers. Approximately half of the mothers (56.7%) can read and write. Meanwhile, 56.7% of the fathers were illiterate. For occupation, most of the mothers (90%)

did not work and about half of the fathers (53.3%). More than half of families (53.3%) have a family number > 5-8 members.

Table 1: Distribution of parents according to their characteristics

Biosocial characteristics of studied parents	No	%
Age	*Mean* ± SD	
Mothers	39.33 ± 7.67	
Fathers	44.93 ± 7.99	
Mother's Education level		
Illiterate	0	0
Read and write	17	56.7
Secondary	8	26.7
University	5	16.7
Father's Education level		
Illiterate	17	56.7
Read and write	9	30.0
Secondary	4	13.3
University	0	0
Mother's occupation		
Work	3	10.0
Don't work	27	90.0
Father's occupation		
Work	16	53.3
Don't work	14	46.7
Family Number		
3-5	9	30.0
>5-8	16	53.3
>8	5	16.7

Figure 1 shows the distribution of families according to their income. It clarified that approximately 73% of families have an insufficient income per month.

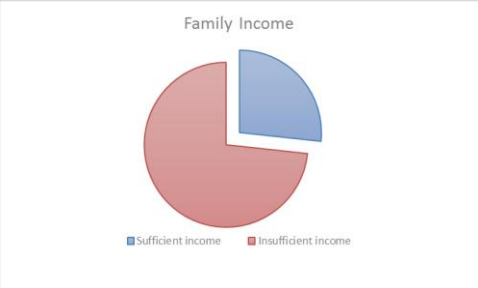

Figure 1: Distribution of families according to Family income

Table 2 shows the characteristics of the developmentally disabled children. It demonstrated that the mean age of children was 7.36 and more than half of them were boys (53.3%). The mean and standard deviation of their weight was 21.53 ± 5.9. For height, it was 99.32 ± 16.12. More than one-third of children diagnosed as mentally retarded.

Table 2: Characteristics of developmentally disabled children

Characteristics of Children	No	%
	Mean ± SD	
Age	7.36 ± 2.98	
Gender		
Girls	14	46.7
Boys	16	53.3
Weight	21.53 ± 5.9	
Height	99.32 ± 16.12	
Diagnosis		
Autism	7	23.3
Down syndrome	7	23.3
Attention Deficient Hyperactivity	5	16.7
Mental Retardation	11	36.7

Figure 2 clarified mothers' perception regarding their children health. It demonstrated that about half of mothers (53.3%) perceive good health of children and only 10 % perceive fair health.

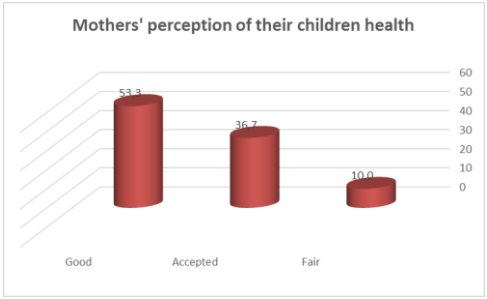

Figure 2: Mothers' perception regarding their children health

Figure 3 shows mothers' knowledge about developmental disabilities at pretest and posttest. It calcified that there was an improvement in knowledge of mothers' about developmental disabilities at posttest than on pretest. For this reason, there was the statistical significant difference at 5% levels of statistical significance.

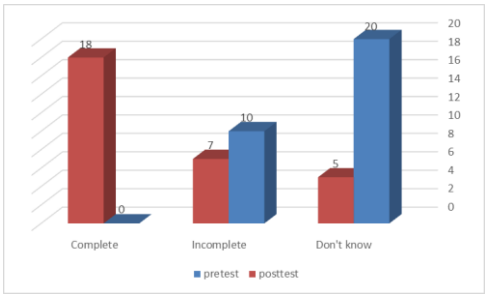

Figure 3: Mothers' knowledge about developmental disabilities at pretest and posttest

Table 3 describes the health problems among children with developmental disabilities. It illustrated that more than half of children (56.7%) have dental problems, more than a third of children (46.7%) have convulsion and more than half of children (60%) suffer from enuresis. Approximately one-third of children (36.7%, 33.3%) has fatigue and pain symptoms. For the sleep problem, 16.5 % of children have sleep problems.

Table 3: Health problems among children with developmental disabilities

Health problems	No (N = 30)	%
Dental problems	17	56.7
Convulsions	14	46.7
Enuresis	18	60
Fatigue	11	36.7
Pain	10	33.3
Sleep problems	5	16.5

Table 4 shows the distribution of common problems among children with developmental disabilities. It revealed that more than half of children (53.3%) have some independence in performing self-care skills and approximately one third (40%) were independent.

Table 4: Distribution of problems among children with developmental disabilities

Health problems	No (N = 30)	%
Self-care skills	12	40
Independent	16	53.3
Some independence	2	6.7
Dependent		
Mean ± SD	14.8 ± 4.86	
Behavioural problems	23	76.7
Self-injuries behaviours	28	93.3
Others- injuries behaviours	15	50
Masturbation	3	10
Mood fluctuation	20	66.7
Social problems	20	66.7
Hyperactivity	19	63.3
Agitation	16	53.3
Fear and anxiety	15	50

The majority of children (76.7%, 93.3%) have behavioural problems and self-injury behaviours. Meanwhile, two-thirds of children (66.7%) have mood fluctuation and social problems. Moreover, more than half of children (63.3%, 53.3%, and 50%) have hyperactivity, agitation and fear and anxiety problems consequently.

Table 5: Means and Standard deviations of Parents Stress on Pretest and Posttest

Parent stress index	Pre-test (n = 30) X ± SD	Post-test (n = 30) X ± SD	ANOVA test	P-value
Parental Distress	38.63 ± 7.13	34.0 ± 5.45	97.33	<0.001**
Parent–Child Dysfunctional Interaction	37.3 ± 6.07	33.63 ± 4.9	31.79	<0.001**
Difficult child	37.3 ± 3.24	33.1 ± 2.26	12.0	<0.001**
Total Parent stress index score	113.33 ± 12.12	101. 46 ± 10.48	15.95	<0.001**

Table 5 demonstrates the means and standard deviations of parental stress on pretest and posttest. It clarified that mothers at posttest had lower levels of parental distress (34.0 ± 5.45), parent-child dysfunctional interaction (33.63 ± 4.9), difficult child (33.1 ± 2.26) and total parent stress index score (101.46 ± 10.48). There were high statistical significant differences between levels of parental stress among mothers at pretest and posttest.

Table 6: Means and Standard deviations of family empowerment on pretest and posttest

Family Empowerment	Pre-test (n = 30) X̄ ± SD	Post-test (n = 30) X̄ ± SD	ANOVA test	p-value
Family	30.0 ± 3.15	42.06 ± 4.79	2.44	0.045 <0.01*
Child's services	28.66 ± 4.06	37.66 ± 8.16	2.67	0.034<0. 01*
parents' involvement in the community	23.6 ± 4.94	25.73 ± 8.14	2.79	0.027<0. 01*
Total Family Empowerment score	82.6 ± 8.33	105.0 ± 16.48	2.87	0.049 <0.01*

*P < 0.05.

Table 6 shows the means and Standard deviations of family empowerment on pretest and posttest. It clarified that mothers at posttest had the highest levels of family empowerment scores (105.0 ± 16.48). Moreover, mothers at posttest demonstrated high levels of empowerment within the three subscales family (42.06 ± 4.79), child's services (37.66+8.16) and parents' involvement in the community (25.73 ± 8.14). Therefore, the difference between the levels of family empowerment among mothers at pretest and posttest was statistically significant.

Table 7: Pearson correlation test between parental stress and family empowerment subscales on the posttest

	Parent stress index Score	
Parameter	R	p-value
Family	-0.51	0.004 <0.001**
Child's services	-0.083	0.66 ns
parents' involvement in the community	-0.476	0.008 <0.001**
Total Family Empowerment score	-0.439	0.015 <0.001**

ns P > 0.05; *P<0.05; **P<0.001.

Table 7 clarifies the Pearson correlation test between parental stress and family empowerment subscales on the posttest. It clarified that there was a highly statistically significant negative correlation between parental stress index score and family empowerment total score (r = -0.439), family (r = -0.51) and parents' involvement in the community (r = -0.476) on posttest at 1% level of significance. Also, parent stress was not statistically correlated to child's service subscale (r = -0.083) of family empowerment.

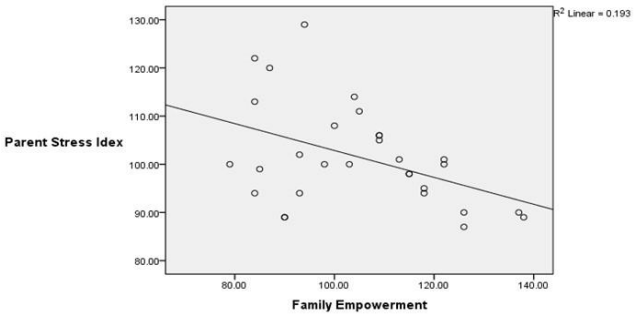

Figure 4: Pearson correlation between parental stress and family empowerment on the posttest

Figure 4 shows the Pearson correlation between parental stress and family empowerment on the posttest. It illustrated that there was a negative correlation at 1% between family empowerment and parental stress index on the posttest.

Discussion

Developmental disability is a major health concern. It includes a wide range of impairments that begin before the age of 18 years. These impairments may affect day-to-day functioning. Developmentally disabled children have high rates of adverse health conditions such as neurological, gastrointestinal disorders and generally, poor health status [9] [10]. Therefore, nurses should promote and coordinate health promotion practices to maximise children potential. However, a family is a central component in

their children's life. Nurses should empower those families to maintain and enhance the existing levels of their children health and thereby being able to live an active and productive life [18]. The current study hypothesised that health promotion toolkit would effectively improve families' empowerment and utilisation of the health promotion toolkit would alleviate parental stress.

Concerning children characteristics, the current study revealed that the mean age of children 7.36 years and more than half (53.3%) of children were boys. These findings are consistent with the finding of a Saudi survey conducted by El-Hzmi et al., [7]. They found that 53.2 % of studied children were males. In agreement with Mash and Wolfe [28] who indicated that the prevalence rate of mental handicapping conditions among boys is about one-half times more than girls are. Also, they demonstrated that the majority of children (70%) were in 5-15 years age group that is consistent with the children's mean age (7.36 years) of the current study.

Regarding parents' level of education, the results of the current study revealed that approximately half (56.7%) of mothers and 30% of fathers were read and write meanwhile, 56.7% of fathers were illiterate. These findings are consistent with Ghoneim et al., [29] who demonstrated that about half (52.7% & 58.1%) of fathers and mothers were without educational qualifications. This reflects that perhaps those families need to know more information regarding developmental disabilities and its care.

Concerning frequency of health problems among children with developmental disability, findings of the current study illustrated numerous problems that were dental problems (56.7%), convulsions (46.7%), enuresis (60%), fatigue (36.7%) and pain (33.3%). In addition, self-injuries behavior (93.3%), behavioral problems (76.7%), social problems (66.7%), mood fluctuation (66.7%), hyperactivity (63.3%), self-care deficit (53.3%) with a mean and standard deviation 14.8 ± 4.86, agitation (53.3%) and anxiety (50%).

These findings are congruent with studies conducted by Moes et al., [30], Abbeduto et al., [31] who reported the great prospect of behaviour problems in children with DD. Moreover, Yousef et al., [32] documented that mentally disabled children had behavioural, emotional, speech and language problems. They stated that impulsivity comprised 55.5% of behavioural problems and 56.3% of children suffered from social problems. Also, Ghoneim et al., [29] reported that mentally disabled children suffered from problems related to growth and development, mood, behavioural and social.

The presence of these problems provides a further challenge and a potential source of stress to the family. Identification of these problems will help in recognising and enhancing parents' abilities to meet their children needs solve their problems and mobilise the necessary resources [33].

Regarding mothers' perception of their children health, the current study documented that more than half (53.3%) of mothers perceived good health for their children and only 10% perceived fair health although those children had numerous health problems.

These findings could be related to traditions that impose mothers to hinder the actual health complaints of developmentally disabled children to protect them from social stigma. Moreover, some mothers still denial that her child has any developmental disability. Another reason could be attributed to inadequate mothers' awareness regarding their children health. This explanation is supported by another finding in the present study that two thirds (66.6%) of mothers did not know adequate knowledge about developmental disabilities before implementing the health promotion toolkit.

Regarding parental stress, findings of the current study revealed that parental distress levels were high in all three subscales and the total score of the Parent Stress Index. The mean and stander deviation of parental distress was (38.63 ± 7.13), parent-child dysfunctional interaction (37.3 ± 6.07), difficult child (37.3 ± 3.24) and total parent stress index score (113.33 ± 12.12). These results are consistent with the findings by Dardas and Ahmed [34]. They clarified that the highest score among the three subscales of parent stress index was PD (40.29) meanwhile; the lowest was PCDI (37.7).

Moreover, the scores of parental stress equal to or higher than 85th percentile that was pathologically high [35]. Also, high mean scores on child domain (132.38 ± 24.01) and parent domain (132.38 ± 26.13) of the PSI were documented [36]. Moreover, in agreement with Estes et al., [37] who proved the evidence for high levels of parent stress and psychological distress among parents of children with developmental disability.

This high level of parental stress could attribute to the fact that families of children with developmental disability have multiple demands on family resources. Another reason could be related to lack of sufficient income or adequate knowledge about developmental disabilities and access to community resources. This explanation was supported by the findings of the present study where the majority of families reported insufficient income and two-thirds of mothers lack adequate knowledge about developmental disabilities.

Concerning family empowerment, the current study revealed that mothers at posttest had significantly high levels of family empowerment total scores (105.0 ± 16.48) and within the three subscales family (42.06 ± 4.79), child's services (37.66 ± 8.16) and parents' involvement in the community (25.73 ± 8.14).

In agreement with Minjarez et al., [38], these findings were consistent with their pretest-posttest study in which they concluded that rating on family empowerment scale showed a significant change from pre to post-treatment indicating an increased level of family empowerment.

These findings are supported by the point of view of Dempsey and Dunst [39] who stated that enabling practices perceived as the prominent predictive variable of families' empowerment regardless of demographics. This could be attributed to the fact that educational programs increase mothers' knowledge and in turn change their practices and attitudes to become more adaptive. In other words, they indirectly empowered to provide adequate care for their children.

About the Pearson correlation test between parental stress and family empowerment subscales on the posttest. There was a high statistical significant negative correlation between parental stress index score and family empowerment total score (r = -0.439), family (r = -0.51) and parents' involvement in the community (r = -0.476) on the posttest. These findings were consistent with Minjarez et al. who reported that parents felt a high level of empowerment when they had a low level of stress [38].

Also, behavioural problems of children associated with high levels of parental stress had a negative effect on family empowerment [36]. Moreover, a correlational analysis between FES and PSI scales' confirmed the presence of a linear inverse relationship between parental stress and empowerment [40].

Finally, the findings of the current study clarified that health promotion toolkit had a positive effect on empowering families as well as lowering parental stress. Therefore, health promotion toolkit should be utilised as an approach for empowering families caring for children with developmental disabilities in Tabuk.

Recommendation

1. Health promotion toolkit should be integrated as a monitoring method of health care needs of health promotional activities for children with developmental disabilities.

2. Scientifically established clinical pathway of managing developmental disabilities using health promotion toolkit should be designed.

3. Home visits programs should be designed for families of developmentally disabled children to assess and meet their health needs based on implementing health promotion toolkit.

4. Establishing a support system for children with developmental disabilities and their families in Umulij and its surrounding villages through coordination between Ministry of Health, Tabuk University, and Shoaa El Aml Day Care Center.

5. Establishing a unit and/or outpatient clinic at Umlij general hospital provide sustained health and medical care for children with developmental disabilities.

6. Establishing societies in Umlij city to care for those families and their children.

7. The current study needs to be applied to a wider range of sample to ensure the generalizability of results.

The current study concluded that mothers of developmentally disabled children had high levels of parental stress and low levels of familial empowerment. Those levels are improved after implementing health promotion toolkit in which parental stress level is decreased, and familial empowerment level is increased. Also, a negative correlation between family empowerment and parent stress had been reported.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1512-1516.
https://doi.org/10.3889/oamjms.2018.285
eISSN: 1857-9655
Public Health

Evaluating Reliability of Theory of Planned Behaviour Questionnaire for Withdrawal of Divorce Petition

Nahid Ardian[1], Seyed Alireza Afshani[2*], Mohammad Ali Morowatisharifabad[1], Seyed Saeed Mazloomy Mahmoodabad[1], Ali Akbar Vaezi[3], Seyed Ali Asghar Refahi[4], Mohsen Askarshahi[5], Masoud Hadjizadehmeimandi[2], Hassan Zareei Mahmoodabadi[6]

[1]Social Determinants of Health Research Center, School of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran; [2]Faculty of Social Sciences, Yazd University, Yazd, Iran; [3]School of Nursing & Midwifery, Research Center for Nursing & Midwifery Care in Family Health, Shahid Sadughi University of Medical Science, Yazd, Iran; [4]Department of Social & Prevent Crime, Yazd, Iran; [5]Department of Biostatistics and Epidemiology, Shahid Sadoughi University of Medical Science, Yazd, Iran; [6]Department of Psychology, Yazd University, Yazd, Iran

Citation: Ardian N, Afshani SA, Morowatisharifabad MA, Mazloomy Mahmoodabad SS, Vaezi AA, Refahi SAA, Askarshahi M, Hadjizadehmeimandi M, ZareeiMahmoodabadi H. Evaluating Reliability of Theory of Planned Behaviour Questionnaire for Withdrawal of Divorce Petition. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1512-1516. https://doi.org/10.3889/oamjms.2018.285

Keywords: Reliability of Questionnaire; Theory of planned behaviour; Divorce

***Correspondence:** Seyed Alireza Afshani. Faculty of Social Sciences, Yazd University, Yazd, Iran. E-mail: aafshani@gmail.com

Received: 20-May-2018; **Revised:** 21-Jun-2018; **Accepted:** 26-Jun-2018; **Online first:** 14-Aug-2018

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Given the increased rate of divorce, it is important to analyse the characteristics of divorce applicants. The theory of planned behaviour (TPB) can provide a suitable framework to predict, explain, and/or change the behaviours. In Iran, no instrument can be found, based on health education models, to investigate divorce petition filing as a behaviour.

AIM: This study was conducted to design a questionnaire on withdrawal of divorce petition based on the TPB and estimate its validity and reliability.

MATERIAL AND METHODS: A qualitative study was conducted in 27 participants involved in the divorce process using directed content analysis. The face and content validity of 58 items, drawn from the qualitative study, were evaluated by 10 experts. The reliability was estimated using Cronbach's alpha coefficient. The SPSS version 16 was used to analyse data.

RESULTS: Estimates of the face and content validity (quantitative and qualitative), revealed that of the 58 items, 48 were valid based on four of the constructs of the TPB. Cronbach's alpha coefficient was also derived greater than 0.6.

CONCLUSION: The designed questionnaire, whose validity and reliability was confirmed in this study, can be used in similar studies. However, the social and cultural differences and their related effects should be considered.

Introduction

Divorce is one of the problems that impose stupendous costs on both the community and individuals [1]. Researchers have suggested several factors as causes of divorce such as marital dissatisfaction, extreme differences in beliefs, personality differences, cultural differences, financial problems, addiction, betrayal, lack of attention to gender identity and sexual orientation, and couples' families differences and meddling [2] [3].

Despite the increased rate of divorce across the globe, certain exceptions have been observed in different societies over time, such as decreased divorce rate and increased marriage length in some countries [4]. To solve couples' problems and reduce the divorce rate, different approaches have been proposed including education and counselling as well as qualitative and quantitative studies [5]. One approach is to investigate social problems is the use of models and theories of health education that can help provide effective communicative strategies to use suitable strategies and theories [6]. Fishbein and Ajzen's theory is based on two presumptions; according to the first one, people make decisions based on their logic and reasonable analysis of available data and the second one states that they consider the consequences of their behavior, Fishbein and Ajzen's theory of planned behavior (TPB) has been used to resolve different social and personal problems [7]. It seems reasonable to select the TPB and its constructs to analyse behavioural intention of

couples to withdraw divorce petition because the withdrawal of divorce is a behavior. The first factor affecting behavioral intention is attitude resulting from positive and negative beliefs about performing a behavior (divorce). Other factor is subjective norms which refer to the influence of the other people who are important to the individual, such as parents, family members, and relatives. The perceived behavioral control refers to one's beliefs regarding personal control over the performance of the behavior and one's belief in their own ability to succeed in performing the behaviour [8]. The perceived behavioral control may be improved by education and skill training, which affects the behavioral intention and behavior change (withdrawal of divorce petition).

To conduct a more comprehensive study and design an appropriate measuring questionnaire, it is recommended to use qualitative methods and obtain a correct perception of experiences [9] [10]. The scales of the TPB should be prepared by a pilot study to ensure the psychometric properties [11]. Despite the need for a valid questionnaire based on the TPB, no study has yet been conducted in Iran to investigate the validity of such a questionnaire.

Because the validity and reliability are affected by changes in the society, the present study sought to design a questionnaire using the results of a qualitative study, leading to a better understanding of withdrawal of divorce. This study is part of a larger study on the use of the TPB in the withdrawal of divorce petition.

Methods

Necessary data were collected in a qualitative study using directed content analysis based on the TPB to design an efficient questionnaire. The study population of the qualitative study included 27 people, 10 of whom were couples who had been referred to the Family Counseling Center, seven were family members of the couples, four were counselors, three were social workers of the Family Counseling Center, and three were Judges and their advisors in the Family Court of the Judicature. The participants were selected by purposive sampling. The only inclusion criterion was providing consent to be interviewed and to collaborate with the study. The exclusion criterion was withdrawing from the interview. Table 1 shows the demographic characteristics of participants. (The qualitative section of the study is going to be published)

In interviewing different groups of samples during the qualitative study, items on the constructs of the TPB or those of the questionnaire were extracted. The pilot questionnaire included 58 items classified as follows: 16 on attitude, 24 on perceived behavioural control, 10 on subjective norms, and 8 on intention). The items were rated on a 5-point Likert scale (from Absolutely agree = 5 to Absolutely disagree = 1), to evaluate the validity, including face and content validity.

Validity refers to the extent of covering the concept a test aims to measure. There are various methods to estimate validity which determine the relationship between a concept (variable) and operative indices selected to measure it. The fundamental methods to evaluate validity are faced validity and content validity. Qualitative face validity indicates whether a questionnaire is appropriate to the study purpose and content area, based on respondents' viewpoints [12]. Participants were first asked to estimate the face validity.

Item clarity: Item clarity addresses the question of how much a test is valid based on respondents' opinions? The pilot questionnaire was filled out by 21 individuals referring to the Family Counseling Center who did not participate in the main study.

After the pilot study, participants were asked to identify any item that was difficult to understand or confusing, express their viewpoints regarding the appropriateness of phrases concerning the questionnaire dimensions, and identify ambiguous items. In the next step, certain items were revised or deleted, or some items were added.

To determine the importance of each item, the item impact method was used. For this purpose, 21 respondents were asked to evaluate the importance of items using a 5-point Likert scale (from 1 to 5):

Impact score = Frequency (%) × importance

Item score indicates the score derived by the item impact method; frequency refers to the percentage of respondents attaining a score of 4 or 5, and importance refers to the average score of the item based on the Likert scale. According to the above formula, those items that attain an item impact score of 1.5 or more remained. According to results, no item was deleted in this step.

The pilot questionnaire was assessed by 10 experts including six university teachers of health education, two university teachers of sociology, and two university teachers of epidemiology. They were asked to examine the questionnaire for grammatical structure, vocabulary, phrases, scoring, and necessity of items, and to see whether it is necessary to add further items. The structure and wording of some items were changed according to experts' comments. Also, the theme of some items was changed, e.g. three items under the intention theme were transferred to the theme of behavioural control.

To determine the CVR, the experts (10 experts participating in the previous part) were asked to judge the necessity and usefulness of all items. The

formula used by the experts to calculate the CVR was as follows: CVR = (n-Ne/2)/(Ne/2).

Where N represents the number of experts judging the items as necessary, and Ne represents the number of evaluators. The CVR greater than .62 was confirmed by Lawshe's table. Items were confirmed or rejected according to the protocol as follows:

If the item CVR were equal or higher than .62, the item would be confirmed; if the CVR was between 0 to 0.62 and the impact ratio was higher than 1.5, the item would be confirmed; if the CVR was less than 0 and the impact ratio was higher than 1.5, the item would be rejected. At the end of this step, 6 items were deleted, and 51 remained. Table 2 shows the CVR scores, numerical means for judgments, and acceptance or rejection of each item.

CVI, which showed the generalizability of judgments made by the 10 experts, indicated the validity of the applicability of the final version of the questionnaire: Simplicity and understandability: 1. The item is not simple; 2. The item is relatively simple; 3. The item is simple, 4. The item is highly simple. Relevance: 1. The item is not relevant; 1. The item is relatively relevant; 3. The item is relevant; 4. The item is fully relevant. Clarity: 1. The item is not clear; 2. The item is relatively clear; 3. The item is clear, 4. The item is fully clear. CVI was estimated by the formula below:

$$CVI = n/Ne \geq 0.79$$

The CVI was calculated as the sum of scores 3 and 4 divided into the total number of scores. Items with CVI more than .79 were accepted, items with CVI between 70% to 79% were considered vulnerable and to need revision, items with CVI less than 70% were not considered acceptable and therefore deleted. The content validity of the questionnaire was confirmed if the CVI scores were acceptable.

By using reliable instruments, we can obtain more dependable results and also similar conclusion if we replicate the study. To estimate reliability, the Cronbach's alpha coefficient was used. Concerning similar studies, the least number of samples to conduct a pilot test to estimate Cronbach's alpha coefficient is 20. The pilot questionnaire was filled out by 21 samples. Because the literacy levels were different in this phase, the interviewer asked the questions and filled out the questionnaire. According to the results, internal reliability was determined by Cronbach's alpha coefficient.

Results

Table 1 shows Personal Characteristics of participants of the qualitative study.

Table 1: Personal Characteristics of participants of the qualitative study

Participants	N (%)	Gender N (%)		Age N (%)		Occupation N (%)		Education N (%)		
		Female	Male	31≤	= 30	Unemployed	Employed	BM and more	Diploma to bachelor	Primary
Couples	10(37)	4(40)	6(60)	7 (70)	3(30)	2(20)	8(80)	3(30)	1(10)	6(60)
Parents	7(25.9)	6(85.7)	1(14.3)	7(100)	0	6(85.7)	1(14.3)	0	0	7(100)
Counselor and social workers	7(25.9)	5(71.4)	2(28.6)	7(100)	0	0	7(100)	5(71.4)	2(28.6)	0
Family judges	3(11.1)	1(33.3)	2(66.7)	3(100)	0	0	3(100)	2(66.7)	1(33.3)	0
Total	27	16	11	24	3	8	19	10	4	13

Of the 52 items, 48 were selected. CVR for each item was estimated (Table 2).

Table 2: CVR scores, the numerical mean of judges, acceptance of items

Acceptance	CVR	Number of confir-mation	Attitude	
Accepted	1	10	To continue a stressful marriage may have negative effects on children	1
Accepted	0.86	9	To continue a stressful marriage may cause physical damages to me.	2
Accepted	0.64	8	To continue a stressful marriage may cause financial losses for me.	3
Accepted	0.64	8	To continue a stressful marriage may cause mental suffering.	4
Accepted	0.64	8	For me, divorce is the last solution to my life problems.	5
Rejected	0.22	6	For me, divorce menace erasing my previous mistake in selecting my spouse	6
Accepted	0.64	8	Thinking on divorce is stressful.	7
Accepted	0.86	9	Divorce is a problem and damage to the society.	8
Accepted	0.64	8	Divorce is a barrier on the way of my progress.	9
Rejected	0.22	6	Divorce is not a beautiful word.	10
Accepted	0.64	8	Society has a negative attitude toward the divorced individuals.	11
Accepted	0.64	8	For me, to think about divorce is also annoying.	12
Accepted	0.64	8	For me, to accept divorce is annoying.	13
Accepted	0.64	8	I think divorce means loneliness and perplexity.	14
Accepted	0.86	9	Divorce is a sort of freedom from the difficulties of the past life.	15
Accepted	0.64	8	To continue a stressful marriage may have negative effects on our children	16
Perceived behavioural control				
Accepted	0.86	9	The problem between my spouse and my parents made reconciliation impossible.	1
Accepted	0.86	9	Since our families are involved in the conflicts, we cannot reconcile.	2
Rejected	0.43	7	Being the only child of the family made for me impossible to decide reconciliation.	3
Accepted	0.86	9	Without meddling of a family of my spouse, I can reconcile.	4
Accepted	0.86	9	Without the help of a family of my spouse in solving our problems, I cannot reconcile.	5
Accepted	0.86	9	After suspicious cases of communicating with the opposite sex, I cannot continue my marriage.	6
Accepted	0.64	8	Dowry and using it as a powerful means, filing a divorce petition by women becomes easier and more possible.	7
Accepted	0.64	8	It is impossible to continue my marriage because my spouse is a pessimist.	8
Accepted	0.86	9	I cannot continue my marriage since my spouse cannot decide independently.	9
Accepted	0.86	9	I cannot continue my life with a spouse who is not responsible for marital life.	10
Accepted	0.86	9	It is impossible to reconcile due to sexual reluctance and coldness of my spouse	11
Accepted	0.86	9	I cannot continue my marriage since my spouse does the violent behaviour.	12
Accepted	0.64	8	I cannot continue my marriage with the one who takes me to court and sends me to the jail.	13
Accepted	0.64	8	Due to the long absence of my spouse, I can not continue my marital life.	14
Accepted	0.64	8	I cannot continue my marital life since my spouse is imprisoned.	15
Accepted	0.86	9	If my spouse changes his/her behaviours, I can continue my marital life.	16
Rejected	0.43	7	Due to appropriate education and counselling, it becomes possible to continue our marriage	17
Rejected	0.22	6	By receiving appropriate counselling and education at other organisations like NGOs, drug rehab centres, etc., it becomes possible to continue our marriage.	18
Rejected	0.43	7	Because of an opportunity to think during the divorce process in Family Counseling Center, I can better think about reconciliation.	19
Accepted	0.64	8	Although I reconciled once by counsellors, I cannot reconcile again.	20
Accepted	0.64	8	As a member of the new generation, I will not tolerate problems in marital life like those belonging to the past.	21
Accepted	0.64	8	Having a forced marriage, I cannot tolerate my marriage.	22
Accepted	0.64	8	Because of my spouse' severe chronicle addiction, I cannot continue my marriage.	23
Accepted	0.64	8	Because of my spouse betrayal, I cannot continue my marriage.	24
Accepted	0.86	9	Due to the unchangeable behaviours of my spouse, I cannot continue my marriage.	25
Subjective norms				
Accepted	1	10	Watching inappropriate satellite programs and misuse of social networks, made adultery more acceptable for my spouse.	1
Accepted	1	10	Increasing the rate of divorce made it easier to decide to divorce.	2
Accepted	1	10	My religious beliefs encourage me to tolerate life problems.	3
Accepted	0.86	9	Counsellors of the Family Counseling centre support me to reconcile.	4
Accepted	0.64	8	My sexual partners encourage me to divorce.	5
Accepted	1	10	My parents support me to divorce.	6
Accepted	1	10	Mothers-in-law have a great influence on encouraging the wife to divorce.	7
Accepted	1	10	My spouse' family encourages my spouse to divorce.	8
Accepted	0.86	9	My spouse' family support us to reconcile.	9
Accepted	0.86	9	My family encourage me to reconcile	10
Intention				
Accepted	0.64	8	I want to reconcile although we decide to divorce together.	1
Accepted	0.86	9	I intend to reconcile since I decide to divorce in a hurry.	2
Accepted	1	10	I intend to reconcile while emotion subsides after a quarrel.	3
Accepted	0.86	9	To reconcile, I intend to be more patient.	4
Accepted	0.86	9	Although my family disagrees, I want to reconcile.	5
Accepted	1	10	I file the divorce petition to punish my spouse.	6

Content validity was confirmed by an estimated CVI of 0.79 according to the above formula.

Table 3 shows the Cronbach's alpha coefficients of the constructs. If the alpha coefficients were equal or higher than 0.6, it was considered appropriate. In this step, three items (items 2, 8, and 10 of subjective norms) were deleted. Finally, 48 items were selected.

Table 3: reliability of items according to constructs

Consistency	Number of items	Themes of questionnaire
0.73	14	Attitude
0.84	21	Perceived behaviour control
0.63	7	Subjective norms
0.91	6	Intention

Discussion

Increased rate of divorce petition filing is a social problem that has forced certain organisations such as the Judicature and Welfare Organization to attempt to reduce. As with some studies, the current study is also theory-based [13] [14]. Although some studies on the questionnaire of marital or family satisfaction have been done in Iran [15] [16] [17] no study has yet been conducted on withdrawal of divorce based on health education models, especially the TPB. This study sought to develop an instrument on withdrawal of divorce petition based on the TPB and to estimate its validity and reliability. To determine the content validity, the designed questionnaire was evaluated by 10 experts of health sciences, sociology, and counselling. However, in the simple cases, fewer experts are involved [18] [19]. Because of the complexity of divorce as a social phenomenon, 10 experts were involved. The expert panel and their different viewpoints, due to differences in their fields of study, made it possible to use their viewpoints in evaluating the qualitative content of the questionnaire. It is noteworthy that this study data were collected in a qualitative study including primary interviews, encoding, and directed content analysis.

Moreover, codes were drawn by interviewing different groups of people involving in divorce, and different experts participated in the evaluation and estimation of the instrument's face and qualitative content validity. In previous studies, fewer experts were involved in validity evaluation [15] [16] [17]. In the studies on marriage satisfaction, CVR and CVI were not taken into account. The present study was first to use these methods to determine people's status, including aspects of attitude, perceived behavioural control, subjective norms, and divorce intention and divorce withdrawal.

The results showed that the designed questionnaire was relatively reliable. Reliability refers to the consistency and coincidence within the constructs of an instrument [20]. In a study conducted in 5 countries, the internal consistency of the TPB was obtained from 0.52 to 0.89 [21] [22]. The results showed that three constructs were significantly reliable, but under the theme of subjective norms, the estimated alpha was 0.63. Although this alpha coefficient represents reliability, it can also be interpreted that probably in different social and cultural contexts, the factors affecting people's subjectivity and decision making are also different and effectiveness of other factors on subjectivity is more apparent.

To evaluate the face validity, people dealing with divorce were asked to fill out the questionnaire in two sections: First, items on four constructs of the TPB; second, 35 items on demographic characteristics. Then, they were asked to detect the inappropriate items. They were also asked to mention the items that seem ambiguous and unnecessary and to introduce new items that they felt they are necessary. To evaluate face validity, quantitative method of impact score was used, as with many other studies [23].

Having reviewed the literature on validity and reliability extensively, we found no questionnaire on divorce and divorce withdrawal. As a limitation of this study was purposive convenience sampling. To obtain better measures of validity and reliability, random sampling can be used. Also, construct validity not estimated because the qualitative method was applied and a limited number of samples participated. In additional studies, more samples should be enrolled to measure this type of validity. Because of drawing items via qualitative interviews and use of personal experiences, some items were deleted after various steps of reliability and validity measurement.

In conclusion, our results showed that the questionnaire drawn by the qualitative method and directed content analysis based on the TPB is relatively valid and reliable. It is a suitable tool to evaluate behavioural intention and may be used to explain divorce behaviour, considering social and cultural differences.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1517-1521.
https://doi.org/10.3889/oamjms.2018.298
eISSN: 1857-9655
Public Health

Physical Activity Stage of Change and Its Related Factors in Secondary School Students of Sarableh City: A Perspective from Iran

Ali Saeidi[1], Amin Mirzaei[1], Behzad Mahaki[2], Asadollah Jalali[1], Mohsen Jalilian[1*]

[1]Department of Public Health, Faculty of Health, Ilam University of Medical Sciences, Ilam, Iran; [2]Department of Statistic and Epidemiology, Faculty of Health, Kermanshah University of Medical Sciences, Kermanshah, Iran

Citation: Saeidi A, Mirzaei A, Mahaki B, Jalali A, Jalilian M. Physical Activity Stage of Change and Its Related Factors in Secondary School Students of Sarableh City: A Perspective from Iran. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1517-1521. https://doi.org/10.3889/oamjms.2018.298

Keywords: Transtheoretical Model (TTM); Physical activity; Secondary School; Adolescent

***Correspondence:** Mohsen Jalilian. Department of Public Health, Faculty of Health, Ilam University of Medical Sciences, Ilam, Iran. E-mail: jalilian91@yahoo.com

Received: 28-Apr-2018; **Revised:** 24-Jun-2018; **Accepted:** 03-Jul-2018; **Online first:** 15-Aug-2018

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Funding: This research did receive financial support from the Ilam University of Medical Sciences

Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Physical activity is highly beneficial to health. These benefits are so important and indispensable for adolescents.

AIM: The aim of this study was to investigate the Physical activity stage of change and its related factors in the male secondary School students of Sarableh city.

MATERIAL AND METHODS: In a cross-sectional study, 261 male secondary School students possessing the eligibility criteria were selected using the simple random sampling technique. After giving their informed consent, the students filled the stage of change questionnaire and the structures of the transtheoretical model in a self-reporting manner. Using SPSS.21, the data were analysed through One-way ANOVA and Pearson correlation test with a 0.05 level of significance.

RESULTS: The mean age of the students was 16.16±0.89 years. According to the stage of change, 26.8 per cent (n = 70) were in preaction stages (precontemplation, contemplation, and preparation) and 73.2 per cent (n = 191) were in action and maintenance stages. The one-way ANOVA revealed that awareness is raising, self-reevaluation, counter conditioning and reinforcing management differed significantly across stages ($P < 0.05$). However, this difference is not significant for other cognitive and behavioural processes ($P > 0.05$). According to the findings, increase in the self-efficacy, pros and decrease in cons was found by students' progress in the Physical activity stage of change ($P < 0.05$).

CONCLUSION: Self-efficacy and processes of change are warranted when designing Physical activity stage interventions in the adolescents.

Introduction

Physical activity (PA) plays a significant role in maintaining health, especially for adolescents. PA has many different benefits including prevention and reduces the risk of cardiovascular diseases, high blood pressure, and diabetes [1]. Moreover, it also has advantages about the improvement of mental health including improving stress management, reducing anxiety and depression, increasing self-confidence, the spirit of cooperation and academic achievement [2]. It should also be noted that PA is the key to energy consumption and weight reduction [3]. Regular PA is necessary for reinforcing motor skills and empowering the musculoskeletal functions [4]. PA is one of the important aspects of a healthy lifestyle, but many adolescents are not active enough to benefits from it [5] [6]. Obesity which is one of the factors closely linked with heart diseases, high blood pressure, and type 2 diabetes, has had an increasing trend during the last 30 years [7]. According to the World Health Organization (WHO), insufficient PA is among the 4 main causes of death in the world and every year about 2 million people die as a result of this factor [5]. The estimations provided by the WHO show that about 80 per cent of adolescents all around the world do not have sufficient PA [8]. The minimum

amount of sufficient PA for adolescents between 15 and 17 years of age is 60 minutes of intense or moderate activity during the day [9]. According to the WHO, in 2013 about 27.1% of secondary school students had less than 60 minutes of PA during the day, and 15.2 per cent of them are physically inactive [7]. In Iran, According to the Caspian study about investigating the state of behaviors related to students' health, it is estimated that 5.4% of elementary school students, 9.2% of the students in the first level and 13.3% in the second level of secondary school did not have at least 30 minutes of PA per day. 21.9% of elementary school students, 15.6% of secondary school students and 14.4% of secondary school students had reported less than 30 minutes of PA per day [10]. According to the evidence, in determining PA behaviours in adolescents, various factors must be considered. Thus, identifying these factors can provide an appropriate framework for implementing effective health education interventions. Training through school-based interventions is so important for increasing the adolescents' PA [11]. Transtheoretical Model (TTM) is one of the models that properly take these factors into consideration, includes precise planning for behaviour change, and is frequently used by researchers for evaluating PA and improving it. The TTM was introduced in 1980 by James O. Prochaska; it is one of the comprehensive models of behaviour change. The major constructs of TTM are the stage of change (soc), self-efficacy, decisional balance and processes of change.

Following the structure of the SOC, in this model people are faced with different stages of preparation for change, and for behaviour change go through the stages of precontemplation, contemplation, preparation, action, and maintenance. According to this model, people use cognitive and behavioural processes to progress in the SOC, and when their assessment of the benefits (pros) of the behaviour is more than its disadvantages (cons), it will be easier and more probable to move to higher stages. Self-efficacy in doing PA, which refers to the person's judgment regarding his ability for performing athletic behaviours under various circumstances, is the major factor that can facilitate the person's move to higher stages [12]. Thus, the present research aimed at investigating the PA SOC and its predictors according to the TTM in the male secondary school students of the Sarableh city.

Materials and Methods

This research was of a descriptive-analytic study which conducted on 261 male students of the secondary schools of Sarableh city located in the west of Iran, in 2017. The students enrolled in the study using a simple sampling method. The eligibility criteria for entering the study included not having physical disabilities, studying in public schools and studying in secondary schools. To collect the data for the study, first of all, the researches took the required certificates to enter the schools. Afterwards, they explained the objective of the study to the students and filled the informed consent form for them. Thus, the students who filled the form entered the study and filled out the questionnaire. So, a self-report questionnaire was used to calculate demographic characteristics and the TTM constructs related to PA. The 5-item questionnaire by Marcus was used to determine the SOC [13].

Moreover, Blanchard et al., [14] decisional balance questionnaire, the Norman et al., processes of change questionnaire [15] and the Nigg et al., [5] scale was also used to assess the self-efficacy in PA. All of the mentioned questionnaires were scored in 5 points Likert scale. It should be noted that these questionnaires have been used in various studies and their validity and reliability have been confirmed in Iran [16][17]. Finally, data were analysed using SPSS.21 with considering a significance level of 0.05 (confidence interval=95%) and conducting statistical tests including descriptive tests, one-way ANOVA and Pearson correlation.

Results

This study involved 261 secondary school students from four schools including Imam Ali (n = 49), Hesabi (n = 20), Razi (n = 85) and Enqelab (n = 107). The age average of the students was 16.16 ± 0.89 years. Of these 261 students, 167 (64%) lived in urban areas and 94 (36%) in rural areas. The distribution of students in the four schools was as follows: 49 students (18.8%) from Imam Ali, 20 students (7.7%) from Hesabi, 85 students (32.6%) from Razi, and 107 students (41%) from Enqelab. Further details are provided in Table 1.

Table 1: Demographic characteristics of the study participants

Variable		Frequency	Per cent
Location type	Urban	167	64
	Rural	94	36
Family income	Low	127	48.7
	moderate	105	40.2
	Imam Ali	49	18.8
School	Hesabi	20	7.7
	Razi	85	32.6
	Enghlab	107	41
	Underweight	68	26.1
	Normal	170	65.1
BMI	Overweight	21	8.0
	Obese Class I	1	0.4
	Obese Class II	1	0.4

The findings of the study demonstrated that 70 of the participants in the study (26.8%) are in preaction stages (precontemplation, contemplation, and preparation) and the other 191 (73.2%) are in the

action and maintenance stages of PA. Comparison of the average and standard deviation of the cognitive and behavioural processes of change, self-efficacy in performing PA, and the benefits and obstacles of PA are represented in table 2, based on the SOC. The findings of a one-way analysis of variance showed that using processes of consciousness-raising, self-reevaluation and the counter conditioning were significantly different within SOC ($p < 0.05$). But this difference is not significant for processes of dramatic relief, environmental-reevaluation, social liberation, helping relationships and stimulus control ($p > 0.05$). A significant difference in the mean of persons' self-efficacy in different SOC was found. So, higher self-efficacy was related to transition upper stages ($p < 0.05$). Also, the findings were demonstrated a significant difference in the mean of the perceived benefits (pros) of PA by stages ($p < 0.05$). But this difference was not significant for the disadvantages (cons) to PA ($p > 0.05$).

Table 2: Mean and SD of the processes of change, self-efficacy, decisional balance (pros and cons) to PA by SOC

Variables	Pc			C			P			A			M			Sig
	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	
Consciousness raising	30	2.5	1.4	12	3.1	1.03	28	2.9	1.2	76	2.9	1	115	3.3	1.2	0.02
Dramatic relief	30	7.7	2.2	12	9.2	3.51	28	8.2	2.4	76	8.6	2.4	115	9.2	2.9	0.44
Environmental reevaluation	30	2.6	1.1	12	3	1.53	28	3.1	1	76	3.1	1.1	115	3	1.4	0.512
Self-reevaluation	30	9.3	2.8	12	11.5	2.5	28	10.3	3.17	76	10.6	2.6	115	11.7	2.7	0.00
Social liberation	30	5.7	2.1	12	6	1.47	28	6.3	1.9	76	6.21	1.9	115	6.6	1.8	0.13
Counter conditioning	30	7.2	2.7	12	8.1	2.88	28	8.4	2.2	76	8	2.8	115	9.2	3	0.00
Helping relationships	30	2.3	1.2	12	3	.95	28	2.6	1	76	2.8	1.3	115	3	1.3	0.06
Reinforcement mangemet	30	2.6	1	12	3.5	1.24	28	2.8	1.2	76	3.1	1.2	115	3.4	1.2	0.00
Self-liberation	30	2.5	1	12	3.2	1.13	28	2.8	1.1	76	3	1.1	115	3.3	1.2	0.02
Stimulus control	30	4.4	1.9	12	5	2.17	12	5	2.1	76	4.8	2.2	115	5.2	2.1	0.24
Self-efficacy	30	28.2	7.5	12	33	9.59	28	28.3	9.8	75	30.1	8.3	115	32.9	9.1	0.01
Pros	30	28	8.3	12	35.5	6.51	28	31.2	7.3	76	32.3	7.1	115	34.4	6.7	0.00
Cons	30	17	5.5	12	14.9	5.01	27	14.7	3.9	76	14.7	3.9	115	15	5.2	0.26

Note. Pc (Precontemplation), C (Contemplation), P (Preparation), A (Action) and M (Maintenance).

Moreover, investigation of the correlation intensity of the processes of change, self-efficacy and pros to PA with the SOC demonstrated that there is a direct and positive relationship between the structures above and the SOC.

Table 3: Correlation between the constructs of the TTM

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Mean	SD
Stage		.17**	.15**	.05	.26**	.15**	.19**	.15**	.20**	.19**	.13**	.17**	.20**	-.07**		
Consciousness -raising	1		.33**	.27**	.49**	.29**	.31**	.25**	.36**	.32**	.25**	.27**	.56**	-.00	3.07	1.2
Dramatic relief		1		.51**	.50**	.37**	.36**	.32**	.39**	.30**	.19**	.28**	.44**	.04**	8.78	2.7
Environmental reevaluation			1	.38**	.31**	.36**	.34**	.39**	.33**	.17**	.31**	.39**	-.02	3.04	1.2
Self-reevaluation				1	.50**	.48**	.38**	.58**	.52**	.26**	.43**	.69**	-.11*	10.9	2.8
Social liberation					1	.45**	.35**	.29**	.29**	.32**	.25**	.45**	.08	6.34	1.9
Counterconditioning						1	.47**	.42**	.36**	.54**	.52**	.47**	-.14**	8.52	2.9
Helping relationships							1	.43**	.28**	.26**	.26**	.38**	-.10*	2.86	1.2
Reinforcement management								1	.53**	.29**	.40**	.53**	-.08	3.21	1.2
Self-liberation									1	.24**	.38**	.45**	-.12*	3.10	1.1
Stimulus control										1	.48**	.24**	-.09	4.97	2.1
Self-efficacy											1	.39**	-.07	31.1	8.9
Pros												1	-.11*	32.8	7.3
Cons													1	15.1	4.8

But the correlation of the cons and progress in SOC was negative and weak ($r = -0.079$, $p > 0.05$). Furthermore, the participants reported a strong and direct correlation in adopting the processes of change and the pros to PA ($p < 0.05$). Moreover, the correlation was significantly negative with the cons of PA ($p < 0.05$); except for the dramatic relief and social liberation ($p > 0.05$).

The findings of the Pearson correlation test showed that there is a positive and strong relationship between self-efficacy and using processes of change ($p < 0.05$). This relation was direct and negative for the cons to PA ($p > 0.05$).

The Fig. 1 shows the findings of the study participants' decisional balance. Our findings show that the perceived benefits of PA increase with progress in the SOC, while the perceived disadvantage (cons) decrease.

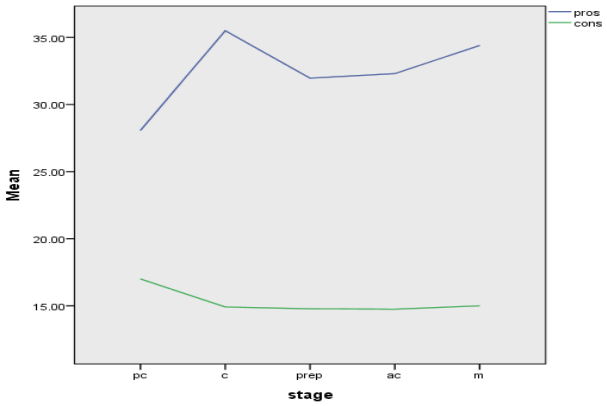

Figure 1: Pros and cons of PA by SOC

Discussion

This study aimed at the evaluation of the relation between the TTM constructs related to PA behaviour in the Secondary School students. Results showed that about 27% of the participants were in the stages of precontemplation, contemplation, and preparation and about 73% were in the stages of action and maintenance of PA. These results are also reported in the study by Aghamolaei et al., (2016) which was carried out on a Sample of Employees in Iran [18]. Considering the general trend of PA among students in the world, the initial expectation is that the percentage of students in the stages of precontemplation, contemplation, and awareness should be high. One of the reasons why the results of this study are different from those of the similar studies can be the fact that the age of the participants is rather low. This factor has also been reported in similar studies such as the study by Rostami et al., (2017), [19]. The impact of the programs by the World Health Organization (WHO) regarding the improvement of PA among the students should also be noted because increasing PA has been one of the concerns of the World Health Organization (WHO) in recent years [5]. This shows how serious the country's

authorities are about the improvement of PA. The reasons for lack of PA in the population under study include low perceived sensitivity, economic problems, physical disability for PA, wastage of free time on school, low level of exposure to the optimal models of PA at school and home (lack of access to optimal models), lack of motivation, influence of friends and classmates activity and self-efficacy in PA, place of residence, lack of access to facilities, and not being aware of the benefits of PA [4]. The earlier studies, including Anderson et al., in 2018, reported the positive role of parents as an effective environmental factor which plays the role of the model for the students in improving PA [1]. These results have also been reported by Teymouri et al., (2008) in which 65% of the students under study had acceptable PA (stages of action and maintenance), [20]. The findings show that using processes of increasing awareness, self-reevaluation, and the counter conditioning significantly varies in different SOC. These findings consist of Rostami et al., (2017) study which was carried out on adolescents [19].

Moreover, Miri et al., study declared that self-efficacy has a negative relation with weight gain in students. This study also shows that obesity can be considered an obstacle to willingness for PA [21]. In this study, it has been reported that perceived self-efficacy, as well as pros and cons, are significantly different in the SOC.

Moreover, a positive correlation between SOC and positive self-concept along the SOC has been reported. This has a positive effect on the tendency to engage in PA and as a result, leads to improving self-efficacy [19]. Accordingly, this finding is confirmed by Charkazi et al., (2011) study on the effective role of reinforcing management on the PA SOC [22]. Moreover, the findings of the study show that the pros along the SOC are significantly different. According to the TTM, this increase from the precontemplation stage to the maintenance stage was expected [23].

So, students in lower stages such as precontemplation and contemplation are less aware of the benefits of PA include the feeling of success, improvement of strength and physical power, improvement of health status, reduction of stress and anxiety, and improvement of academic performance. In other words, it can be said that people experience these benefits in the stages of action and maintenance, and as a result, have a better understanding, insight, and enthusiasm for maintaining this PA behaviour. These results have been reported by Rostami et al., (2017), [19]. Regarding the improvement of self-efficacy along the transition from SOC, there are similar results in other studies. For example, it has been reported in the study by Kim et al., [24] that self-efficacy can have an increasing trend along the passage from the SOC towards higher stages [24]. Furthermore, the study by Jalilian et al., (2012) also reported similar results about the self-efficacy along the SOC [25].

In conclusion, according to the PA SOC, about 70% of the students were physically active, and about 30% were in the stages preceding PA, which means they are still inactive. Thus, it is necessary to design and conduct further interventional studies to improve PA in students. Moreover, the results of the study showed that about 30% of the students have a BMI higher than the normal level, and this points to the importance of planning for the improvement of PA and reduction of BMI in this group through designing suitable interventions. Moreover, Designing and implementing interventional programs emphasising the role of self-efficacy, and the inclusion of parents in the programs can promote regular PA in students [1].

Acknowledgement

This research was a part of the thesis in master degree of health education. We are grateful to the Ilam University of Medical Sciences for financial support, and to all the study participants.

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https://doi.org/10.3889/oamjms.2018.284
eISSN: 1857-9655
Public Health

Gestational Diabetes Mellitus Knowledge Assessment among Saudi Women

Ahmad S. Alharthi[1]*, Khalid A. Althobaiti[1], Khaled A. Alswat[2]

[1]Taif University, School of Medicine, Taif, Saudi Arabia; [2]Department of Internal Medicine, Taif University, School of Medicine, Taif, Saudi Arabia

Citation: Alharthi AS, Althobaiti KA, Alswat KA. Gestational Diabetes Mellitus Knowledge Assessment among Saudi Women. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1522-1526. https://doi.org/10.3889/oamjms.2018.284

Keywords: Diabetes mellitus; Gestational diabetes; Awareness and knowledge

***Correspondence:** Ahmad S Alharthi. Taif University, School of Medicine, Taif, Saudi Arabia. E-mail: a7mad.al0rabi@gmail.com

Received: 04-May-2018; **Revised:** 14-Jun-2018; **Accepted:** 20-Jun-2018; **Online first:** 19-Aug-2018

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: IDF estimates that 16.2% of women giving live births in 2015 had some form of hyperglycemia during pregnancy. In Saudi, a study estimated that the prevalence of gestational diabetes mellitus (GDM) is 39.4%.

AIM: We aimed to assess Saudi women's GDM knowledge and awareness.

METHODS: A cross-sectional study was conducted between August and December 2016 in Saudi Arabia using a validated questionnaire that included 12 questions focused on awareness and knowledge about GDM. Their responses were scored, and participants were divided poor knowledge (≤ 4/12) fair/good knowledge (≥ 5/12).

RESULTS: A total of 9002 adult female participated. Mean age was 27.8 ± 7.9, and they were mainly married urban residents with bachelor's degrees or higher. The mean overall score was 5.5 ± 2.5 with most of them in the fair GDM knowledge category. Participants were mostly aware of the GDM risk factors (54%) while they were least aware of the GDM diagnosis (15.9%). Multigravida and a prior history of GDM were the two risk factors about which participants were most aware (67.7%). Compared to those with poor knowledge, those with fair/good knowledge were more likely to live in urban areas, live in the central region of Saudi Arabia, work in medical fields, and be married, educated, and have personal and/or family histories of chronic diseases (all P values < 0.001).

CONCLUSION: Our study showed a high prevalence of poor awareness and knowledge, mainly in those areas relating to GDM diagnosis.

Introduction

According to the Saudi General Authority of Statistics, the total number of births in the Ministry of Health hospitals in 2014 was 267,455 with about 3335 stillbirths [1]. According to the Central Intelligence Agency, the birth rate in Saudi Arabia was 18.4 births/1,000 people [2].

Gestational diabetes (GDM) is defined by the American Diabetes Association as glucose intolerance that was not present before pregnancy. During pregnancy, the placenta secretes diabetogenic hormones, including placental lactogen and progesterone, which contribute to insulin resistance and subsequent hyperglycemia [3] [4].

There are several factors have shown to have a role in developing of GDM. The most common risk factors include previous personal and family history of GDM, history of the macrocosmic baby, unexplained stillbirths, and family history of type 2 diabetes (T2D). In-between pregnancy weight gain is considered one of the most common modifiable risk factor for GDM [5] [6] [7]. Advanced maternal age and cigarette smoking are also risk factors for GDM [8].

According to the American Diabetes Association (ADA) guideline, the time for GDM screening depends on the presence of risk factors. If the pregnant women had any risk factors, then the screening should be done in the first prenatal visits while if the pregnant women have no risk factors, then the screening should be done between 24–28 weeks of gestations. Oral Glucose Tolerance Test (OGTT) used for diagnosis of GDM [13].

In 2015, the IDF estimated that 16.2% of women giving live births had some form of

hyperglycemia in pregnancy with an estimated 85.1% due to GDM, 7.4% due to other forms of newly diagnosed diabetes, and 7.5 % due to the history of diabetes detected before pregnancy [9]. In 2002, a prospective study of 808 pregnant women in Saudi Arabia estimated the prevalence of GDM to be 12.5% [10]. Another prospective study involving pregnant women was conducted from 2011 to 2014 using the oral glucose tolerance test (OGTT), which showed that 39.4% of them had GDM [11]. A cross-sectional study of 13,627 pregnant women, who were subject to fasting plasma glucose, showed that the prevalence of GDM was 36.6% [12]. GDM caused an increase in maternal and fetal complications that include preeclampsia, Caesarean section, neonatal hypoglycemia, respiratory distress, and perinatal mortality [14] [15].

Most women with GDM will not need to continue on insulin after delivery, but it is very important to repeat the OGTT after delivery. Regular screening for diabetes after delivery is very important as these women at risk to have earlier GDM in next pregnancies and about 50% of them will develop type 2 diabetes in the next 5-10 years [16] [17]. Both the ADA and the American College of Obstetricians and Gynecologists recommend that women with GDM should be screened at 6-12 weeks postpartum for persistent high glucose level [18] [19] [20]. Regarding post-delivery management, breastfeeding, doing exercise for 150 minutes per week at a moderate intensity and dietary changes have shown to decrease the incidence of type 2 diabetes and promote postpartum weight loss [21].

Knowledge and awareness about this chronic disease will translate to an increase in self-care as a result of early diagnosis and treatment, which ultimately will contribute to complication reduction. GDM knowledge allows patients and those at risk to undertake early interference and thus prevent many complications by making simple lifestyle changes that include an increase in physical activity and diet control [22].

Despite the high prevalence of GDM in our society, there are lacks of studies that estimate the awareness level. Our primary study goal was to assess the awareness and knowledge of Saudi women about GDM and its possible related maternal and neonatal complications.

Methods

We conducted a cross-sectional study from August to December 2016 in different Saudi regions to assess the awareness and knowledge of women about GDM and its possible complications. Any Saudi females above the age of 18 years were invited to participate in the study through an online invitation. We excluded male and non-Saudi participants and incomplete responses. The study proposal was submitted to Taif University School of Medicine Ethical Committee and was approved. All completed online questioners were collected in an Excel spreadsheet and exported to the Statistical Package for the Social Sciences (SPSS) file.

The questionnaire included 13 questions about the baseline characteristics that included sociodemographic data, educational level, personal experience in or living with someone who works in the medical field, living with someone who has diabetes, history of pregnancy or GDM, and history of any chronic illnesses. Weight and height were self-reported by the participants, and body mass index (BMI) was calculated.

To assess GDM knowledge and awareness, we used a validated questionnaire that included 12 questions focusing on general awareness and knowledge about DM and GDM risk factors, diagnosis, treatment, and consequences/ complications [23]. The answer options provided were yes, no, and don't know. Each correct response was given a score of 1, and each woman was scored out of a total of 12. A score of 0–4 was considered poor knowledge, 5–8 was fair, and 9–12 was good GDM knowledge. Participants were divided into two groups consisting of poor knowledge and fair/good knowledge.

Data were analysed using the SPSS software version 20. Frequencies and percentages were used for each variable. The chi-square test was used to study the relationship between variables, and the T-test was used for comparison between means. A p-value ≤ 0.05 was considered statically significant.

Result

A total of 9002 adult females participated in the study and were included in the final analysis. Participants' mean age was 27.8 ± 7.9, and they were mainly urban residential, married, had bachelor's degrees or higher, and the mean BMI was in the overweight range (Table 1). Eighty per cent of the participants were from the central or western region of Saudi Arabia. Less than one-third of the participants were either working or living with someone who worked in the medical field, but the majority was unemployed. Half of the participants reported living with diabetic patients. Almost 90% of the participants had no personal history of any chronic illness, but in those with chronic illnesses, hyperlipidemia was most observed, and thyroid disorders were the least common. Around two-thirds knew a female with a

history of GDM, but only 8.1% had a personal history of GDM.

Table 1: Baseline characteristics of the whole cohort

Baseline characteristics (N=9002)	
Mean age (yrs)	27.8 ± 7.9
Urban residence (%)	89.7
Single (%)	50.6
Married (%)	45.7
High school or less (%)	17.1
Bachelor degree (%)	76.3
Mean weight (Kg)	63.5 ± 14.2
Mean BMI (Kg/m^2)	25.1 ± 5.4
Central region of Saudi (%)	40.6
Eastern region of Saudi (%)	9.6
The western region of Saudi (%)	39.3
North region of Saudi (%)	4.9
South region of Saudi (%)	5.6
Work in the medical field (%)	21.7
Living with someone who works in the medical field (%)	32.9
Unemployed (%)	67.6
Chronic illness history	
Living with someone who has diabetes (%)	50.7
No previous personal history of any chronic illness (%)	89.6
Personal history of diabetes (%)	3.0
Personal history hypertension (%)	3.0
Personal history hyperlipidemia (%)	3.6
Personal history of thyroid disease (%)	0.9
Pregnancy-related history	
Mean numbers of previous pregnancy	1.4 ± 2.2
Previous history of GDM (%)	8.1
Know someone who had GDM (%)	67.0
Knowledge about GDM risk factors	
Increase the number of pregnancies increases the risk of developing GDM (%)	72.6
Prior personal history of GDM increases the risk of future GDM (%)	62.9
Weight gain preconception increases the risk of developing GDM (%)	24.7
The family history of GDM increases the risk of future GDM (%)	54
Excessive weight gain in pregnancy increase the risk of future GDM (%)	57.1
Knowledge about GDM diagnosis	
OGTT is the gold stander test to screen for GDM (%)	9.7
The optimal time to do OGTT is 24-28 weeks (%)	22.2
Knowledge about GDM treatment	
Lifestyle and diet modifications are part of the GDM management plan (%)	65.4
Insulin is one of the appropriate GDM management plan (%)	23.7
Knowledge about GDM consequences/complications	
GDM usually disappears after delivery (%)	59.7
Untreated GDM increases the risk of neonatal complications (%)	47.6
GDM increases the risk of future type 2 diabetes (%)	47.3
GDM knowledge	
Mean of the total score out of 12 points	5.5 ± 2.5
Poor diabetes knowledge (%)	33.8
Fair diabetes knowledge (%)	54.8
Good diabetes knowledge (%)	11.4

Regarding overall GDM knowledge assessment, the mean overall score was 5.5 ± 2.5 with most of them in the fair GDM knowledge category. Participants were mostly aware of the GDM risk factor component while they were least aware of the GDM diagnosis-related component. Increased numbers of pregnancy and the prior history of GDM were the two risk factors about which those participants were most aware.

When compared to females with poor knowledge, those with fair/good GDM knowledge were more likely to be older (P < 0.001), urban residents (P < 0.001), married (P < 0.001), have a bachelor's degree and be employed (P < 0.001), have higher BMI (P < 0.001), live in the central region of Saudi Arabia (P < 0.001), work or live with someone who works in the medical field (P < 0.001), live with diabetics (P < 0.001), report chronic illnesses (P<0.001), have higher number of pregnancies (P<0.001), report history of personal GDM (P < 0.001), and know a female(s) with GDM history (P < 0.001) (Table 2).

Table 2: Groups based on the overall GDM knowledge score

Variables	Poor knowledge	Fair/Good knowledge	P value
Number of participants (%)	33.8	66.2	n/a
Mean of the total score out of 12 points	2.7 ± 1.3	6.9 ± 1.6	<0.001
Mean age (yrs)	27.0 ± 7.6	28.2 ± 8.1	<0.001
Urban residence (%)	87.5	90.8	<0.001
Single (%)	54.3	48.7	
Married (%)	42.3	47.4	<0.001
High school or less (%)	20.4	15.4	
Bachelor degree (%)	74.2	77.4	<0.001
Mean weight (Kg)	62.1 ± 14.1	64.3 ± 14.2	<0.001
Mean BMI (Kg/m^2)	24.5 ± 5.4	25.4 ± 5.3	<0.001
Central region of Saudi (%)	35.3	43.3	
Eastern region of Saudi (%)	9.2	9.8	
Western region of Saudi (%)	43.9	36.9	<0.001
North region of Saudi (%)	4.4	5.1	
South region of Saudi (%)	7.2	4.9	
Work in the medical field (%)	19.3	22.8	<0.001
Living with someone who works in the medical field (%)	29.2	34.7	<0.001
Unemployed (%)	75.7	63.5	<0.001
Chronic illness history			
Living with someone who has diabetes (%)	47.6	52.3	<0.001
No previous personal history of any chronic illness (%)	91.9	88.4	<0.001
Personal history of diabetes (%)	1.8	3.6	<0.001
Personal history hypertension (%)	2.4	3.3	0.015
Personal history hyperlipidemia (%)	2.5	4.1	<0.001
Personal history thyroid disease (%)	0.6	1.1	0.009
Pregnancy-related history			
Mean numbers of previous pregnancy	1.4 ± 2.1	1.5 ± 2.2	0.018
Previous history of GDM (%)	4.1	10.2	<0.001
Know someone who had GDM (%)	56.5	72.4	<0.001
Knowledge about GDM risk factors			
Increase the number of pregnancies increases the risk of developing GDM (%)	42.1	88.2	<0.001
Prior personal history of GDM increase the risk of future GDM (%)	31.2	79.0	<0.001
Weight gain preconception increases the risk of developing GDM (%)	5.3	34.5	<0.001
The family history of GDM increases the risk of future GDM (%)	23.2	69.7	<0.001
Excessive weight gain in pregnancy increase the risk of future GDM (%)	26.4	72.8	<0.001
Knowledge about GDM diagnosis			
OGTT is the gold stander test to screen for GDM (%)	2.8	13.2	<0.001
Optimal time to do OGTT is 24-28 weeks (%)	9.6	28.7	<0.001
Knowledge about GDM treatment			
Lifestyle and diet modifications is part of the GDM management plan (%)	37.6	79.6	<0.001
Insulin is one of the appropriate GDM management plan (%)	8.0	31.7	<0.001
Knowledge about GDM consequences/complications			
GDM usually disappears after delivery (%)	41.9	68.8	<0.001
Untreated GDM increase the risk of neonatal complications (%)	19.7	61.8	<0.001
GDM increase the risk of future type 2 diabetes (%)	18.0	62.2	<0.001

Regarding the overall GDM knowledge assessment and when compared to females with poor knowledge, those with fair/good GDM knowledge were more likely to be aware about all assessed GDM-related risk factors (P < 0.001), all assessed GDM-related diagnostic strategies (P < 0.001), all assessed GDM-related treatment plans (P < 0.001), and all assessed GDM-related consequences/ complications (P < 0.001).

Partial correlations were made adjusting for region, age, BMI, marital status, education, and knowing someone or working in the medical field between GDM knowledge score and personal history of diabetes (r - 0.046, P < 0.001), between GDM knowledge score and number of pregnancies (r-0.024, P < 0.025), and between GDM knowledge score and previous personal history of GDM (r= -0.139, P < 0.001).

Discussion

One of the major study findings is the lack of GDM-related knowledge and awareness among the participating women despite the high prevalence of pregnancy and GDM in Saudi Arabia. When compared with the prevalence of GDM in India, which is about 39.4% varying between 3.8% and 41%, the mean overall score for GDM knowledge in Saudi Arabia was 5.5 ± 2.5 with most of them in the fair GDM knowledge category; in India, the median knowledge score was 7, and the level of awareness and knowledge about GDM was mostly fair [23] [24]. Even though most of our study participants had bachelor's degrees or higher while most of the Indian study participants had secondary education, our participant's knowledge and awareness was still not optimal, which may be related to the lack of any direct GDM-related educational programs and campaigns . The overall score was low for our participants, but questions about risk factors revealed that most of them have good knowledge and were aware of GDM, especially those with histories of prior pregnancies and those with a personal history of GDM; this finding was different when compared with the study done in India [24]. This finding was likely related to the high number of pregnancies in Saudi Arabia when compared with India.

Most of our participants were unaware of the optimal test to screen for GDM (OGTT) or the recommended time to do the OGTT. They were also unaware about the lifestyle and diet modifications as the first line treatment for GDM despite documented maternal and fetal harm of uncontrolled GDM [25]. This may explain the high observed prevalence of undiagnosed GDM nationally, which was estimated to be around 50% along with the high prevalence of uncontrolled GDM-related complications [26].

Few studies have evaluated the impact of health campaigns on the population's disease-specific awareness. An English study that measured the impact of public awareness campaigns for cancer symptoms concluded that public behaviours were influenced by those campaigns. They also observed an increase in early-stage cancer diagnoses along with a decrease in the late stage cancer diagnoses [27]. Another study done in the United States that measured the influence of health promotion on the diagnosis and management of diabetes concluded that chronic disease detection and management could be improved by health campaigns [28]. For that purpose, we have recommended a national plan for educational programs and health campaigns to promote and improve GDM knowledge and awareness among Saudi women.

Our study strengths include the large sample size, diversity, and national distribution of participants. Our limitations include the electronically distributed questionnaire.

In conclusion among the participating Saudi women, our study showed a high prevalence of poor awareness and knowledge, mainly in those areas relating to GDM diagnosis.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1527-1532.
https://doi.org/10.3889/oamjms.2018.303
eISSN: 1857-9655
Public Health

Improving Nursing Care Documentation in Emergency Department: A Participatory Action Research Study in Iran

Seyed Majid Vafaei[1], Zahra Sadat Manzari[2*], Abbas Heydari[2], Razieh Froutan[2], Leila Amiri Farahani[3]

[1]PhD Candidate in Nursing, School of Nursing and Midwifery, Mashhad University of Medical Sciences, Mashhad, Iran; [2]Department of Medical-Surgical Nursing, School of Nursing and Midwifery, Mashhad University of Medical Sciences, Mashhad, Iran; [3]Department of Reproductive Health and Midwifery, School of Nursing and Midwifery, Iran University of Medical Sciences, Tehran, Iran

Citation: Vafaei SM, Manzari ZS, Heydari A, Froutan R, Amiri Farahani L. Improving Nursing Care Documentation in Emergency Department: A Participatory Action Research Study in Iran. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1527-1532. https://doi.org/10.3889/oamjms.2018.303

Keywords: Documentation; Health Services Research; Action Research; Nursing; Emergency Service; Hospital

***Correspondence:** Dr. Zahra Sadat Manzari. Assistant Professor, School of Nursing and Midwifery, Mashhad University of Medical Sciences, Mashhad, Iran. E-mail: Manzariz@mums.ac.ir

Received: 26-May-2018; **Revised:** 22-Jul-2018; **Accepted:** 27-Jul-2018; **Online first:** 19-Aug-2018

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Standardization of documentation has enabled the use of medical records as a primary tool for evaluating health care functions and obtaining appropriate credit points for medical centres. However, previous studies have shown that the quality of medical records in emergency departments is unsatisfactory.

AIM: The aim of this study was improving the nursing care documentation in an emergency department, in Iran.

MATERIAL AND METHODS: This collaborative action research study was carried out in two phases to improve nursing care documentation in cooperation with individuals involved in the process, from February 2015 to December 2017 in an affiliated academic hospital in Iran. The first phase featured virtual training, an educational workshop, and improvements to the hospital information system. The second phase involved the recruitment of human resources, the implementation of continuous codified training, the establishment of an appropriate reward and penalty system, and the review of patient education forms.

RESULTS: The interventions improved nursing documentation quality score of 73.20%, which was the highest accreditation ranking provided by Iran's Ministry of Health and Medical Education in 2017. In other words, this study caused a 32% improvement in the quality of nursing care documentation in the hospital.

CONCLUSION: The appropriate practices for improving nursing care documentation are employee participation, managerial accountability, nurses' adherence to documentation standards, improved leadership style, and continuous monitoring and control.

Introduction

In the nursing profession, documentation is a critical part of quality improvement [1], which in turn, is a major factor that affects the transparency of nursing practices [2]. Documentation plays a vital role in the appropriate planning of nursing care services, the accurate recording of daily events, and the satisfaction and well-being of patients [3] [4]. It can also be used to plan and evaluate patient care [3]. The documentation of nursing services is important for some other reasons. It facilitates communication and collaboration [5], organises the nursing care chain [6], smoothens decision making about patient care and safety, ensures professional accountability [7], and provides regulatory and observatory standards that facilitate evidence-based processes [8] [9]. Documentation likewise serves as a tool for research, qualitative assessment, and the provision of records for medical jurisprudence. Written evidence of patients' progress is essential in nursing care because such evidence ensures the organisation of nursing services based on logical thinking for clinical decisions [10].

Despite the criticality of documentation, however, studies conducted in Iran showed that the quality of medical records in the country's hospitals is unsatisfactory [8] [11]. Research that compared world standards with those of Iran revealed that nurses in other countries exhibit more desirable quality regarding adhering to documentation principles and

standards [12]. The insufficient quality of nursing service documentation remains one of the main challenges in Iran's nursing profession [13], with years of clinical interventions failing to achieve significant change [14].

To address this issue, the current study was conducted to improve the nursing care documentation in the emergency department of an affiliated academic hospital in Iran.

Material and Methods

The study was carried out in a 233-bed academic affiliated hospital located in Khorasan Razavi Province. The hospital's emergency department is the major trauma centre in the province and comprises 24 active beds as well as admission, outpatient, radiology, and triage sectors that admit an average of 470 elective patients and 89 inpatients daily. The emergency medical staffs include 32 nurses, 12 nursing assistants, 9 general physicians, and 3 emergency physicians. The documentation system for nursing services is a paper and an electronic system. The 20-year presence of researchers in nursing management positions in this hospital failed to contribute to the total accreditation score of the hospital, which gained only 42.2% in 2014.

The study was of a participatory action research design based on Kemmis's model [15]. For the action research process, the model features a circular structure, with each circle containing four steps: problem definition and planning, action, observation, and reflection. We systematically reviewed 14 articles, among which the action research conducted by Lees et al., [4], Okaisu et al., [16], Corben et al., [17], and Vabo et al., [18] involved the use of revision methods in the correction of evaluation tools, workshops, and documentation forms to improve the documentation of nursing services. The interventions implemented in these works provided valid data and created procedural unity for the process of intervention in nursing care documentation in the examined organisations. The authors indicated that the organisations satisfied the minimum predetermined standards for documentation. As described by Corben et al., [17], the auditing of documentation services has been critical to the legal aspects of documentation and has improved the quality of records. The author believed that auditing is needed to develop legal documentation and improve its quality because such documentation provides evidence that is necessary for care planning and provision and enables access to information. Before auditing was implemented in the organisation examined by the author, interventions and major changes to more than 90% of organisational documents were evaluated as accurate, professional, and concise.

In the studies conducted by Dehghan et al., [19], Darmer et al., [20], Ofi et al., [21], Gugerty et al., [22], Lees [4], and Corben [17], a nursing service auditing method was used to provide valid, clear, and encoded data and consequently improve patient care (Lees) and coordination in the process of recording nursing interventions by the treatment team Ofi et al., [21]. Asserted that a nursing audit is an important part of risk management and quality assurance processes because the appraisal of patient records reduces errors and improves poor standards. Documentation is also a powerful tool for improving the quality of nursing care as it paves the way for formulating global standards for care. Ning claimed that electronic documentation designed to enhance the quality of nursing care is somewhat better than paper-based documentation, but such evaluation requires more comprehensive consideration. In the research of Vabo et al., [18] and Lees et al., [4], nursing staff training was found to play an important role in improving the documentation of nursing services. Feng found that although the use of clinical care classification may generate numerous results, it is, in fact, ineffective in obtaining findings regarding the effects of the nursing intervention on patient care quality. The nurses participating in the study use free text to evaluate nursing care outcomes. This intervention style, according to the author, has been unsuccessful in improving the documentation and development of nursing services.

To determine the status quo in the case hospital, an instrument used to evaluate the quality of nursing care documentation was extracted from Esmaeilian et al., [23]. The reliability of the instrument was determined through concurrent validation and comparison with previous instruments in a survey administered to 200 patients discharged from the emergency department of the investigated hospital (Cronbach's alpha=82%). [1] The validity of the instrument was evaluated based on comments from professors at Mashhad University of Medical Sciences and Iran University of Medical Sciences. Validity was confirmed after modifications to the instrument were made. The instrument was scored based on four categories of documentation: white = 0 (no documentation), incomplete = 1 (one required items), illegible = 2, and complete = 3 (full compliance).

After the examination of nursing documentation quality in the second phase [i.e., qualitative study with content analysis (the methods used in the present study) and conventional content analysis (as a data analysis method)], the existing problem and its causes and possible solutions were investigated on the basis of the perceptions and actual experiences of the hospital personnel and the individuals involved in documentation. For this purpose, 22 semi-structured interviews were held with 13 interviewees (In some cases, two or more

interviews were conducted with the participant) at 45-minute sessions [13].

Table 1: Classification of examined indices based on the minimum score obtained by 200 subjects

No.	Index of concern in records	White	Incomplete	Illegible	Complete
1	The full demographic information of patients (name, age, place of birth, date of birth) appears on the file cover, and all information is completely documented.	0	100	12	99
2	File documents are arranged by the order issued by the Medical Documents Center (admission letter, physician's prescriptions, nursing reports, para-clinical tests, content letter, history, and patient training).	0	186	0	14
3	All documents on para-clinical measures are attached and checked according to the date in the relevant file.	1	175	1	23
4	A physician's instructions along with the number of items in letters and the time and date come with a signature.	0	183	8	9
5	A physician's instructions are terminated with a straight underline so that nothing more can be added.	0	180	0	20
6	Vital signs are accurately recorded in specified fields on a chart sheet in red (temperature), blue (pulse), black (blood pressure), and green (breath).	3	161	2	34
7	The information requested is completely and accurately documented in tables below the vital signs chart.	4	190	0	6
8	The intervals for checking vital signs registered on a patient's chart sheet should be consistent with the instructions written in the corresponding file.	3	182	0	12
9	Nursing reports are legible with mistakes.	3	81	65	51
10	Nursing reports are written in succession with no blank spaces among them.	0	105	1	94
11	Nursing reports are signed and contain the name of the nurse in charge, his/her position, and documentation time.	0	13	15	172
12	If there is a mistake in the nursing report, it must be marked and then signed and stamped.	0	20	0	171
13	The exact time of specific measures (tests, radiography, physician's visits) is indicated.	124	40	33	3
14	Ambiguous words, such as "good," "normal," and "medium," are not used in the report.	0	42	39	119
15	In the nursing report, the cause, type of disease, and type of referral are mentioned.	0	106	0	94
16	Only the abbreviations approved by the institute are used in medical records.	0	131	28	41
17	There are enough explanations about the general status of a patient (vital signs, level of consciousness, objective and subjective symptoms).	197	3	0	0
18	Sufficient explanations are provided about a patient's excretion conditions (number of times, colour, consistency of symptoms and patient's complaints).	198	2	-	-
19	The report is closing with a straight underline so that nothing more can be added.	186	4	0	0
20	The nutritional status of a patient is denoted with measurable benchmarks (amount of food, total food intake per day).	198	2	0	0
21	Notes on invasive treatments (urinary catheterisation, nasogastric tube, etc.) are provided, along with usage time, the instructor, patient response to the treatment, and follow-up points in the subsequent shift.	9	141	37	13
22	A patient's training sheet is completed and signed according to the measures taken.	193	6	0	1
23	Nursing procedures, including nursing diagnosis, nursing interventions (a type of intervention, patient's behaviour, intervention time), and evaluation of actions (patient's response), are recorded in documentation reports.	93	85	14	8
24	Exact drug prescriptions are documented by mentioning the drug, consumption method, and timing of medication. A nurse's signature should appear in the document.	0	75	14	111
25	Nursing diagnosis is written, and the nursing process is specified at the end of each assessment form.	128	52	14	6
26	The orders in a file accord with a physician's instructions.	0	119	10	71
27	Patient's profile, medical and nursing diagnosis are stored in the file.	0	102	4	94
28	Telephone orders are signed by two people, and the exact time is included.	0	137	3	60
29	A patient's electrocardiography contains the patient's profile and date and is attached to a special sheet.	0	122	0	60
30	Consent forms include explanations about the risks and benefits of treatment or surgical intervention, other treatment alternatives, and measures. It provides some evidence of the fact that a patient or his lawyer are fully satisfied with the surgery or treatment.	8	157	13	22

Results

In the accreditation process implemented by Iran's Ministry of Health and Medical Education in December 2014, the emergency department of the investigated hospital received a score of only 42.2% out of 100%. This outcome, the assessment of the quality of nursing service documentation in the quantitative stage of this study, the explanation of documentation experiences by the emergency department staff in the qualitative stage, the 20-year experience of researchers regarding the observed imperfections of nursing service documentation, and interviews with the head nurse, matron, and nurses working in the emergency department revealed numerous problems in the hospital's documentation process. Accordingly, this action research was conducted to improve the quality of nursing service documentation in the examined emergency department.

Table 2: Main themes and sub-themes extracted from interviews

Main Themes	Sub Themes
Documentation competency	The necessity of effective training
	Need to train documentation standards
	Need to increase skills in reporting
Job burnout	Job stress
	Work pressure
Perceived control	Planned control
	Effective control
Intra-organizational coordination	Improvement of health information system
	Documentation time management
Legal barrier to documentation	Escaping from the law
	Legal liabilities

To plan the quantitative and qualitative stages of the research, a brainstorming session was held with the deputy head of the hospital's Treatment and Care Department, the head of the hospital, the head nurse of the emergency department, the matron, two male and female representatives of the nursing staff, the educational supervisor, the head nurse of the emergency department, and representatives of the quality promotion and accreditation committees. The following interventions were established: virtual training for the nursing staff; staff Management Based On Performance: Application Of A Work Measurement, conducted in three different shifts in the emergency department; a review of any necessary modifications to the hospital information system (HIS); and cooperation regarding implementing continuing codified education.

The five stages of an intervention planned for the first phase are described as follows.

Virtual training was aimed at retraining individuals involved in the process of nursing care documentation regarding documentation standards, which were loaded into the hospital educational system. After 21 days, a written test was administered to evaluate the knowledge of the employees, who obtained a mean score of 87.23% out of 100%. Given that a score greater than 80% was achieved by the employees; the next stage of intervention was carried out.

The work performed by six nurses in the morning shift, two nurses in the evening shift, and two

nurses on the night shift was observed. The nurses' performance in a given work shift was recorded by a chronometer, after which the data collected were analysed (Table 3).

Table 3: the Average activity of nurses in three different shifts (in minutes)

Direct care	121
Indirect care	178
Miscellaneous (rest, tea, etc.)	58
Documentation in the system	23
Documentation in the case	31
Total	420

The HIS was investigated to reduce the time spent on electronic documentation. Specifically, the system's deficiencies were identified and recorded following the observation of the nurses as they entered records in the HIS and interviews with the users throughout a week.

This stage involved the creation of a specialised team, who conducted a one-day investigation and determined that the average admission time in the emergency department within three working shifts was eight minutes.

A two-day workshop aimed at improving the emergency department staff's standard documentation skills was one of the measures adopted in the first phase of the research. The workshop was intended to train the employees on documentation standards, reviewing and criticising current documentation, carrying out group work, and reviewing nursing documentation and legal regulations on such documentation. Finally, the participants were assigned a standard documentation exercise to evaluate their performance.

The intervention process and its interim and final results were examined in a meeting, during which a decision was reached to run a second phase of the research given that a documentation quality score of 57.2% out of the target 70% was achieved in this stage. The impediments to effective nursing service documentation at this stage were the lack of specific policies regarding the documentation process, the lack of a reward and penalty system for enhancing documentation, the necessity of reviewing nursing documentation forms, the absence of appropriate interaction between the medical and nursing teams, and the lack of support from the nursing director in efforts to enhance the documentation process.

The interventions implemented in the second phase of the research are discussed as follows.

A decision was made to establish continuous documentation monitoring by a team in three working shifts. The team was also tasked to submit their weekly reports to the matron and nursing director. A channel was created on the Telegram instant messaging service to coordinate control and monitoring actions.

A two-day specialised workshop was planned to review standard documentation and eliminate the deficiencies identified in the first phase of the research. The workshop was conducted with the full cooperation and assistance of the Neyshabur Department of Medical Sciences and its faculty members.

Following consultations with the head of the hospital's human resources department and approval from the deputy, applicants requesting for a renewal of the available staffing, especially emergency department employees, were accepted. Hence, four nurses were provided a plan renewal and two nurses working in other departments of the hospital were instructed to initiate work in the emergency department.

To establish a structured reward and penalty system by collective wisdom, a decision was made to assign a point to the employees' total work and annual evaluation scores for the quality of nursing service documentation. Two points out of the 17 points in the worksheets were assigned for annual evaluation scores, and 5% of the professional skill score in the annual evaluation was allocated to quality.

The previous version of the patient education form was based on compulsory selection scores, and the nurses used to complete this form carelessly and attach it to files. To increase accuracy and improve patient education, a new version of the form was developed. The new version contains fields for recording the intervention education that nurses provide to patients (i.e., descriptions of interventions). The new version was sent to the Ministry of Health and Medical Education for approval.

A meeting was held with the nursing staff and emergency department physicians. The challenges facing the health team was raised and discussed in free exchange. Another meeting was held with the head of the emergency department, the matron, the researchers, and a representative of emergency department nurses to improve interactions between physicians and nurses.

Throughout the intervention process and at the end of the second phase of the research, the interventions and their interim and final results were evaluated and analysed in a meeting held with the process owners. Because of the performance of the participants at the end of the second phase, the documentation quality score of the hospital increased from 41.75% to 72%.

Discussion

Organizational culture is a system of common understanding by members toward an organisation

and distinguishes organisations from one another. Argyris defined organisational culture as a living system characterised by the behaviours that individuals manifest, the way they think and feel, and the method through which they interact [17]. In Iran's hospitals, organisational culture is a combination of providing an appropriate environment for enhancing employee creativity, knowledge, and productivity. Managers play the most critical role in establishing a desirable organisational culture because their behaviours significantly affect such culture [19]. This study endeavoured to create fundamental change in the organisational culture of the investigated hospital by enhancing collaboration between the employees and nurses by the principles of Kemmis's model [15]. The specific achievements of the research in this regard were the improvements to cooperation and coordination between the managers of the organisation and the adherence of nurses to documentation standards through continuous training workshops and the employment of experienced nurses in the emergency department.

Given the lengthy process of admission in the investigated emergency department, the process of documentation was equally accompanied by delays and errors. The research involved improving the patient admission process through interventions such as enhancing computer software, installing a new printer, and informing patients regarding the availability of a national ID number for patients. These interventions reduced admission time from 8.1 minutes to 3.2 minutes. The key factors that affected the performance improvement of novice employees were the nature of the workplace, sufficient empirical and scientific support for the novice employees, their willingness to learn, adaptation to work cultures, and adaptability to others' expectations [19]. In this study, the scientific and practical potential of the novice employees regarding standardised documentation was increased by enlisting the help of experienced staff and assigning them to different shifts alongside the non-experienced employees. With the return of two experienced nurses to the emergency department and the elimination of the human resource problem, the issues arising from the new nurses' lack of experience were partially resolved. In the current setup, one experienced nurse is assigned to three novice nurses in each work shift.

With reference to the comments of the nursing staff, the brainstorming meetings held with the hospital authorities, and the viewpoints of the faculty members of the hospital's Department of Nursing and Midwifery, the patient education form was checked in terms of the compulsory selection process, the signing of a form, and its attachment to a file. Training was provided for this purpose, but continuing registration inaccuracies and evaluations from patients admitted to the emergency department indicated the low effectiveness of the training. Correspondingly, the form was revised through a professional process based on international standards into a descriptive report form and sent to the Ministry of Health and Medical Education for final approval. The experiences of the nurses in the qualitative stage and the work observation sessions revealed that the average time spent on completing electronic documentation in the hospital is longer than the regional standard level (an average of 4 minutes). In two meetings with the hospital's information technology officials, the matron, the director of the radiology department, and the director of the laboratory and the pharmacy of the therapeutic center, the existing panels were examined, non-practical panels for the emergency department were removed from the system, and a number of nursing care packages were developed and added. These solutions, along with the improved computer systems and the installation of updated operating systems, reduced documentation time to 3.2 minutes.

About the qualitative results, one of the problems identified was the lack of control and supervision by the authorities. This problem was solved in the two phases, namely, the coordination of and reflection on the comments provided by the nurses and individuals involved in the documentation process and the formation of a supervisory and control committee consisting of researchers, four nurses with more than 5 years of work experience, and the matron. In all the working shifts, the committee assigned one selected nurse to control and monitor the work process and submit a report to the committee every other two weeks. These interventions directly affected the assessment of payments and salaries and influenced the nurses' awareness and understanding of the significance of documentation.

In conclusion, the results of this study indicated that the staff training and awareness development effectively improved the quality of documentation provided by the nurses in the examined emergency department. Continuous and planned monitoring and control, along with incentive policies, influenced the continuation of staff behaviours. Changing the authorities' attitudes toward the importance of improving documentation was also a fundamental factor. Finally, the interventions were confirmed to be successful by credible evidence and the experiences of the staff working in the emergency department.

Acknowledgements

The authors would like to express their gratitude to the Mashhad Department of Medical Sciences, the nurses, and the other individuals who assisted.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1533-1544.
https://doi.org/10.3889/oamjms.2018.217
eISSN: 1857-9655
Review Article

Effect of Stress, Depression and Type D Personality on Immune System in the Incidence of Coronary Artery Disease

Saideh Masafi[1], Seyed Hassan Saadat[2], Katayoun Tehranchi[3], Roohollah Olya[1], Mostafa Heidari[4], Saied Malihialzackerini[3], Mahdi Jafari[5*], Ehsan Rajabi[6]

[1]Department of Psychology, Kish International Branch, Islamic Azad University, Kish Island, Iran; [2]Behavioral Sciences Research Center, Lifestyle Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran; [3]Department of Psychology, Kish International Branch, Islamic Azad University, Kish Island, Iran; [4]Department of Psychology, Saveh Branch, Islamic Azad University, Saveh, Iran; [5]Department of Clinical Psychology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran; [6]Shahid Beheshty University of Medical Science, Tehran, Iran

Citation: Masafi S, Saadat SH, Tehranchi K, Olya R, Heidari M, Malihialzackerini S, Jafari M, Rajabi E. Effect of Stress, Depression and Type D Personality on Immune System in the Incidence of Coronary Artery Disease. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1533-1544. https://doi.org/10.3889/oamjms.2018.217

Keywords: Psychoneuroimmunology (PNI); Stress; Depression; Type D Personality; Coronary Artery Disease (CAD)

***Correspondence:** Mahdi Jafari, MD, MPH, PhD in Health Psychology, Department of Clinical psychology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Tel: +989122166934. E-mail: drmjafari@sbmu.ac.ir

Received: 19-Apr-2018; **Revised:** 08-Jul-2018; **Accepted:** 18-Jul-2018; **Online first:** 01-Aug-2018

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Psychoneuroimmunology (PNI) is the study of the interaction between psychological processes and the nervous and immune systems of the human body. The impact of psychological factors on the immune system and the role of this system in Coronary Artery Disease (CAD) are confirmed. Coronary Heart Disease (CHD) is arisen due to the failure of blood and oxygen to the heart tissues.

AIM: The present study aimed to describe psychoneuroimmunological processes which contribute to CAD and CHD progression.

METHOD: Such psychological risk factors like stress, depression and type D personality were investigated here. Psychoneuroimmunological pathways of all three mentioned risk factors were described for CAD.

RESULTS: The studies review indicated that stress could be accompanied with myocardial ischemia and help to rupture. The depression involves in the transfer of stable atherosclerotic plaque to unstable, and type D personality is effective in the initial stages of a CAD.

CONCLUSION: As more information on cardiovascular immunity becomes available, this will provide a better understanding and thus act as the foundation for the potential development of new treatment strategies for treatment of cardiovascular disorders.

Introduction

About 3 decades ago, some evidence was obtained that showed immune system interacts with the central nervous system and endocrine system, and such evidences indicated the impact of psychological factors on these systems. This awareness led to scientific findings and quick growth of a field called “psychoneuroimmunology” [1] [2]. The life of this field crystallised by the publication of brain, behaviour and immunity magazine in 1987 [1]. The psychoneuroimmunology studies the mutual relations between psychological factors, immunity and Neuroendocrine mechanisms as well as the application of the findings related to such relations in health and disease [2]. Also, it tries to present a picture of mutual relations between behaviour and immunity to explain mechanisms of the autonomic nervous system and Hypothalamic-Pituitary-Adrenal axis (HPA or HTPA axis) to relate central nervous system and immunity responses [2]. The relation between psychological factors and immunity system performance indices were seen from the common cold to the immune response to vaccination [3].

The impact of psychological interventions on immunity indicators was also seen in previous studies [4]. Therefore, psychological factors are related to some factors of immunity system that play a considerable role in the aetiology of coronary heart syndrome [5]. The brain influences immune responses through the HPA axis. This axis enhances/suppresses inflammatory responses through secretion of Corticotropin-releasing hormone (CRH) and Adrenocorticotropic-releasing hormone, respectively from the hypothalamus and pituitary glands, and the secretion of cortisol from the adrenal. However, the

paths between the brain and the immune system have not well been known [1].

The pathophysiological mechanisms effective among psychological factors and CAD progress can be related to immunological processes [6]. Recent research findings indicate that CAD is an important clinical appearance of psychoneuroimmunological mechanisms in heart disease progression and acute coronary syndromes. Kop classifies psychological risk-factors into three groups based on the duration, that is, their persistent or temporary presence [7]: Acute triggers such as psychological stress and anger [8], episodic factors with duration of few weeks to 2 years such as depression and exhaustion [1], and Chronic Factors such as negative personality characteristics (enmity in Personality Type A and Personality Type D) and low socioeconomic level.

The review studies which relate psychoneuroimmunological factors to coronary heart disease will provide helpful information to understand the psychoneuroimmunology of heart diseases. In the present paper, we will study the works which take advantage of the role of stress, depression and Personality Type D on the immune system while these factors lead to CAD progression. The results here allow physicians and specialists to realise the importance of the immune system as a relation between mind and cardiovascular system and pay more attention to mental health maintenance to prevent coronary artery disease.

Therefore, the present paper will study the following items:

- Stress effect process in the immune system and the incidence of coronary artery disease
- Depression effect process in the immune system and the incidence of coronary artery disease
- Personality Type D effect process in the immune system and the incidence of coronary artery disease

And finally, to find a response to the below question:

Do Stress, Depression and Type D Personality have different impacts on the immune system and incidence of coronary artery disease?

Materials and Methods

The present study was carried out as a review work. The search was conducted on the platforms associated with medical and psychiatric journals based on such keywords as stress, depression, type D personality, immune system and Coronary Artery Disease (CAD). This process took three months during which total of 38 papers and 108 authoritative abstracts were collected through Pubmed and Google Scholar. They ultimately were used to write and prepare for this review.

Results

Emotional stress is harmful to the heart. Statistical and clinical studies show that stress can increase the mortality associated with acute myocardial infarction. Of every seven adult Americans who suffer heart attacks, one person is experiencing stress. Tobacco and caffeine can increase heart rate up to 14 beats per minute, and if they are along with stress, the increase will reach to 38 beats per minute. Immune system responses to stress can potentially help to form Atherosclerotic plaque and avulsion or detachment of plaque. Most of the studies on psychoneuroimmunology show increased CD8+ cells, decreased CD4+ cells, increased blood viscosity and stimulated the immune system versus acute psychological challenges [7] [9].

Psychological stress activates the SNS, which regulates heart rate and release of catecholamines, and the HPA axis, which regulates the release of corticosteroids from the adrenal glands [10]. In acute psychological stress, catecholamines predominantly affect natural killer (NK) cell circulation. The relationship between acute stress, SNS and leucocytes are illustrated in Figure 1. In chronic stress, the activity of the HPA axis may decrease, leading to fatigue and increased activation of immune-mediated inflammation [11] [12].

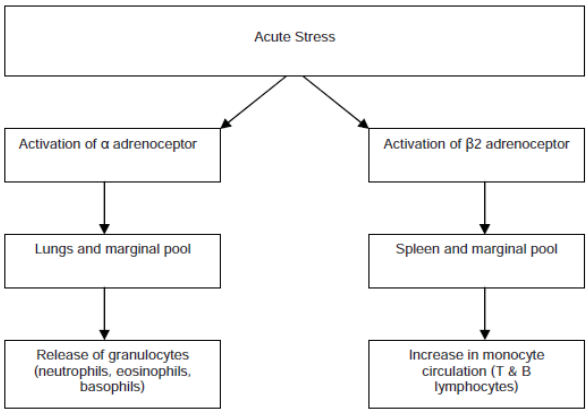

Figure 1: The relationship between acute stress, the sympathetic nervous system and the white blood cells

Owen and Steptoe studied the associations between NK cells, proinflammatory cytokine stress responsiveness and heart rate in humans. Increases in NK cell counts the following stress were positively

associated with heart rate responses and individual differences in sympathetically-driven cardiac stress responses were associated with NK and proinflammatory cytokine responses to psychological stress [13].

An acute psychological stressor increases proinflammatory cytokines including mononuclear cell IL-1β gene expression and plasma interleukin-6 (IL-6). The increased IL-1β gene expression was positively correlated with heart rate and systolic blood pressure reactivity [14]. The cytokines also affect the brain and evoke feelings of malaise, sickness and tiredness [15] [16]. These cytokines can induce the proliferation and migration of smooth muscle cells by stimulating other growth factors that lead to coronary lesions [5] [17]. Mann suggested that the short-term expression of stress-activated cytokines within the heart may be an adaptive response to stress, whereas long-term expression of these molecules may be frankly maladaptive by producing cardiac decompensation [18].

Chronic psychosocial stressors increase both haemostatic factors (e.g. Factor VII) and acute phase proteins (e.g. Fibrinogen) [19]. Lonely individuals also displayed greater fibrinogen response to stress [20]. Fibrinogen is thought to promote atherosclerosis by promoting platelet aggregation, enhancing the release of endothelial-derived growth factors, stimulating smooth muscle cell proliferation and increasing plasma and whole blood viscosity [14] [21]. Acute and chronic stress may activate the coagulation cascade and lead to thrombus formation and myocardial infarction (MI). There is robust evidence from epidemiological studies and meta-analyses that higher levels of acute phase proteins such as CRP and fibrinogen predict future cardiovascular death and are associated with low socioeconomic status. Psychological stress is associated with increased platelet activation and increases the risk of cardiovascular disease [20].

The relationship between depressive symptoms and coronary artery disease (CAD) is mediated in part by immune system parameters. This review describes research on the psychoneuroimmunological pathways accounting for the association between depression and CAD and addresses conceptual and methodological issues [21].

The Immune-Cytokine Model of Depression (ICMD) is an entirely new concept for understanding the riddle of depression. This is the only model of depression to bridge the conceptual and diagnostic gap between physical and mental disorders [22]. ICMD views depression to be any number of chronic physical-biological disorders that have mental-emotional symptoms. From the perspective of ICMD, depression isn't a disease, but rather a multifaceted sign of chronic immune system activation. During chronic immune system activation, greater than normal amounts of various cytokines are secreted. The cytokines produce the multifaceted signs and symptoms of depression [23].

Cytokines are at the heart of the immunological basis of depression since they provoke a wide spectrum of neuropsychiatric symptoms when given to human volunteers. The profound effects of cytokines on mood though, and behaviour was first discovered in the early 1980's. For the first time in history, physicians had found molecules made by the human body which, when given to humans, produced all the symptoms necessary for the diagnosis of depression [24].

Depressed patients, compared to healthy controls, have an elevated white blood cell count. A high white count is called leukocytosis. The white blood cells (leukocytes) include all of the immune cells found in the blood; consequently, leukocytosis is a reliable sign of an activated immune system [24].

Increased numbers of monocytes in the blood (called monocytosis) of depressed patients were first reported by Maes et al. and recently confirmed by Seidel et al. Monocytes are found in the blood, which makes them easy to sample and measure. They are the chief source of IL1, IL6, TNF and INFα in the blood [25] [26].

Monocytes migrate from the blood into solid tissues where they are transformed into macrophages. Macrophages never return to the blood. This means they are rarely evaluated in humans because almost all immune system analyses are done on blood. Nevertheless, in animal experiments, whenever there is monocytosis, there is macrophage activation someplace in the body. Thus, the monocytosis exhibited by depressed patients indicates that macrophages are activated someplace in their bodies [24].

Maes two papers on monocytes cited above also found high levels of neutrophils (a condition called neutrophilia) in the blood of depressed patients. The most severely depressed individuals had the highest numbers of neutrophils. Neutrophils, the most plentiful of the white blood cells, are members of the inflammatory arm of the immune system. Neutrophilia is a well-established sign of immune system activation. Thus the discovery of neutrophilia in depression is another persuasive piece of evidence showing that depressed individuals have activated immune systems [24].

The total number of lymphocytes does not appear to be increased in depressed patients. Nevertheless, within the various types of lymphocytes, there are very important changes. In a recent study by Maes et al., of 106 subjects, there was a significantly increased number and percentage of B-lymphocytes in depressed subjects compared to controls [27]. This was confirmed in another study of depressed patients [28]. B-lymphocytes are the antibody-producing cells. (They are called B-lymphocytes because they are

matured in bone.) Increased numbers and percentages of B-lymphocytes are clear signs of immune system activation [24].

The T stands for the fact that these lymphocytes mature in the thymus. By secreting regulatory cytokines like IL-2 and INFγ, T-lymphocytes exert remarkable control over immune system activity. Immunologists have identified many different types of T-lymphocytes. Two of the most important is the T-helper lymphocytes (these are identified by the so-called CD4 antigen on their cell surface) and the T-suppressor lymphocytes (these are identified by the so-called CD8 antigen on their cell surface) [24].

Maes et al., in one of his many landmark papers on depression, reported extraordinarily consistent evidence of T lymphocyte activation in depressed patients. Healthy controls were compared to 101 depressed inpatients consecutively admitted to the Psychiatric Ward of the University Hospital of Antwerp. Depressed patients had significantly higher percentages of T-helper lymphocytes and lower percentages of T-suppressor lymphocytes than healthy controls. The T-helper/T-suppressor ratio was significantly elevated in depressed patients. The patients with the most severe depression had the highest percentage of T-helper lymphocytes and the highest T-helper/T-suppressor ratio [28].

A high percentage of T-helper lymphocytes combined with the finding of monocytosis in depression means that both the lymphocyte and the macrophage arms of the immune system are activated. The reduced percentage of T-suppressor lymphocytes is another clear sign of the immune system is energised. The high T-helper/T-suppressor ratio is a reliable indicator of immune system activation. In the same paper, Maes et al. provided additional evidence of lymphocyte activation [28].

Recently Müller et al. investigated the lymphocyte subsets of severely depressed patients. Their results were very similar to Maes et al. 's findings. Müller et al. 's paper provided independent confirmation of over-active immune systems in severely depressed patients. Several earlier papers by other scientists have also reported a high T-helper/T suppressor ratio in depressed patients [29].

Another reliable sign of lymphocyte activation in the presence of interleukin2 receptors on the outer surfaces of lymphocytes. Maes et al. reported that increased interleukin2 receptors on lymphocytes are a hallmark for major depression. This is further independent evidence of immune activation with depression [30].

The usual antibodies made by activated B-lymphocytes will clump and identify foreign proteins. As soon as a foreign protein is tagged with an antibody, it will be devoured by macrophages and killer lymphocytes. In this way, the immune system can quickly identify and destroy foreign invaders. In sharp contrast, autoantibodies, clump and identify self-proteins (that is, proteins which are an integral part of your own body). Self-proteins, after they are tagged with autoantibodies, will be attacked and devoured by macrophages and killer lymphocytes. In other words, when autoantibodies are produced, the immune system begins attacking the very body it is supposed to defend. Diseases which are caused by the immune system attacking the body are called autoimmune diseases. Another profound similarity between depression and autoimmune disease is the very high incidence of depression with autoimmune diseases.

Typically, biomedical scientists either have no explanation for the high rates of depression occurring with autoimmune diseases or very convoluted explanations. In sharp contrast, the immune-cytokine model of depression has a clear and direct explanation, i.e., the activated immune systems in persons with autoimmune disease secrete excessive amounts of cytokines. Excessive cytokines provoke the symptoms and signs of depression [31].

Evidence suggests that these associations can be affected by a) the clinical characteristics of depression (e.g., typical depression versus atypical depression and exhaustion), b) the duration and severity of depressive symptoms, and c) the stage of an underlying CAD. Depressive symptoms are hypothesised to affect the transition primarily from stable CAD to acute coronary syndromes via plaque activation and prothrombotic processes and may play an additional role in response to injury at early stages of coronary atherosclerosis [24].

Type D personality is a behavioural model in which people experience negative emotions such as depression, anger, hostility and anxiety while they refuse to express it. Denolt (2000) identifies type D personality in the long-term by an increased risk of the first myocardial infarction [32]. Type D personality can lead to increased fatigue or depression among the people with such a personality type. Therefore, these factors will be correlated with increase reactivity to acute stresses [33].

Type D personality is specified by a combination of two fixed personality structure: negative affectivity and social inhibition [34][35]. Negative affectivity is the tendency to experience negative emotions constantly such as restlessness, boredom, fear and irritability in all times and situations. Social inhibition is the intendancy to inhibit expressing the emotions, high levels of insecurity experience in social situations and extreme control of self-revelation for fear of others' displeasures [32]. Type D personality is relatively common. The estimations show a range of 21-28% of cardiovascular patients and 53% of the people with high blood pressure among the public population [35] [36]. Type D personality theorists believe that the synergistic effect of high negative affectivity and high social

inhibition predict less health and especially poor prognosis in the heart [34].

Previous studies indicate that type D personality predicts severe heart disease and it may be associated with psychological and physiological indicators of poor prognosis in patients with heart disease [37] [38]. Type D personality is parallel to psychological distress in patients with CHD including signs of social alienation, depression, anger, anxiety, paranoia and vital exhaustion [39]. The patients with type D are more likely to commit maladaptive health behaviours such as smoking and a poor diet. The people with type D personality use the solutions for dysfunctional coping strategies in response to disease [40]. Therefore, type D personality can lead to a poorer prognosis by affecting the selection of lifestyles among the patients with CVD [41]. Also, the studies show the relationship between anger (as one of the negative affectivity components in type D personality) and the increased cardiovascular diseases [42] [43].

Type D individuals tend to experience negative emotions such as depressed mood, anxiety, anger, hostile feelings, and to inhibit these emotions while avoiding social contacts [44] [45] [46]. Situations involving fear, anxiety, helplessness, and loss of control result in the release of cortisol [47] [48]. The relationship between negative affect and cortisol activity has been documented in several studies using structured laboratory stressors, such as public speaking and mental arithmetic [49] and aversive stimulation [48], and in the scientific literature related to changes in the hypothalamic-pituitary-adrenal (HPA) axis in depressed patients [50] [51]. A recent study has documented relationships between negative affect, positive affect and cortisol in response to naturalistic stressors [52]. Both the experience of a current stressor and anticipating a stressor were associated with increased salivary cortisol levels. Negative affect was associated with higher cortisol levels, and positive affect was associated with lower cortisol levels. Another study also found that stressful daily events were associated with increased cortisol secretion in healthy volunteers [53]. Distress, as reflected by the mood states 'negative affect' and 'agitation', was associated with higher cortisol levels. Mood plays a mediating role in the relationship between stressful events and cortisol secretion [52] [53]. Negative affectivity is not just a confounder but is related to elevated cortisol secretion during normal daily activities. In a recent study, both type D dimensions (negative affectivity and social inhibition) were associated with greater cortisol reactivity to stress [46], although the results were not significant in more stringent regression analyses. However, it is reasonable to suggest that there is a difference in HPA regulation in type D individuals and people with other personality types.

Depression appears to be an independent risk factor for the development of coronary heart disease and osteoporosis and affects the prognosis of these and other medical disorders [54] [55]. Considerable evidence suggests an association between depression and hypertension, peptic ulcers, and diabetes [54] [55]. Elevated cortisol may be a mediating factor in these relationships. Cortisol has many effects that promote coronary heart disease. For example, cortisol inhibits the growth hormone and gonadal axes. Growth hormone deficiency is associated with a higher relative risk for premature cardiovascular disease in adults [56] [57]. Cortisol is a potent stimulus to visceral fat. Inhibition of the growth hormone and gonadal axes exacerbates visceral fat accumulation. Excess visceral fat leads to dyslipidaemia and, along with hypercortisolism, to insulin resistance, hyperinsulinism, and their sequelae [58]. Similar mechanisms may increase the vulnerability of type D individuals to cardiac and other medical illnesses. Elevated cortisol may be a mediating factor in the association between type D personality and the increased risk for coronary heart disease and, possibly, other medical disorders. It is important to note that cortisol is not the only mediating factor in this association. A recent study suggests that type D personality is associated with increased circulating levels of cytokine tumour necrosis factor α and its soluble receptors 1 and 2, which are predictors of mortality in chronic heart failure [59].

Depression is associated with impairment in feedback control of the HPA axis, contributing to higher cortisol levels during episodes of depression [50] [60]. Prolonged exposure to elevated cortisol levels may be neurotoxic, especially for brain regions rich in corticosteroid receptors, and may mediate neuronal vulnerability to stressors. Recurrent depression is associated with atrophy of the hippocampus and amygdala [61] [62] as well as the prefrontal cortex [63]. A gradual deterioration of hippocampal feedback inhibition of the HPA axis due to down-regulation of glucocorticoid receptors from repeated stress has been demonstrated [64] [65]. Evidence suggests that age and/or length of depression and/or the number of depressive episodes affect HPA regulation in depressed patients [51] [61] [62]. The potentiating or additive effect of age in conjunction with depression on pituitary-adrenocortical activity was suggested by some studies [51] [62] [67]. Mean 24-h cortisol level increases with age in depression [68]. Elderly depressives who are cortisol non-suppressors after dexamethasone need more time for pituitary adrenocortical normalisation to occur than do younger subjects [69]. An increase in post-dexamethasone cortisol levels with age has been reported in major depressive disorder [70]. A significant effect of age on cortisol release in depressed patients has been observed during the combined dexamethasone-corticotropin-releasing hormone test: older patients had higher post-dexamethasone cortisol levels [71]. In patients with endogenous depression, advancing age leads to higher baseline cortisol and a greater likelihood of being a dexamethasone non-suppressor [72]. Cortisol

responses to fenfluramine administration in depressed patients increased with the number of major depressive episodes [51]. Other authors have reported similar observations [66] [68] [73]. However, some authors suggest that age does affect HPA regulation in healthy humans [74] [75]. Differences in the results of studies have been be explained by differences in a sample size, screening criteria, and some other factors, such as differences in sleeping patterns [51] [76].

Equivocal results of these studies may be, in part, related to a different prevalence of type D individuals in study samples: i.e. some type D individuals may have alterations within the HPA axis that are similar to HPA axis changes in depressed patients [77]. Future studies of HPA function should control for the presence of type D individuals. Type D individuals should perhaps not participate in psychobiological studies as healthy controls. Studies of HPA function should also control for other personality traits that may affect the HPA axis. For example, individuals with borderline or antisocial personality features may have HPA axis abnormalities [78] [79] [80].

Results

To clarify the discussion, it is necessary to have a glance at some studies associated with psychological risk factors including depression, stress and type D personality on CAD. The stress is also addressed by plenty of researchers. Lots of studies report changes in quantity and ratio of T and B cell as well as changes in Natural Killer (NK) Cells and cykotines and failure in functional responses due to acute psychological stresses. Nevertheless, recent studies often focus on the relationship between street with other psychological factors and inflammatory markers [34].

Bosch et al. (2003) show a considerable amount of Chemokine receptor incidence by T cells caused by induced stress [81]. Mills et al., (1995) show that immune responses caused by stress are strengthened among the people with high blood pressure [82]. Besides, Fuligni et al., (2009) show an increased CRP level due to increased experience of daily stresses, considering CRP as one of the inflammatory indices for cardiovascular diseases [83]. Benson et al., indicate that acute stress leads to a significant increase of CRP and IL-6 in fat women [84]. Steptoe et al. (2007) conclude, in a review study on interface mechanisms between psychological factors and cardiovascular diseases risk that IL-1 and IL-6 increase after acute stress [9].

Also, Mommersteeg et al., (2008) indicate the relationship between hostility and Cytokine/ Chemokine clusters [85]. They also confirm the relationship between hostility and increased proinflammatory and anti-inflammatory cytokines [85]. Bacon et al., (2006) address that mental stress leads to heart pain and myocardial ischemia among the patients with cardiovascular patients [86]. Decreased plasma volume may be a mechanism by which potential mental stress is increased for acute cardiovascular events [87]. Bosch et al., (2003) also show that acute stress causes to move T cells which were initially induced for response to inflamed endothelium [81]. Acute stressors can help to absorb circulation of immune cells under endothelium and hence to accelerate plaque formation and lead to the effects caused by acute stressors. This mechanism can help to express the relationship between stress and cardiovascular diseases [88].

Also, the depression risk factor is addressed by plenty of researchers. Masselman and Freedland (2002) show biological processes associated with the role of depression on CHD risk in women [89]. They conclude that the depression causes to increase blood pressure and also elevate the risk of coronary arteries occlusion by platelet aggregation and accumulation of steroid hormones [89]. Ladwig et al., (2003) and Miller et al., (2003) show both the relationship between depressive disorders and inflammation markers as well as significant relations among the people with overweight [90] [91]. Miller et al., (2003) use Structural Equation Modeling to determine the role of leptin regarding depression, obesity and CRP and IL-6 markers. Von Kanel et al., (2008) indicate that depression signs-as one of the cardiovascular diseases risked factors-predict increases TNF-α level and decreased IL-4 as well as the elevated ratio of IL-4 to TNF-α [92]. Ranjit et al., (2007) show, in a study on psychosocial risk factors for cardiovascular disease, the positive relationship between severity of depression and an increase of IL-6 level [93]. Personality type risk factor is also studied in several papers. Denollet et al., (2003) address type D personality for chronic negative affectivities and present some evidence on increased TNF-α among the patients with congenital heart failure having type D compared to those without type D [94]. Gridon et al., (2003) investigate the conventional chemical indices among the admitted patients without acute coronary syndromes and observe that chronic psychological risk factors are associated with increased white blood cell count and lymphocyte percentage [95]. Pedersen and Middel (2001) recognised type D personality independent of such factors as disease severity and argue it causes to increase the risk of bad prognosis up to 2-5 times, decrease life quality and arise factors of stress and depression [96].

Although there exists low information about harmful impacts of type D personality in clinical results, these can be some possible causes: immune system, the behaviours associated with health including smoking and refusal of medical commands.

Pederson et al., (2004) the study impact of type D personality on the occurrence of side effects among the patients with Ischaemic Heart Disease (IHD) after Percutaneous Coronary Intervention (PCI) by Sirolimus-Eluting Stent (SESs) or Bare Metal Stent (BMSs) for nine months [97]. Regardless of the stent type, the patients with type D personality are more likely to be on the subject of death and MI compared to those without type D personality for nine months [97]. The patients with depression or type D personality are in the subject of the risk of improper response to treatment by the stent. The patients with type D personality often expect unsuitable clinical consequences in IHD along with Left ventricular dysfunction [97].

Also, some studies focus on stem cells. Recent studies in this regard have expanded our knowledge on Haematopoietic Stem Cell (HSC) niches which are important to maintain and conduct renewal and differentiation the HSC. Osteoblasts, Mesenchymal Stem Cells (MSCs) and CXCL12-Abundant Reticular (CAR) cells are components of the bone marrow microenvironment and are associated with HSCs which are specified in the performance of body immune system and Homeostasis. It is noteworthy to say that cell populations of the bone marrow microenvironment send a message for different and proper functions of the immune system through G Protein-Coupled Receptors (GPCRs) [98].

MI is the main mortality cause in industrial countries. Therefore, stem cells-based therapeutical approaches are an important necessity for MI in Regenerative Medicine and coronary arteries. The experimental studies show that stem cells derived from Bone marrow endothelial progenitor cells can improve the coronary performance after Myocardial Infarction. Phases I and II studies started quickly to transfer these concepts to the clinical stage. However, impacts of stem/ progenitor cells on MI in a clinical stage have not met the expectations so far. Therefore, a better understanding of the common limitation causes is necessary for cell therapy approaches. It is again noteworthy to mention that quantity and performance of endothelial progenitor cells is decreased among the patients with cardiovascular risk factors or CAD. These observations may provide the opportunities to optimise and amend cell therapy approaches. In present review study, a summary of current evidence on the role of stem/progenitor cells in pathophysiology and treatment of ischemic diseases is presented including properties of the cells, regeneration capacity in the colony of stem/progenitor cells. Also, stem/progenitor cells delivery methods, their implantation adjustment as well as potential approaches to start employment of stem/ progenitor cells in cell therapy methods are explained for cardiovascular diseases [99].

While the requests are increasing considerably for effective therapeutic choices for chronic coronary failure, the recent identification of physiologic and pathologic changes of myocytes in the adult human's heart has presented the fundamental base of regeneration therapy. Different methods have been represented experimentally in this regard among which some selected cases were used. This history starts with skeletal myoblasts and bone marrow-derived cells and then proceeds already with stem/Mesenchymal stromal cells inside the heart. Among them, C-KIT (positive) cardiac stem cell transplantation caused leading results with long-term impacts without side effects in the patients with chronic ventricular dysfunction. For more optimisation of present methods, we should identify different factors including the target disease, cellular population and quantity of injected cells as well as cell transfer method. Identification of former clinical tests results allows us to predict an ideal cell therapy for different cardiovascular disorders [100].

Discussion

The connection between heart and mind is a deep and prolonged bond. Advances in modern behavioural medicine have shifted psychology specialists towards the key role of abiotic factors in CHD. The researches on this disease have paid psychological factors into attention for a while, and the relationship between immune system parameters and psychological factors is an important topic of today studies on the progression of a CAD. In this regard, the present study aimed to investigate the effect of three psychological factors including depression, stress, and Type D personality on the immune system in coronary artery disease. Generally, the research findings discussed in this review confirm the validity of the hypothesis that psychoneuroimmunologic processes involved cardiovascular diseases. A set of these findings which have been published earlier are based on hypotheses about the potential role of psychoneuroimmunologic pathways in the pathogenesis of cardiovascular diseases.

Figure 2 which is derived from Kop's theory shows three categories of psychological factors (acute, episodic and chronic), immune system parameters related to CAD progression and progression stages of heart diseases and pathologic changes/lesions in coronary arteries, respectively from left to right [7]. As it can be seen at the right edge of the figure, the initial stages of coronary arteriosclerosis are specified by monocytes deposition in arteries wall that in this process, adhesion molecules play an important role. In the next stages of CAD, cytokines involved in the activation of T cells and the formation of macrophage foam cells. In this stage, the performance of Endothelial will diminish and thereby its dilation, and contractile properties will be lowered to respond to blood flow and other arteries

vasodilatation stimuli.

After initial vascular lesions, Smooth muscle cells will proliferate and migrate to plaque surface and finally contribute to form a fibrous coating with a stable ration on atherosclerotic lesions. In severe CAD mode, several factors may cause to stimulate the plaque and result in lesion instability and thinning of the fibrous coating. The plaque rupture leads to partial or complete occlusion of coronary arteries. This lesion often is caused by thrombus formation resulting from blood contact with collagen. Sudden occlusion of coronary arteries can cause cardiac ischemia and chest pain while complete and continuous clotting results in myocardial infarction [101] [102]. More precise studies on this complex process can be followed up in several types of research. Psychological risk factors are effective through differentiated immunologic processes in the pathophysiological trend of heart.

Pathophysiologic mechanisms involved in stress include Catecholamines increase due to the sympathetic nervous system, increased heart rate and blood pressure, decreased plasma volume and coronary vasoconstriction [86]. Immune system responses to stress can potentially help Atherosclerotic plaque rupture. Most of the studies on psycho-immunology show increased CD8+ cells and decreased CD4+ cells, as well as increased blood viscosity and stimulating the immune system in response to acute psychological challenges [7] [9]. These responses are the same as acute phase reactivity and relate to Hemodynamic responses to acute stresses [1].

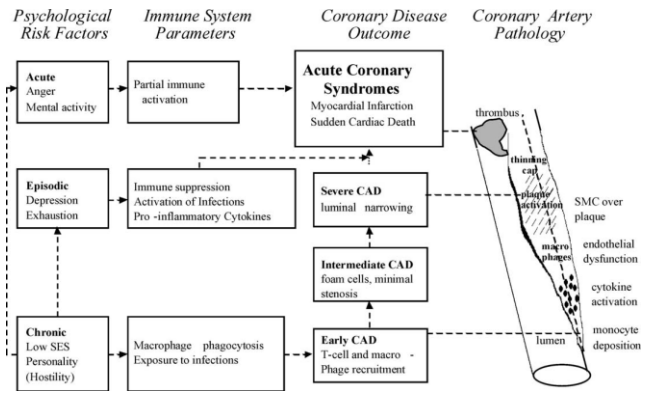

Figure 2: Acute, episodic and chronic psychological risk factors model by the immune system parameters involved in Coronary Arteries Disease

The value of depression- as the predictor variable for unsuitable long-term cardiac consequences- relates to its relapsing nature [1] [103]. In major depression, secretion of Corticotropin-Releasing Hormone (CRH) and repeated activation of HPA axis and thereby irregularity in this axis are seen [103] [104] [105]. Since psychological risk factors of depression have temporary nature. In most of the studies, no significant correlation is found between these factors and CAD severity [103]. Therefore, the processes involved in atherosclerotic plaques transition from stable to unstable mode are the probable factors which contribute on the formation of predictor role for depression and other episodic factors in acute coronary syndromes [1] [6] [106]. Immunologic correlates of depression include increased leukocytes of peripheral blood (mainly neutrophils and monocytes), decreased lymphocyte count, elevated serum cytokines (IL-6 and TNF-α), reduced indices of cell function and increased viral antibodies (e.g. cytomegalovirus) [7] [106].

Stable conditions such as type D personality are related to increased risk of first myocardial infarction in a long-term period [107]. Pro-atherogenic processes-which cause to increase lipid deposition and inflammatory processes due to the sympathetic nervous system-are of the well-known pathophysiologic pathways among chronic risk factors and initial stages of CAD [7]. Also, chronic psychological factors involved in the appearance of episodic risk factors. For instance, these factors can be associated with an increased incidence of depression or exhaustion [104]. Therefore, the prediction power of chronic psychological factors is somewhat influenced by their relation with episodic and acute [sychological risk factors for CAD. Kop refers to Jeron et al., in which neurohormones are proved as the potential factors for cytokine myocardial (IL-6) adjustment using an experimental model [7].

Regarding the mechanism of type D personality components effectiveness on coronary arteries narrow, the effects they make on the coronary system can be referred. Negative affectivity causes to increase Cortisol levels. Therefore, the people who experience negative affectivities are more prone to increased blood pressure and heart failure. In other words, stress hormones like Cortisol may be adjusted unsuitably among the patients with type D personality [43] [108]. This leads to increase blood pressure and blood vessel blockages. The arteries occlusion does not allow the blood full of oxygen to reach sufficiently the heart. On the other hand, the patients with type D personality may have a more active immune system with more inflammation which may damage blood vessels.

Briefly, the present study aimed to describe psychoneuroimmunological processes which contribute to CAD and CHD progression. Such psychological risk factors as stress, depression and type D personality were investigated here. Psychoneuroimmunological pathways of all three mentioned risk factors were described for CAD. The studies review indicated that stress could be accompanied with myocardial ischemia and help to rupture. The depression involves in the transfer of stable atherosclerotic plaque to unstable, and type D personality is effective on initial stages of a CAD.

However, most of the statistical indices in this

regard have a small effect size which is likely to be due to the role of different risk factors role in cardiovascular disease progression. Therefore, more researches are required on psycho-immunological mechanisms along with other cardiovascular risk factors including blood pressure, obesity, insulin resistance and age. Several studies in this regard show that other risk factors play as contributors in the relationship between psychological factors and immune system parameters associated with CAD [7]. As a result, some studies should be carried out by advanced methodologies including structural equations modelling. Also, to show clinical application of the reported relations, longitudinal studies are proposed to be conducted. Future clinical measures and researches on the integration of cardiovascular behavioural medicine and psycho-immunology can lead to increase classification accuracy of vulnerable patients as well as potentially improve intervention strategies.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Aug 20; 6(8):1545-1553.
https://doi.org/10.3889/oamjms.2018.294
eISSN: 1857-9655
Review Article

The burden of Diabetes, Its Oral Complications and Their Prevention and Management

Muhammad Ashraf Nazir[1*], Lamiah AlGhamdi[2], Mariam AlKadi[2], Noura AlBeajan[2], Latifah AlRashoudi[2], Mai AlHussan[2]

[1]Department of Preventive Dental Sciences, College of Dentistry, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia; [2]College of Dentistry, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

Citation: Nazir MA, AlGhamdi L, AlKadi M, AlBeajan N, AlRashoudi L, AlHussan M. The burden of Diabetes, Its Oral Complications and Their Prevention and Management. Open Access Maced J Med Sci. 2018 Aug 20; 6(8):1545-1553. https://doi.org/10.3889/oamjms.2018.294

Keywords: Diabetes; Oral complications; Prevention

***Correspondence:** Muhammad Ashraf Nazir. Department of Preventive Dental Sciences, College of Dentistry, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia. E-mail: manazir@iau.edu.sa

Received: 11-May-2018; **Revised:** 27-Jun-2018; **Accepted:** 03-Jul-2018; **Online first:** 15-Aug-2018

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Diabetes mellitus (DM), chronic disease, is a public health problem that affects 8.5% adult population worldwide. The number of adults with DM has risen sharply from 108 million in 1980 to 422 million in 2014. In 2012, 1.5 million individuals died because of DM and an additional 2.2 million deaths occurred because of high blood glucose level resulting in cardiovascular and other systemic diseases. DM brings huge economic loss to patients, their families, and healthcare systems. Globally, the cost of DM was US$1•31 trillion in 2015.

AIM: This review article utilised the prevalence data of diabetes mellitus from the World Health Organization and International Diabetes Federation to provide a comprehensive picture of the disease in different parts of the world.

METHODS: Electronic databases such as Google Scholar, Medline via PubMed, Scopus, and Web of Science were used to search the literature. The library resources of Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia were used to retrieve studies on the topics of the present review.

RESULTS: Systemic complications of DM include heart attack, kidney disease, limb loss, blindness, and peripheral nerve damage. More than 90% of diabetic patients were found to have oral manifestations. It is known that DM severely damages oral tissues causing periodontal disease, tooth loss, xerostomia, caries, burning mouth disorder, taste and salivary gland dysfunction, delayed wound healing, lichen planus, geographic tongue, and candidiasis. The evidence is mounting about a strong bidirectional relationship between DM and periodontal disease. Unfortunately, many diabetic patients are unaware of the association between DM and oral health, and only a small percentage of them visit the dentist for routine dental check-ups. Changes in lifestyles (control of blood glucose levels and self-care practices), regular dental check-ups with emphasis on periodontal assessment, and reinforcement of oral health instructions can effectively prevent oral complications of DM. Scaling and root planning are effective in improving glycemic control among diabetic patients.

CONCLUSION: Dental professionals should be part of the multidisciplinary team that helps individuals with diabetes.

Introduction

Diabetes mellitus (DM) is a heterogeneous group of clinical and genetic metabolic disorders recognised by abnormally high levels of glucose in the blood. It is classified broadly into two types – diabetes mellitus type 1 (DM I) and diabetes mellitus type 2 (DM II). There is an absolute reduction in insulin secretion due to β-cell destruction in DM I. DM II, also known as non-insulin dependent, is the most common form of DM that results from a progressive defect in the secretion of insulin and or resistance to the effects of insulin [1]. Separate global estimates of prevalence for DM I and II do not exist, because sophisticated laboratory tests are usually required to distinguish between both conditions [2]. There were 30.3 million people with DM (about 9.4% of the total population), out of which 23.1 million were diagnosed, and 7.2 million were still undiagnosed in the U.S. [3].

Damage and failure of various organs of the body such as the heart, blood vessels, kidneys, eyes, and nerves are caused by chronic hyperglycemia. Hence, cardiovascular diseases, chronic kidney disease, acquired blindness, and non-traumatic limb loss are complications of DM [4]. Diabetic patients manifest a high prevalence of oral problems such as dental caries, xerostomia, periodontal disease, sensory disorders, taste problems, salivary gland dysfunction, and oral infections [5]. Periodontal disease is one of the most common chronic inflammatory conditions which is characterised by the destruction of connective tissue surrounding the teeth,

and the condition gradually leads to tooth loss. Furthermore, the periodontal infection can predispose individuals to the complications of DM [5]. Increasingly, younger populations are now suffering from DM due to westernised lifestyles, poor eating habits, and the increased prevalence of obesity [4].

This review article utilised the prevalence data of diabetes mellitus from the World Health Organization and International Diabetes Federation to provide a comprehensive picture of the disease in different parts of the world. Also, the original studies and reports were used to describe the burden of diabetes, its oral manifestations and complications, and prevention and management.

Methods

Electronic databases such as Google Scholar, Medline via PubMed, Scopus, and Web of Science were used to search the literature. The library resources of Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia were used to retrieve studies on the topics of the present review. Various combinations of following keywords were used to search the studies. Keywords included “diabetes mellitus”, “oral”, “dental”, “epidemiology”, “prevalence”, “incidence”, “burden”, “costs”, “clinical”, “manifestations” “signs”, “symptoms”, “complications”, “xerostomia”, “Burning mouth syndrome”, “periodontal disease”, “dental caries”, “infections”, “lichen planus”, “healing” “halitosis”, “bad breath” “candidiasis”, “salivary”, “dysfunction”, “malfunction”, “prevention”, “control”, “treatment”, and “management”. Also, the reference lists of retrieved articles were also searched to find more relevant articles.

For the present review, recent studies were used to synthesise the latest information to update the healthcare professionals on the topic.

Results

Burden of Diabetes

DM is one of the most common metabolic disorders as it affects about 2-5% of the European and 20% of world populations [6]. Globally, the number of diabetic adults has increased to 422 million in 2014 from 108 million since 1980 [2]. The standardised age prevalence of DM was 9.8% for men and 9.2% for women in 2008 which increased from 8.3% and 7.5% respectively in 1980 in the world [7]. A recent study reported that the rate of diabetic death increased by 60.7%, from 12.1 per 100,000 population in 1990 to 19.5 per 100,000 population in 2013 [8]. By the year 2035, an estimated 592 million people will be diagnosed with DM [9].

Recent data were collected from World Health Organization and International Diabetes Federation (IDF) to provide a comprehensive picture of the prevalence of DM among selected high, middle and low-income countries [10] [11]. It can be seen from Figure 1 that Saudi Arabia has the highest prevalence of DM whereas Australia has the lowest prevalence rate among high-income countries. The average prevalence of DM in these countries is 8.61 (Figure 1).

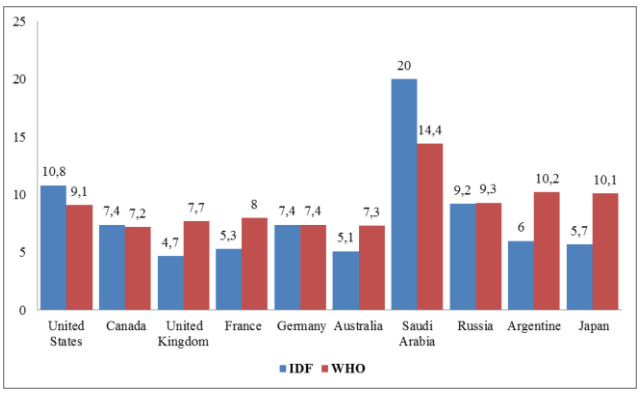

Figure 1: Prevalence of diabetes in high-income countries [10] [11]

Figure 2 shows the highest estimates of DM prevalence of Egyptians followed by Turks and Mexicans. Among the selected middle-income countries, Nigeria has the lowest prevalence of DM.

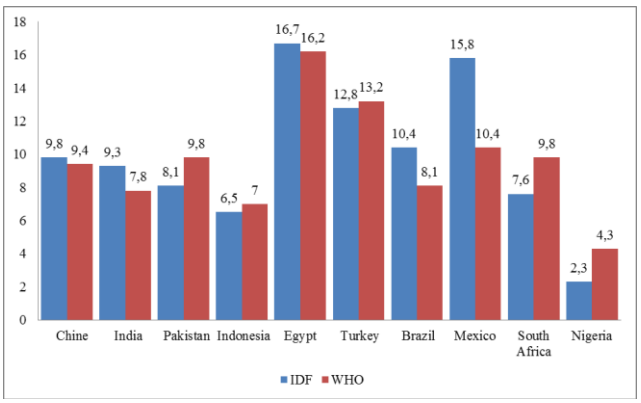

Figure 2: Prevalence of diabetes in middle-income countries [10] [11]

The average prevalence was 9.76 among these middle-income countries. Afghanistan has the highest prevalence of DM, and the average prevalence among these low-income countries was 5.3 (Figure 3).

There is no drastic difference in the average prevalence of DM in high and middle-income countries. However, the average prevalence of DM is considerably lower in low-income countries compared with high and middle-income nations. According to WHO, the Eastern Mediterranean Region has the

highest prevalence of DM (13.7%) in the world [2]. Based on WHO estimates, the countries with the highest prevalence rates in this region include Egypt (16.2%), Kuwait (14.7%) and Saudi Arabia (14.4%). While according to IDF data, Kuwait (20%), Qatar (20%), Saudi Arabia (20%), Bahrain (19.6%), and United Arab Emirates (19.3%) have highest DM prevalence in the region (Figure 4).

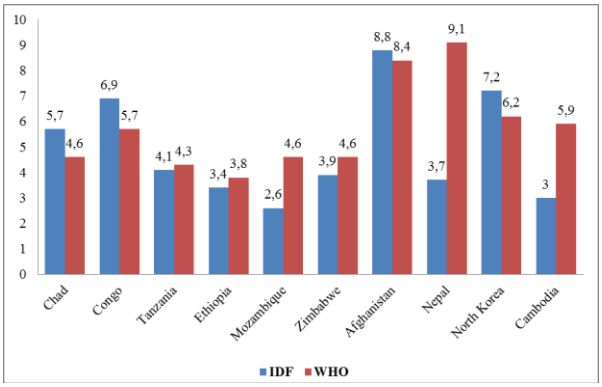

Figure 3: Prevalence of diabetes among low-income countries [10] [11]

DM is considered one of the principal causes of premature illness and deaths in most countries [12]. The patients with DM have increased the risk of cancers of the pancreas, liver, breast, urinary tract and colon [13]. Similarly, smokers are at greater risk (30%-40%) of developing DM than non-smokers [14]. It was also reported that HIV patients had a higher prevalence of DM than the adults from the general population [15]. DM is a major risk factor for coronary artery disease, and 75% of patients with DM die because of cardiovascular disease [16].

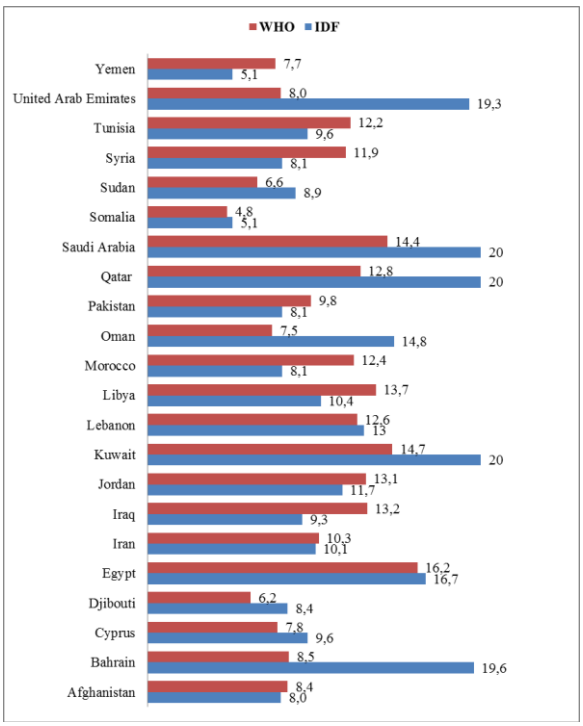

Figure 4: Prevalence of diabetes in the Eastern Mediterranean Region [10]

DM brings huge economic loss to patients, their families, and healthcare systems including loss of productivity and pressure on national economies [2]. In the U.S., an estimated cost of diagnosed DM was US$ 245 billion in 2012 out of which US$ 176 billion was spent for direct cost and US$ 69 billion was attributed to a reduction in productivity. There was an increase of 41% in the cost from the previous estimates of US$ 174 billion in 2007 [17]. Globally, the economic burden of DM was US$ 1·31 trillion in 2015, and indirect cost accounted for 34·7% of the total cost of the disease [18].

Oral manifestations and complications

Numerous oral complications are observed in both types of DM, which include periodontal diseases, oral candidiasis, tooth loss, xerostomia, halitosis, delay wound healing, burning mouth syndrome, salivary and taste dysfunction, tooth decay, lichen planus, geographic tongue, and complications associated with dental implants [19] [20].

DM is a risk factor for periodontal disease that affects its prevalence and incidence, and the degree of periodontal tissue destruction is determined by the level of metabolic control and the duration of DM [21] [22]. The prevalence of periodontitis is high (34%-68%) in diabetic patients, and deep pockets and attachment loss are common in patients with poorly controlled diabetes [21] [23]. The risk of alveolar bone loss is 11 times higher among patients with poorly controlled diabetes compared to healthy individuals [5].

The production of antibodies in response to microorganisms in periodontal tissues, and the presence of natural killer T cells, autoreactive B-cells, heat shock proteins, autoantibodies, and predisposing genetic factors provide the basis for an autoimmune role in the pathogenesis of the periodontal disease [24]. In diabetic patients, autoimmunity occurs in response to some defect in the usual self-control process resulting from chronic infection or tissue breakdown. The autoimmune process can target and destroy insulin-producing cells (β-cell), causing their destruction and impairing the production of insulin and promoting hyperglycemia. β-cell destruction occurs due to the production of autoantibodies by lymphocyte (B cell), and a T-cell mediated autoimmune response [25]. Epidemiologically, the link between periodontal disease and diabetes (DM I) is also attributed to the existence of autoimmune components in both these conditions [26].

There is a bidirectional relationship between periodontal disease and DM, and appropriate periodontal care can produce beneficial effects on metabolic outcomes among DM patients [27]. A meta-analysis of 9 randomised clinical trials found a moderate reduction in Haemoglobin A1c among DM patients after non-surgical periodontal treatment [28].

Another meta-analysis demonstrated that there was 0.29% reduction in Haemoglobin A1c with periodontal care at 3-4 months [29]. Moreover, a meta-analysis of 9 clinical trials observed that periodontal treatment resulted in the reduction of inflammatory biomarkers such as tumour necrosis factor alpha and C-reactive protein in diabetic patients [22]. However, robust evidence is lacking about the long-term positive effects of periodontal therapy on diabetes and a reduction in the prevalence of its complications [30].

One-quarter of diabetic patients were shown to have oral candidiasis which is one of the early and non-specific signs of uncontrolled DM [23]. Although, *Candida* is a normal commensal of the oral cavity, however, hyperglycemia, immune dysfunction, and acid production promote candidal infection among people with diabetes [31]. Also, increased salivary glucose levels in diabetic patients can enhance candidal proliferation and increase the possibility of acquiring oral candidiasis [31]. Dental plaque contains predominantly Candida albicans species that give rise to infection in the mouth [32]. It has been shown that diabetic patients have higher candidal colony-forming units in saliva than non-diabetic subjects which are associated with increased salivary glucose [31]. It was found that increased salivary glucose was significantly related to increased serum glucose levels. Therefore, it was suggested that salivary glucose levels could be used to evaluate the diabetic status of the patients and to monitor their glycemic control [33].

Tooth loss is highly prevalent among diabetic patients. A recent cross-sectional study identified that 15.3% of diabetic patients lost all their teeth and only 6.4% retained all natural teeth, and tooth loss was associated with older age and diabetic retinopathy [34]. It was reported that the diabetic patients had 1.46 times higher odds of having one tooth removed compared with individuals without DM [35]. Diabetic patients were found to underutilise dental care services although it is known that regular dental visits are beneficial for them [36]. The increased tooth loss among diabetic patients is related to the severity of periodontal disease that leads to alveolar bone destruction consequently resulting in tooth removal [34] [37]. Smoking and bruxism are significantly associated with tooth loss related to periodontal disease [37].

Xerostomia can affect oral functions and compromise patient's wellbeing, and its aetiology has been linked, among other factors, to the existence of systemic diseases, including DM [38] [39]. A recent study of diabetic patients (65-91 years) found that 92.5% had reduced salivary flow [40]. In a meta-analysis of 32 studies, it reported that the prevalence of xerostomia was 46.09% among diabetic patients and salivary flow rates were lower in DM patients than in non-DM population [38]. Salivary secretions are vital to oral health, as they assist in mechanical cleaning and aid in protective functions through physiological and biochemical mechanisms [40]. Hyposalivation can negatively affect the quality of life of the patient by compromising their eating habits, nutritional state, speech, and tolerance to dental prostheses. It can also enhance the risk of oral infection such as candidiasis, and increase patient's susceptibility to dental caries, periodontal disease, and tooth loss [38] [39]. Because of the complex aetiology of xerostomia, its treatment entails an interdisciplinary approach that should focus on reducing possible complications and improving quality of life [38].

It was found that 52% of patients suffered from halitosis which was the second most common oral complications among people with diabetes and even higher prevalence (76%) was observed among patients with uncontrolled diabetic [41]. On the other hand, a study identified 16% of diabetic patients with halitosis [23]. Diabetic patients with chronic periodontitis were found to have a significantly higher concentration of odoriferous microorganisms in tongue coating and subgingival plaque than non-diabetic patients. These odoriferous microorganisms produce volatile sulfur compounds which are responsible for oral malodor in diabetic patients [42].

Burning mouth syndrome affects 1.3 million Americans. Burning painful sensation in the mouth is often linked with dysgeusia and xerostomia. Classically, its symptoms improve in the morning, worsen during the day, and diminish at night [43]. The condition is characterised by a burning sensation in the tongue or other oral sites. Depression, various nutritional deficiencies, and DM increase the risk of burning mouth syndrome [44]. Though oral mucosal conditions such as candida infections, lichen planus, and dryness can cause burning sensations in diabetic patients, however, a neuropathic basis explains the burning sensations often accompanied by an alteration in taste (dysgeusia) or other sensory distortions. Therefore, the patients with peripheral diabetic neuropathy are susceptible to have burning sensations in the oral tissues [45] [46].

DM disturbs the hemostasis of the oral cavity by altering salivary function and composition even in well-controlled patients [47]. Dysfunction of salivary glands is a common phenomenon in DM [48] [49]. It has been reported that saliva of diabetic patients contains the higher concentration of proteins than controls [50]. Similarly, significantly high glucose and potassium concentrations are found in people with diabetes. Salivary dysfunction alters the taste sensation, and its effect is pronounced especially in poorly controlled diabetic patients [49].

A high prevalence of dental caries is found in diabetic patients compared to non-diabetics. Accumulation of microbial plaque flora causes demineralisation and dental caries. In diabetic patients, decreased flow of saliva reduces cleansing and buffer capacity besides diminishing levels of calcium that promotes tooth decay [51]. The reduction

of saliva also decreases the resistance to caries producing bacteria [52]. Moreover, saliva contains high glucose levels in diabetic patients that increase the amounts of fermentable carbohydrates for oral bacteria. The available current evidence on the association between DM and dental caries are mainly from studies conducted on DM I patients; however, there is a higher prevalence of caries in DM II patients compared with non-diabetic patients [47] [53].

It's a disorder that occurs due to the inflammation of mucous membrane and skin and is characterised by the chronic recurrent rash [49]. It was found that 62%- 85% of lichen planus patients had diabetes and they had an abnormal glucose tolerance test [54]. It was reported that oral lichen planus was found in 5.76% of type I and 2.83% of type II diabetic patients [55]. Similarly, higher prevalence of oral lichen planus was reported in diabetic patients compared to controls [56]. Oral lichen planus has been shown to have an autoimmune mechanism and frequently occurs in DM I which is also an autoimmune disorder [49].

Geographic tongue is an inflammatory disease associated with DM [57]. The lesion, found on the dorsum and margins of the tongue, causes pain, discomfort, and burning sensations, and is more common in male than female subjects. There is atrophy of the filiform papillae that leaves an erythematous area with a white, yellow or faintly grey elevated peripheral zone, and ill-defined spiky pattern of the tongue [58].

DM is associated with poor bone healing, increased risk of bone fracture, osteoporosis and diminished bone regeneration [59]. However, the patients who have controlled DM or are on hypoglycemic medication may not be at risk of increased of delayed wound healing after tooth extraction [60]. Delayed wound healing among diabetic patients is attributed to reduced cellular response including diminished macrophage functions, impaired production of growth factors, decreased angiogenesis and insufficient blood supply [61]. Also, impaired response to injury in DM can occur due to reduced substance P (nerve-derived mediator) [62].

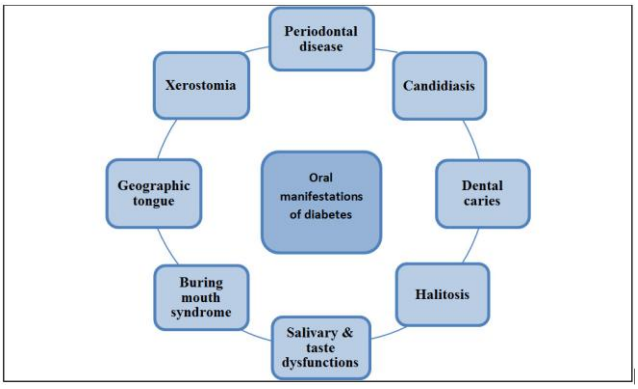

Figure 5: Oral manifestations among diabetic patients [23] [41]

Prevention of oral manifestations

The current body of evidence is not enough to prevent type 1 DM [2]. Oral manifestations of type 2 DM can be prevented through several approaches that are aimed at ensuring proper brushing and flossing behaviours, encouraging patients to visit the dentist for a routine check-up and controlling blood glucose levels [63].

Many DM patients are unaware of the relationship between DM and oral health [64]. There is a lack of awareness about the importance of maintaining oral health among patients with DM [65]. Additionally, the only a small percentage of patients diagnosed with DM visit their dentists for periodontal check-ups [66]. Every diabetic patient is assumed to be at risk for periodontal disease and should be referred for periodontal screening and educated on the importance of oral health and regular dental visits [67]. It was reported that more than 90% of DM patients had oral manifestations due to lack of periodic dental check-ups [68]. It has been suggested that individuals with high educational levels were more concerned about preventing and controlling the disease [69]. Therefore, providing education will raise awareness that will help prevent oral complications of DM.

The involvement of oral health care professionals in strategies to recognise individuals at risk for DM will strengthen preventive and screening efforts required to prevent oral diseases. Better treatment outcomes can be achieved if the dental practitioners are aware of dental implications and risk factors of DM [70] [71]. DM patients should be encouraged to visit the dentist for reinforcement and instruction on oral health information through diabetic and dental care centres. Systemic health is related to oral health particularly in diabetic individuals, which increases the need for dental and medical management of the patient. For improving the general and oral health of diabetic patients, collaborative relationship between patients, physicians, and dentists should be developed.

Dentists should provide advice about the use of fluoride mouthwash to prevent caries and antiplaque mouthwash to prevent periodontal problems. Tooth brushing with fluoride toothpaste twice a day and dental floss once a day should be emphasised to ensure plaque control. The patients with dentures should be advised to remove dentures at night and keep them properly cleaned [63]. Giving oral health education to relatives and friends could be beneficial as well because more than 55% of DM patients could be influenced by them [64]. The Internet can be used to educating DM patients because of its growing use among people [72]. Oral health educational material having accurate and updated information needs to be available to DM patients through different channels of communications. Dental practitioners should

participate in educational activities at an organisational level to raise awareness about oral health matters with diabetic people. Preventing harmful complications by raising awareness through different campaigns is the responsibility of dental professionals as well as government agencies.

Several programs could be designed to prevent or reduce oral manifestations of DM. The program such as lifestyle Change Plus Dental Care (LCDC) was developed based on the Health Belief Model, Social Cognitive Theory, and Cognitive Behavioral Theory [73]. In the program, lifestyle changes and periodontal care were given particular attention to avoid dental complications. The program significantly improved glycemic control and periodontal health by improving periodontal status parameters such as plaque index, gingival index, pocket depth, clinical attachment loss, and bleeding on probing. LCDC program was effective in increasing awareness and modifying DM patients' attitudes about oral health care [74].

Having increased physical activity, eating healthy food, reducing body weight, and managing blood pressure, cholesterol levels, and emotional issues can prevent complications of DM. Also, high-risk individuals who have impaired glucose tolerance or fasting glucose are susceptible to developing DM; however, standard lifestyle changes, proper diet, regular exercise and use of antidiabetic drugs can help prevent DM in them [75]. It was reported that prediabetic patients were found to have deteriorated periodontal health as demonstrated by worse periodontal parameters, and glycemic control was shown to reduce the severity of these parameters [76]. Thus, an oral assessment should be part of routine measures and dentist should be a part of the multidisciplinary team that helps diabetic patients.

To sum up, the oral manifestations of DM could be reduced by several preventive measures that include blood glucose control, self-care practice about DM, and maintenance of proper oral health. These measures can also minimise health-care spending for diabetic patients.

Early identification, assessment, and management of patients who at risk of developing DM require a dentist's active role in diagnosing the condition in previously undiagnosed individuals. Diabetic patients should visit the dentist regularly, every 3 months [67]. Oral cavity examination and detailed history taking are necessary before undertaking any dental procedure [77]. The dentist should be familiar with the medical management of DM and the recognition of signs and symptoms of the undiagnosed or uncontrolled disease.

The regular self and professional periodontal care can benefit patients with periodontitis and DM [78]. Poor glycaemic control over a prolonged time can lead to impaired healing, and severe periodontal disease, as high levels of postprandial plasma glucose (PPg) and HbA1c are the leading cause of oral complications [79]. Appointments timing should be scheduled in the early morning, to reduce the disturbances in patients' medical regimens [71]. Vasoconstrictors such as adrenaline present in dental local anaesthetic cartridge could deregulate the blood glucose level. Therefore, adrenaline should be reduced in poorly controlled insulin-dependent diabetic patients [80]. The patients on insulin or other antidiabetic medications should take these with drugs as usual before visiting a dentist. These medications along with some snack or meal should be brought to the dental office [63].

It has been reported that diabetic patient's periodontal health status can be improved by scaling and root planning, with or without antibiotics [20]. Scaling and root planning is effective in reducing haemoglobin A1c (a marker of average blood sugar level) by 0.29% (3-4mmol/l) for up to three months [81]. Poorly controlled DM is considered a relative contraindication for implant therapy [27]. However, maintaining the blood glucose level of implant patients can help suppress the progression of periodontal destruction, bone loss and improve osteoblastic function [82] [83]. Dental clinicians should provide 6 months of peri-implant and periodontal maintenance phase which includes the provision of oral health instructions and full mouth scaling and root planning around natural teeth and implant [81].

Conclusion

In conclusion, DM is a public health crisis and health care professionals should play their roles to prevent and control the disease and its oral and other systemic complications. There is a high prevalence of DM, especially in high and middle-income countries. Eastern Mediterranean region has the highest prevalence of DM in the world. In addition to millions of people diagnosed with DM, a considerable proportion of the population is undiagnosed. The condition causes huge economic and financial burden to the healthcare systems in addition to increased morbidity and mortality. Oral complications of DM are numerous and include periodontal disease, hyposalivation, dental caries, halitosis, delayed wound healing, taste and salivary dysfunctions, candidiasis, and burning mouth syndrome.

Increasing awareness and knowledge about the DM, its association with oral health including oral complications among patients can help prevent DM and improve their quality of life. Diabetic patients should be emphasised to take good care of their oral hygiene and control DM by maintaining appropriate glucose levels to prevent oral complications. Health care providers can develop certain programs to help patients control DM and its oral complications. The patients should be encouraged to regularly visit dentists, and they should be part of an

interdisciplinary team of health providers. Routine periodontal assessment and provision of scaling and root planning are necessary to maintain optimal periodontal health among diabetic patients. Health care providers should update their knowledge about the oral manifestations of DM and work collaboratively to control DM and prevent its complications.

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