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Basic Science

Correlation of Immunohistochemistry and Fluorescence *in Situ* Hybridization for HER-2 Assessment in Breast Cancer Patients: Single Centre Experience

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Abstract

BACKGROUND: Accurate assessment of HER-2 is imperative in selecting patients for targeted therapy. Most commonly used test methods for HER-2 are immunohistochemistry (IHC) and fluorescence in situ hybridisation (FISH). We evaluated the concordance between FISH and IHC for HER-2 in breast cancer samples using Food and Drug Administration approved tests.

MATERIAL AND METHODS: Archived paraffin tissue blocks from 73 breast cancer patients were used. HER-2 immunostaining was performed using Ventana anti–HER-2 monoclonal antibody. The FISH assay was performed using PathVysion™ HER-2 DNA Probe Kit.

RESULTS: Of the 73 cases 68.5% were IHC 0/1+, 15.07% were IHC 2+ and 16.44% were IHC 3+. Successful hybridisation was achieved in 72 cases. HER-2 FISH amplification was determined in 16.67% cases. Ten IHC 3+ and two IHC 2+ cases were FISH positive. Two of the IHC 3+ cases were FISH negative. Concordance rate was 100%, 18.18% and 83.33% for IHC 0/1+, 2+ and 3+ group, respectively. Total concordance was 84.72%, kappa 0.598 ($p < 0.0001$). The sensitivity of IHC in detecting IHC 2+ and IHC 3+ cases was 16.7% and 83.3%, and the specificity was 85% and 96.67%, respectively.

CONCLUSION: The consistency between the methods was highest for IHC negative and lowest for IHC equivocal cases. The immunohistochemistry showed high sensitivity for IHC 2+/3+ cases and high specificity for IHC 3+ cases. Our results support the view that false-positive rather than false-negative IHC results are a problem with HER-2/IHC testing, and that IHC should be used as an initial screening test, but IHC 2+/ 3+ results should be confirmed by FISH.

Introduction

The human epidermal growth factor receptor gene HER-2 (also known as HER-2/neu, c – *erb*B-2) is located on chromosome 17q12 and encodes a member of the epidermal growth factor receptor (EGFR) family with tyrosine kinase activity that is responsible for cell-cell or cell-stroma communication through the process of signal transduction [1]. Activation of the protein receptor is associated with increased cell proliferation, tumour invasiveness, progressive regional and distant metastases, increased angiogenesis and reduced apoptosis [1]. HER-2 gene amplification is the primary mechanism of protein overexpression and is found in nearly 15 to 20% of breast cancer patients [1] [2]. HER-2 gene amplification or protein overexpression are molecular targets for specific targeted therapies associated with good results in early and metastatic HER-2 positive breast carcinomas [3] [4] [5]. Therefore, accurate assessment of HER-2, using reliable, highly sensitive and specific test is imperative in the selection of patients for the therapy [3] [4] [5].

To date, there is still no single, universally accepted test for HER-2 assessment. Two most commonly used techniques are immunohistochemistry (IHC) and in situ hybridisation (fluorescence in situ

hybridization-FISH and bright field in situ hybridization-BRISH), performed on formalin fixed paraffin embedded (FFPE) tissue samples [6] [7] [8]. Immunohistochemistry uses antibodies to detect HER-2 protein expression on the surface of tumour cells, while FISH is a molecular method that uses fluorescently labelled DNA probes, to determine HER -2 gene copy number. Although both methods are widely used in the routine analysis, both have advantages and disadvantages. Immunohistochemistry is relatively cheap and fast method that uses the light microscope for analysis. Conversely, a FISH method is technically more demanding, time-consuming and expensive assay [9], but is more consistent and more objective [9]. Numerous studies that evaluated the consistency between the IHC and FISH, as well as their effect on the response to trastuzumab therapy, showed contradictory results [10].

In this study we evaluated the concordance between FISH and IHC for HER-2 assessment in breast cancer tissue samples, using Food and Drug Administration (FDA) approved tests.

Material and Methods

In this retrospective study, we used FFPE tissue blocks from 73 patients diagnosed with invasive breast carcinoma, non-special type (NST), during 2014-2015. Patients who underwent radical mastectomy and did not receive neoadjuvant therapy were included.

All the cases were stained and analysed in the standard procedure to determine the histologic type and grade of a tumour, lymph node status and the stage of the disease. Tumour grade was determined based on the recommendations of the Nottingham grading system [11], while the stage of the disease was determined according to the criteria of the American Joint Committee on Cancer (AJCC) [12]. The patients' age and tumour dimension were obtained from medical records. HER-2/IHC was performed in parallel with ER, PR, and Ki-67 as part of the daily routine work at our Institute. Regardless of the IHC results, additional FISH testing was done in all cases, using parallel sections from the same tissue block as for IHC.

Using 4 micron thick sections mounted on silanized microscopic slides, HER-2 immunostaining was performed on BenchMark GX automated staining instrument (Ventana Medical Systems, Inc., USA) using Ventana anti–HER-2 rabbit monoclonal primary antibody, clone 4B5 and UltraVIEW universal DAB Detection Kit (Ventana), according to the manufacturer's recommendations. Briefly, after deparaffinization with EZ Prep, slides were pretreated with Cell Conditioning 1 for 36 minutes at 100°C and then incubated with anti–HER-2 primary antibody for 20 minutes at 37°C. The antibody was detected using DAB and then counterstained with hematoxylin and bluing reagent, for 4 minutes in both steps.

The ER, PR and Ki67 immunostainings were performed using DAKO monoclonal antibodies (clone EP1, dilution 1:50; clone PgR 636, dilution 1:100 and clone Mib1, dilution 1:150, respectively), by semiautomated PT Link immunoperoxidase technique. Shortly, after deparaffinization and rehydration, samples were pretreated using Target Retrieval Solution for 20 minutes at 97°C and then incubated with primary antibody for 20 minutes at 25°C. Antibodies were detected using visualisation system (EnVision FLEX, DAKO) for 20 minutes at 25°C and chromogen (di–amino-benzene-DAB) for 5 minutes, also at 25°C. After that slides were counterstained with hematoxylin.

Semiquantitative evaluation of HER-2 protein expression included evaluation of membrane positivity according to the criteria of American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) [7]. The expression of ER, PR and Ki67 was evaluated as a percentage of positive cells of the total number of cells. One percent was the cut-off point for hormone receptors [13], while 20% was taken as a cut-off point that distinguishes cases with low and high proliferative index (Ki67-low/Ki67-high) [14]. The slides were analyzed with a light microscope, Nikon 80i Eclipse (Nikon Instruments, Austria).

The FISH assay was performed by using PathVysion™ HER-2 DNA Probe Kit (Abbott/Vysis, IL, USA) containing two fluorophore-labeled DNA probes allowing simultaneous detection of HER-2 and chromosome 17 (CEP 17) gene copy numbers: Spectrum Orange-labeled DNA probe for HER-2 gene locus and Spectrum Green labelled DNA probe for CEP 17. Samples were tested using two different paraffin pretreatment kits in two different FISH protocols, Vysis/Abbott Paraffin Pretreatment Reagent Kit (40 samples) and DAKO Histology FISH Accessory Kit (33 samples), described in details in a previous paper [15]. Briefly, 4 μm thick tumour sections were mounted on a positively charged microscopic slide, air dried and baked in the oven at 56°C, overnight. After deparaffinization and pretreatment, slides were incubated with protease/pepsin. Then slides were washed, dehydrated and DNA probe was applied. After denaturation (5 minutes at 72°C) and hybridisation (16 hours at 37°C), the slides were washed in preheated post-hybridisation buffer, counterstained with DAPI, and cover slips were applied.

For accurate localisation of the invasive tumour component, the FISH assays were viewed in conjunction with H&E sections, and DAPI counterstain was used to identify tumour nuclei. Signals were

analyzed at x1000 magnification, using an appropriate filter. The results were interpreted according to recommendations of ASCO/CAP, where HER-2 status is defined as positive when the HER-2/CEP17 ratio is greater than 2, and negative when the ratio is less than 2 [7]. The tests were analysed using Olympus BX43 fluorescence microscope (Olympus Corporation, Japan) equipped with appropriate filters. Each case was photographed and documented with Olympus XM10 monochrome camera and analysed using the Olympus cell Sens Standard software, Version 1.15.

Analyses were performed by using SPSS for Windows 17.0. The results of HER-2 status by FISH and IHC were compared, and concordance, sensitivity, specificity, negative and positive predictive values were evaluated considering FISH as the gold standard. Kappa test was used to determine the concordance between the methods. Also, Fisher's exact two-tailed test and Chi-square tests were used to determine the correlation of HER-2 status with ER and PR status along with various clinical and histology parameters. The p-value < 0.05 was considered statistically significant.

Results

The HER-2/ IHC results showed that most of the samples 32 (43.84%), were classified as IHC 0, followed by 18 (24.66%) classified as IHC 1+, 12 (16.44%) classified as IHC 3+ and 11 (15.07%) classified as IHC 2+ (Figure 1A). From 73 cases included in this study, 72 showed successful hybridisation. HER-2 FISH gene amplification was determined in 12 (16.44%) of the cases, while 60 (82.19%) of the cases were FISH negative (Figure 1B and Figure 2). One case with failed hybridisation was excluded from the study. The FISH failure rate was 1.37%.

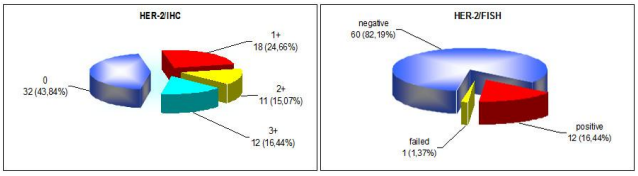

Figure 1: Distribution of HER-2 according to A, IHC (left) and B, FISH (right)

Of 12 HER-2 FISH amplified cases, 10 (83.3%) were scored IHC 3+, 2 (16.7%) were scored IHC 2+ and none was scored IHC 1+ or 0. Among the 60 FISH-negative cases, only 2 (3.3%) had IHC score 3+ (Figure 3), and the other samples were either indeterminate 9 (15%) or negative 49 (81.7%). The two IHC 3+ cases that were negative for FISH showed polysomy for chromosome 17.

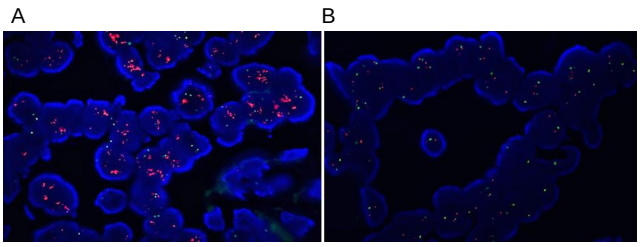

Figure 2: Typical examples of FISH-positive and FISH negative case. A, FISH amplified case, HER-2/Chr 17 > 2 (DAPI counterstain x 1000); B, FISH non amplified case, HER-2/Chr 17 < 2 (DAPI counterstain x 1000)

In Table 1 we present the rate of concordance for HER-2 results obtained by IHC and FISH. The concordance rate was high (100%) for negative IHC 0/1+ group and low (18.18%) for undetermined IHC 2+ group. The concordance rate for IHC 3+ group was 83.33%.

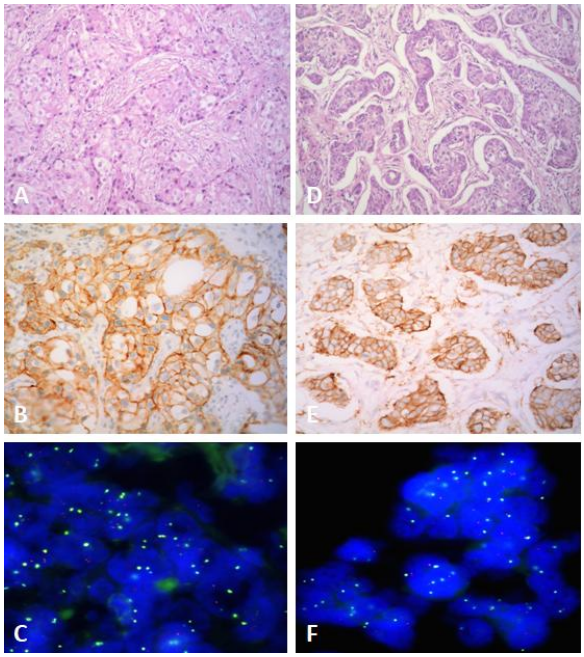

Figure 3: Discordance between IHC and FISH in two cases. Case 1: A, Invasive breast carcinoma (H&E x 200); B, HER-2/IHC 3+ (HER-2 x 200), C, FISH, HER-2/Chr 17 < 2, non amplified (DAPI counterstain x 1000). Case 2: D, Invasive breast carcinoma (H&E x 200); E, HER-2/IHC 3+ (HER-2 x 200); F, FISH, HER-2/Chr 17 < 2, non-amplified (DAPI counterstain x 1000)

When IHC 2+ and 3+ positive tumours were grouped, the total concordance between IHC and FISH was 84.72% (61/72), and the Kappa coefficient was 0.598, with a statistical significance of p < 0.0001. After excluding the IHC 2+ cases, the concordance rate improved to 96.72% (59/61).

Table 1: Comparison of HER-2 results determined by IHC and FISH

IHC scoring	HER-2/FISH positive	HER-2/FISH negative	Concordance by IHC	Discordance by IHC
0/1+ (n=49)	0	49	(49/49) 100%	(0/49) 0%
2+ (n=11)	2	9	(2/11) 18.18%	(9/11) 81.82%
3+ (n=12)	10	2	(10/12) 83.33%	(2/12) 16.67%

Table 2 presents the diagnostic performances of the IHC method in determining the HER-2 status using the FISH method as a gold standard. As indicated, the positive predictive value for positive IHC 3+ cases was 83.3%, and for 2+/3+ cases was 52.2%. The immunohistochemical method showed the sensitivity of 100% and 83.3% in detecting IHC 2+/3+ and 3+ cases, and specificity of 81.67% and 96.7% in detecting IHC 2+/3+ and IHC 3+ cases, accordingly.

Table 2: Sensitivity, specificity, positive and negative predictive values of IHC according to FISH as gold standard

IHC scoring	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)
2+/3+ positive	100(75.8-100)	81.67(70.1-89.4)	52.2(33.01-70.8)	100(92.7-100)
3+ positive	83.3(55.2-95.3)	96.7(88.6-99.1)	83.3(55.2-95.3)	96.7(88.6-99.1)
2+ positive	16.7(4.7-44.8)	85(73.9-91.9)	18.2(5.1-47.7)	83.6(72.4-90.8)

In table 3 we present a correlation between HER-2 amplification and clinico-pathological characteristics. The mean age of the patients included in the study was 57.98 ± 10.3 years (range, 41-86 years), and the mean tumour size was 27.01 ± 14.8 mm (range, 8-75 mm). There was no significant correlation between HER-2 amplification and patients' age, tumour size, tumour grade (G), nuclear grade (NG), tumour status (pT), lymph node status (pN) or stage of the disease. A significant correlation ($p < 0.05$) was detected between HER-2 and biological markers (ER, PR, Ki 67). ER and PR were more commonly detected in the HER-2/FISH negative than in HER-2/FISH positive tumours (90% vs 58.33%; 78.33% vs 41.67%, respectively). Conversely, the high proliferative index was more frequently found in HER -2 positive tumours (91.67% vs 56.67%).

Table 3: Correlation of clinical and pathological features with HER-2 amplification status

Variable		Total n (%)	HER-2/FISH		p-value
			Negative (n = 60)	Positive (n = 12)	
Age	≤50	23 (31.94)	17 (28.33)	6 (50)	0.18
	>50	49 (68.06)	43 (71.67)	6 (50)	
Tumor size (mm)	≤20	24 (33.33)	19 (79.17)	5 (20.83)	0.5
	>20	48 (85.42)	41 (85.42)	7 (14.58)	
G	1	3 (4.17)	3 (5)	0	0.26
	2	19 (26.39)	18 (30)	1 (8.33)	
	3	50 (69.44)	39 (65)	11 (91.67)	
NG	1	1 (1.39)	1 (1.67)	0	0.12
	2	15 (20.83)	15 (25)	0	
	3	56 (77.78)	44 (73.33)	12 (100)	
pT	1	24 (33.33)	19 (31.67)	5 (41.67)	0.34
	2	41 (56.94)	35 (58.33)	6 (50)	
	3	2 (2.78)	1 (1.67)	1 (8.33)	
	4	5 (6.94)	5 (8.33)	0	
pN	0	27 (37.56)	24 (40)	3 (25)	0.13
	1	21 (29.17)	18 (30)	3 (25)	
	2	12 (16.67)	11 (18.33)	1 (8.33)	
	3	12 (16.67)	7 (11.67)	5 (41.67)	
Stage	I	16 (22.22)	14 (23.33)	2 (16.67)	0.66
	II	30 (41.67)	26 (43.33)	4 (33.33)	
	III	26 (36.11)	20 (33.33)	6 (50)	
ER	N	11 (15.28)	6 (10)	5 (41.67)	0.015
	P	61 (84.72)	54 (90)	7 (58.33)	
PR	N	20 (27.78)	13 (21.67)	7 (58.33)	0.016
	P	52 (72.22)	47 (78.33)	5 (41.67)	
Ki 67	high	45 (62.5)	34 (56.67)	11 (91.67)	0.025
	low	27 (37.5)	26 (43.33)	1 (8.33)	

G- histological grade, NG- nuclear grade, pT- tumour status, pN- lymph node status, ER- estrogen receptor, PR- progesterone receptor, Ki67- a marker of proliferation.

Discussion

Breast cancer is the most common malignant tumour and second leading cause of cancer death in women [16]. Prognosis and treatment of breast cancer patients depend on several factors, such as histological type, grade, stage, hormone receptor status and HER-2 status. Determination of HER-2 status is a strong indicator of response to treatment with trastuzumab [17] [18]. Considering the benefits and side effects that patients would have from targeted therapy, the use of the appropriate test for HER -2 assessment is essential in selecting patients for treatment [3] [5]. Immunohistochemistry and FISH are most widely used routine test methods in pathology laboratories. Both methods have their advantages and disadvantages. To date, it is still under debate which single method is the best for HER-2 determination. According to some authors, the use of IHC and FISH methods in combination is the most effective strategy even though it is not cost effective [19] [20]. Immunohistochemistry is widely used, relatively inexpensive and easy to perform test method for HER-2. It is affected by variations in tissue fixation and processing and variations in testing methodologies that can influence the final results [21]. Other disadvantages of IHC are subjectivity in interpretation of the results and absence of internal control, which calls into question the reliability of the analysis, especially when the HER-2/IHC results are negative [3] [21] [22].

Fluorescence in situ hybridisation is expensive, technically demanding molecular assay that requires special equipment for evaluating the results, and its performance is limited to a smaller number of pathology laboratories [9]. However, the FISH method has several advantages over immunohistochemistry: it is less affected by artefacts associated with tissue processing, is more objective because the results are quantitative, and there are internal positive controls [9]. Fluorescence in situ hybridisation is a method of choice when selecting patients for HER-2 targeted therapy regarding accuracy, reproducibility, and predictivity [3]. It provides 96.5% sensitivity and 100% specificity for detection of HER-2 gene amplification in breast cancer patients [23].

In this study, we evaluated the concordance between FISH and IHC for HER-2 detection in breast cancer patients using FDA approved tests. Most of our cases (68.5%) were classified IHC 0/1+, 16.44% were classified IHC 3+ and 15.07% were classified IHC 2+. None of IHC 0/1+ cases was FISH positive. 16.67% of cases in our study showed amplification for HER-2: two cases of IHC 2+ group were FISH positive, and two cases from IHC 3+ group were FISH negative. Concordance rate in our study was 100%, 18.18% and 83.33% for IHC 0/1+, 2+ and 3+ group, respectively. When 2+ and 3+ positive tumours were

grouped together, the concordance rate between IHC and FISH was 84.72%, kappa 0.598 ($p < 0.0001$), but after excluding the IHC 2+ cases form the group, the total concordance rate improved to 96.72%. According to literature, concordance rate between IHC and FISH ranges from 79% to 100% for 3+ cases [24] [25] and from 12% to 36% for 2+ cases [26] [27].

Gokhale et al., [10] showed high concordance between FISH and IHC 3+ groups and poor concordance in the 0, 1+ and 2+ groups. Contrary to these results, other authors [28] [29] have shown that the concordance rate between IHC and FISH is highest for the IHC negative cases and lowest for the IHC 2+ and 3+ cases. Our results also confirmed high concordance rate in IHC 0/1+ group, followed by IHC 3+ group with the lowest concordance rate in the IHC 2+ group. Other authors reported low concordance rates of only 51% between IHC and FISH [21] owing to subjectivity in interpretation, chromosome 17 aneuploidy and technical aspects of tissue processing and IHC. Sarode et al. [30] showed significant improvement in concordance rate in 10 year period due to an overall improvement in standardisation of pre-analytic and analytic variables and experience in HER-2 scoring. The finding of IHC 3+ staining without gene amplification is attributed to false -positive immunostaining when using an unstandardized or unvalidated immunohistochemical method, or chromosome 17 polysomy [31] [32]. Several studies have shown that chromosome 17 polysomy is responsible for discrepancies between protein expression and gene amplification [23] and that these patients have similar clinical outcomes to patients without the HER-2 gene alteration. The rate of discordance in our study may be the result of variability in tissue fixation (time to fixation and time in fixative) because almost half of the cases included were from other city hospitals where tissue fixation started. However, we cannot exclude the influence of aneusomy 17 because two IHC 3+/FISH- cases in our study, showed chromosome 17 polysomy.

Taking the FISH method as a gold standard, sensitivity rate in our study was 16.7% and 83.3% for IHC 2+ and 3+ cases. The specificity rate was 96.67% and 85% for 3+ and 2+ cases, respectively. When 2+/3+ cases were analyzed as a group, the sensitivity was 100%, but the specificity was 81.67%. The positive predictive value of positive IHC 3+ and IHC 2+/3+ cases was 83.3% and 52.2% respectively, and negative predictive value for negative IHC 0/1+ cases was 100%. The immunohistochemical method showed the highest sensitivity of 100% in detecting IHC 2+/3+ positive tumours as one group. Other authors reported high specificity (94%), but low sensitivity (43%), of immunohistochemistry [10].

According to some authors, the lowest cost - effective HER-2 testing is to screen all breast cancer patients with immunohistochemistry (because of its high negative predictive value) and to confirm only IHC 2+ and 3+ scores with the FISH assay [29]. Although FISH testing is much more expensive than IHC, it never exceeds the cost of treating patients who are not likely to benefit because of a false-positive IHC [29]. Our findings support the view that false - positive rather than false-negative IHC results are a major issue with HER-2 IHC testing. HER-2 positive status is a bad prognostic marker, and these tumours are associated with high histological grade, negative hormone receptor status and positive regional lymph nodes at the time of diagnosis [30] [33] [34]. Our results did not show a significant correlation between HER-2 amplification and other clinico-pathological parameters like patient's age, tumour size, tumour grade, nuclear grade, lymph node status and stage of the disease. Although statistically non-significant HER-2 amplified, tumours were more frequently poorly differentiated with high nuclear grade, positive lymph node status and high postoperative stage indicating biologically more aggressive tumours. Other authors found no association between HER-2 and patients age [35] [36] [37] [38] tumor size [35] [36] [37] or lymph node status [35], too. Contrary to our results, other authors noted significant correlation of HER-2 with tumour size [39] tumour grade [35] [39] or lymph node metastasis [40] [41].

Statistically significant association in our study was detected between HER-2 positive tumors and negative estrogen ($p = 0.015$), progesterone receptor ($p = 0.016$) status, and high proliferative index Ki67 ($p = 0.025$). Some authors also reported significant correlation with negative hormone receptor status [35] [39] and high proliferative index [30] [37] [38], while other authors showed correlation with positive hormone receptor status [41] [42] or low Ki67 [41].

In conclusion, the overall concordance between IHC and FISH was 84.72%. The consistency between the two methods was highest for IHC negative and lowest for IHC equivocal cases. With FISH as the gold standard, the positive predictive value of positive (IHC 3+) cases was 83.3%, and negative predictive value for negative (IHC 0/1+) cases was 100%. The immunohistochemical method showed high sensitivity in IHC 2+/3+ cases and high specificity in IHC 3+ group. Our results support the view that false -positive rather than false-negative IHC results are a bigger problem with HER-2/IHC testing, and that IHC should be used as an initial screening test, but that FISH should confirm IHC 2+ and 3+ results. Standardization of tissue processing is necessary to improve the specificity of the IHC assay.

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Basic Science

Impact of Silver Nanoparticles on Gene Expression in *Aspergillus Flavus* Producer Aflatoxin B1

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Abstract

AIM: In this study, we evaluated the effect of silver nanoparticles (AgNPs) on the production of aflatoxin B_1 (AFB_1) through assessment the transcription activity of aflatoxin biosynthesis pathway genes in *Aspergillus flavus* ATCC28542.

MATERIAL AND METHODS: The mRNAs were quantitative by Real Time-polymerase chain reaction (qRT-PCR) of *A. flavus* grown in yeast extract sucrose (YES) medium containing AgNPs. Specific primers that are involved in the AFB_1 biosynthesis which highly specific to *A. flavus*, O-methyltransferase gene (*omt-A*), were designed and used to detect the fungus activity by quantitative PCR assay. The AFB_1 production (from *A. flavus* growth) which effected by AgNPs were measured in YES medium by high-pressure liquid chromatography (HPLC).

RESULTS: The AFB_1 produced by *A. flavus* have the highest reduction with 1.5 mg $^{-100\ ml}$ of AgNPs were added in media those records 88.2%, 67.7% and 83.5% reduction by using AgNP HA1N, AgNP HA2N and AgNP EH, respectively. While on mycelial growth give significantly inhibitory effect. These results have been confirmed by qRT-PCR which showed that culture of *A. flavus* with the presence of AgNPs reduced the expression levels of *omt-A* gene.

CONCLUSION: Based on the results of the present study, AgNPs inhibit growth and AFB1 produced by Aspergillus flavus ATCC28542. This was confirmed through RT-PCR approach showing the effect of AgNPs on omt-A gene involved in aflatoxin biosynthesis.

Introduction

Aflatoxins are secondary polyketide metabolites mainly produced by aflatoxigenic fungi of *Aspergillus flavus* and *Aspergillus parasiticus*. Among the aflatoxins that have been identified, aflatoxin B_1 (AFB_1) is most prevalent form, presents the highest potential toxic [1]. AFB_1 is classified as group 1 carcinogenic to mammals by the International Agency for Cancer Research (IARC) [2]. AFB_1 has potential hepatic [3], carcinogenicity [4], cytotoxicity, genotoxicity [5] [6] [7] and immunotoxicity [8].

Swallowing of AFB_1 has a toxic to the immune system works [9] and mainly reduces the function of cell-mediated immunity [8]. Biochemical pathways and gene regulation it is vital to aflatoxin (AF) also characterized. Many studied already were published in genetics about the AF biosynthesis [10] [11]. Approximately Twenty Seven enzymatic and at least 25 genes involved in the AF biosynthesis in *A. flavus*, this genes are cluster within 75 kilobytes (kb) in the fungal genome [12] [13]. Most of that seems to be co-organized by the DNA binding protein AflR, encoded by the genes aflR [14]. Yabe and Nakajima [15] demonstrated that the three genes pksA, ver-1 and *omt-A* encode enzyme proteins were involved in the AF biosynthetic pathway. On the other hand, gene aflR is a regulatory gene whose product regulates

transcription of some genes; pksA, ver-1 and *omt-A* [16]. Many strategies have been developed to reduce aflatoxins contamination, either by preventing the growth of aflatoxigenic fungi or by blocking the production of the toxin after infection [17]. Silver nanoparticles (AgNPs) is recent advancements in the field of nanotechnology were used as a novel therapeutic agent as antibacterial, antifungal, antiviral, anti-inflammatory and anti-cancer agents [18] [19]. The effect of Silver nanoparticles on growth and production of other types mycotoxins by toxigenic fungi were studied but; the effect Silver nanoparticles AgNPs on gene expression of AFs biosynthesis pathway genes still need more studies. This study aims to evaluate the effects of three types AgNPs on the production of AFB_1 through assessment the transcription activity (gene expression) of AFB_1 biosynthesis pathway genes in *Aspergillus flavus* ATCC28542.

Material and Methods

Fungal strain

Fungal Strain: a Toxigenic strain of *Aspergillus flavus* (ATCC 28542) was obtained from Microbial Research Center, Faculty of Agriculture, Ain Shams University Cairo, Egypt (MIRCEN).

Chemicals and solvents

Potato dextrose agar (PDA) and yeast extract sucrose (YES) liquid growth medium and Sodium sulphate anhydrous were obtained from Sigma-Aldrich, France. Aflatoxin B_1 (AFB_1) standard was purchased from Sigma, Chemical Co. (St. Louis, MO, U.S.A). Stock solutions and standard were prepared and assayed according to Association of Official Analytical Chemists (AOAC) [20] Method 990.33A. Methanol and acetonitrile HPLC grade were produced by BDH, England. The water was double distilled with Millipore water purification system (Bedford, M A, USA).

Synthesis and characterised of AgNPs

AgNPs in this study were synthesised by *Aspergillus terreus*HA1N (KR364880) and *Penicillium expansum* HA2N (KR269857) which isolated and identified according to Ammar and El-Desouky [21]**.** Also, the characterisation of AgNPs has been done by UV-Visible Spectrophotometer, Dynamic Light Scattering (DLS), Fourier Transform Infrared Spectroscopy (FTIR), and Transmission Electron Microscope (TEM) in the previous study [21]**.** On the other hand, in the same study, we synthesised the AgNPs by Egyptian honey (EH) preparation and production according to El-Desouky and Ammar [22].

Inhibition of A. flavus growth and aflatoxin B_1 production in the presence of silver nanoparticles

A hundred ml of YES medium were put in a 500 ml flasks and then autoclaved at 120°C for 15 min. Inoculation was carried out by adding 1 ml of a suspension of spores (10^5 spores) of a toxigenic *A. flavus* ATCC28542 strains without AgNPs (control) or with 0.5, 1.0 and 1.5 mg/100ml YES medium of one of the tested AgNPs (6a, 6b, 6c AgNps HA1N were synthesized by *Aspergillus terreus* (KR364880),3a, 3b, 3C AgNps EH were synthesized by Egyptian honey, and 1a, 1b, 1C AgNps HA2N were synthesized by *Penicillium expansum* (KR269857). The flasks were incubated in the dark for 14 days at 28°C. After the incubation period, extraction of AFB_1 from in the YES culture according to the method of Munimbazi and Bullerman [23]. Where, the mycelium of each flask contained YES medium was harvested by filtration through Whatman paper (No. 4), then extracted with 100 ml chloroform. The chloroform extract was dried by addition of anhydrous sodium sulfate. The residue was transferred to a vial and evaporated off using a stream of nitrogen at a temperature below 60°C. The dry film was used for the detection and determination of AFB_1 by (HPLC) according to (Deabes et al., [24,6] the retention time of AFB_1 standard separation is 4.061. The percentage of inhibition of AFB_1 is calculated using equation: % inhibition = (control- treatment /control) X100

Effect of AgNPs on biosynthesis pathway genes of AFB_1 in Aspergillus flavus ATCC28542

DNA extraction

DNA was extracted from 25 mg of the harvested mycelia, which was frozen in liquid N2 and ground in a mortar, according to the protocol recommended for the DNA Tissue purification mini kit (Qiagen). The genomic DNA was checked by agarose gel electrophoresis, and the concentrations of the purified total genomic DNA were determined with a NanoDrop 1000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and stored at -20°C for further use.

Primer design

Primers presented in Table 1 were selected according to the sequence of the *omt-A* gene of *A. flavus* from GenBank database (http://www.ncbi.nlm.nih.gov/).

Table 1: Primer used in this study for amplification of AFB_1 gene

Primer	5'-3' nucleotide sequence
omt-F	GACCAATACGCCCACACAG
omt-R	CTTTGGTAGCTGTTTCTCGC

Polymerase Chain Reaction amplification

PCR reactions were carried out in a total reaction volume of 20 µl, containing (10 µl of 2 X Go Taq master mix (Promega Corporation, Madison, WI) and 10 pmol of each primer of 50 ng template DNA. Amplification was performed in a T100-Bio-Rad Gradient Thermal cycler. The following programmer was used to amplify the DNA: 5 min at 94°C (1 cycle); 1 min at 94°C, 1 min at 59°C, and 1 min at 72°C (35 cycles); and 10 min at 72°C. A 10 µl aliquot of PCR products were separated on a 1.5% agarose gel stained with ethidium bromide (0.1 mg/l) and photographed under Gel Doc™ XR+ Gel Documentation System. Thermo Scientific GeneRuler 100 bp DNA Ladder was used as a size standard.

Gene Expression Analysis

Extraction of mRNA and cDNA synthesis

mRNA was extracted from selected *A. flavus* isolates treated with nanoparticles using mRNA Isolation Kit (Roche Applied Science).

Quantitative Real Time-PCR (qRT-PCR)

A Step One Real-Time PCR System (Applied Biosystem, USA) was used to assess the copy of the cDNA of *A.flavus* strain treated with AgNps HA1N, AgNps HA2N and AgNps EH, to detect the expression values of the tested genes according to El-Baz *et al.* [25].

Statistical analysis

All data were statistically analysed using the General Linear Model procedure of the SPSS ver. 18 (IBM Corp, NY).The significance of the differences among treatment groups was determined by Waller–Duncan k-ratio [26]. All statements of significance were based on the probability of $P < 0.05$.

Results

The present study was undertaken to investigate the antifungal activity of silver nanoparticles on *A. flavus* in vitro study. The addition of AgNPs including (AgNps HA1N, AgNps HA2 N and AgNps EH) individually to the (YES) growth medium at a level of 0.5, 1.0 and 1.5 (mg/100ml). The percentages of AFB_1 reduction with AgNPs HA1N were 22.8, 50.7 and 88.2% after treating by 0.5, 1 and 1.5 mg AgNPs /100mL medium, respectively. On the other hand, in case of AgNps HA2N AFB_1 reduced to 13.3, 37.3 and 67.7%, while AgNps EH reduced $AFB_1$to 22.1, 42.9 and 83.5% as (Fig. 1).

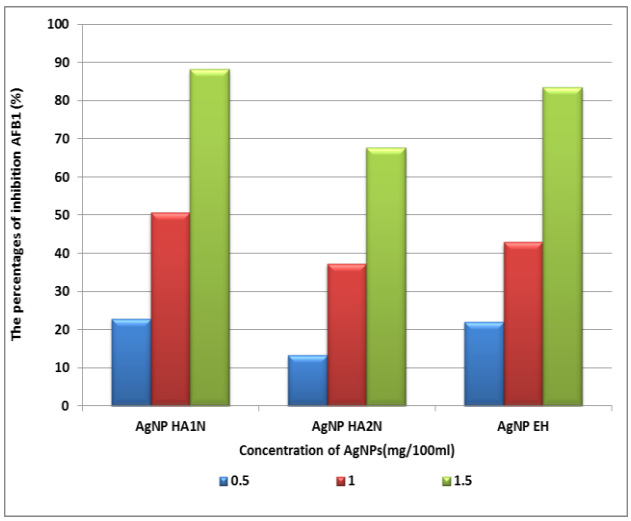

Figure 1: The percentages of inhibition of AFB1 production by Aspergillus flavus ATCC 28542 in YES medium

The results have also indicated that the AgNps HA1N at concentration 1.5 mg/100 mL medium gave the highest reduction of AFB_1 were determined by HPLC (Fig. 2).

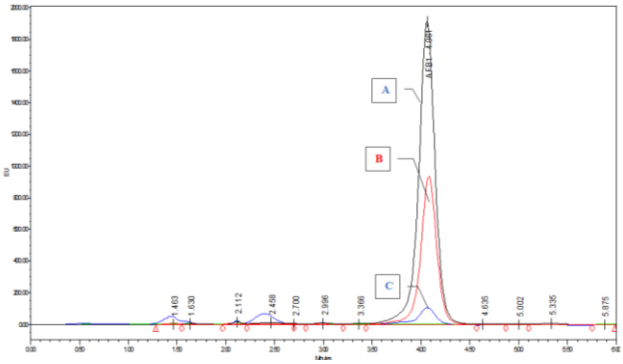

Figure 2: HPLC chromatogram of AFB1 with different concentration of AgNPs HA1N. A = AF; B1 treated by 0.5 mg AgNp HA1N/100 ml media; B = AFB1 treated by1 mg media AgNp HA1N/100 ml media; C = AFB1 treated by 1.5 mg AgNp HA1N/100 ml media

The obtained data in (Table 2) showed the AFB_1 concentrations in YES medium treated with three types of AgNPs, which showed the significant differences between the different concentrations as well as between the types of AgNPs (Table 3). On the other hand, data in (Table 4) display the effect of AgNPs on mycelial production from *A. flavus* ATCC 28542. The reduction on fungal growth and AFB_1 creation was dependent on the concentration of AgNPs. The antifungal activity of AgNPs was demonstrated by the diminution of AFB_1 production by *A. flavus.* This reduction is due to the interfering with

growth and fungal proliferation that AgNPs are altering protein activity and leads to cell death.

Table 2: Effect of AgNPs on AFB_1 produced by *Aspergillus flavus* ATCC 28542

Concentration of AgNPs (mg)/100ml media	AFB_1 (µg/100 ml media)* AgNP HA1N	AgNP HA2N	AgNP EH	Mean for concentration
0.5	0.826 ± 0.051	0.928 ± 0.041	0.833 ± 0.044	0.863 ± 0.063[c]
1.0	0.602 ± 0.010	0.746 ± 0.029	0.685 ± 0.018	0.677 ± 0.065[b]
1.5	0.126 ± 0.054	0.346 ± 0.036	0.176 ± 0.058	0.216 ± 0.109[a]
Mean for type AgNPs	0.518 ± 0.312[a]	0.673 ± 0.259[c]	0.564 ± 0.301[b]	

Control = 1.07 ± 0.11; *Mean ± SD.

This action may be attributed to the associated with the cation of silver, and it's the soluble complexes [27] [28] [29] [30]. With the result of Ionic silver (Ag+) binding to the thiol groups in NADPH enzyme, and hamper the bacterial respiratory chain creating interactive oxygen species that cause oxidative stress and cell damage [30].

Table 3: Analysis of variance of the effect of type and different concentration of AgNPs on AFB_1 produced by *Aspergillus flavus* ATCC 28542

Source	SS	df	MS	F	*P*
Intercept	9.258	1	9.258	5423.688	0.000
Type Nano	0.114	2	0.057	33.377	0.000
Con. Nan	1.996	2	0.998	584.762	0.000
Type Nano x Con. Nan	0.016	4	0.004	2.376	0.091
Error	0.031	18	0.002		
Total	11.415	27			

SS - the sum of squares; df - degree of freedom; MS - mean square; *P* - probability at confidence 0.95.

Some previous research suggests that the metabolic activity of fungi is also overlapped with AgNPs by decreasing the mycotoxins production, cytotoxicity and organic acid production. Moreover, a change in the extracellular enzyme profile is observed [31]. The antimicrobial effects of AgNPs depend on their size and silver rate release ion, and also the decrease of the size of AgNPs increased the antimicrobial activity [32] [33] [34].

Table 4: Effect of AgNPs on mycelial production from *Aspergillus flavus* ATCC 28542

Concentration of AgNPs (mg)/100ml media	The weight of mycelial production (mg/ml)* AgNP HA1N	AgNP HA2N	AgNP EH
0.5	11.7±1.25	17.2±0.76	14.5±0.5
1.0	8.5±1.32	13.2±0.77	10.6±1.27
1.5	4.4±1.15	8.6±0.87	6.5±0.51

*Control=25mg/ml; Mean ±SD

The obtained data recorded in Fig. **3** showed that the bands of the *omt_A* gene fragments which could be visualised at 300 bp. The patterns showed in all treatments isolates indicating that the presence of this structure gene, *omt-A*, enclosed in aflatoxin biosynthetic pathway which regulates the production of aflatoxin *aflR* gene [16].The results of the gene expression analysis using quantitative real-time RT-PCR are summarised in (Fig. 4). The gene encoding AFB_1 was determined in all isolates of *A. flavus* (ATCC 28542) after treatment with three types of AgNPs at 1.5 mg/100 ml media 6a AgNps HA1N, 1C AgNps EH, and 3c AgNps HA2N.

Discussion

The results found that samples of *A. flavus* treated by AgNps HA2N (1C) were very lower expression levels of AFB_1 gene than those collected from AgNps HA1N (6A) treatment and AgNps HA2N (3C). In control sample (without treated) isolate increased significantly ($P < 0.01$) the expression levels of AFB_1 gene with highest up-regulation action of the gene compared with the other treatments.

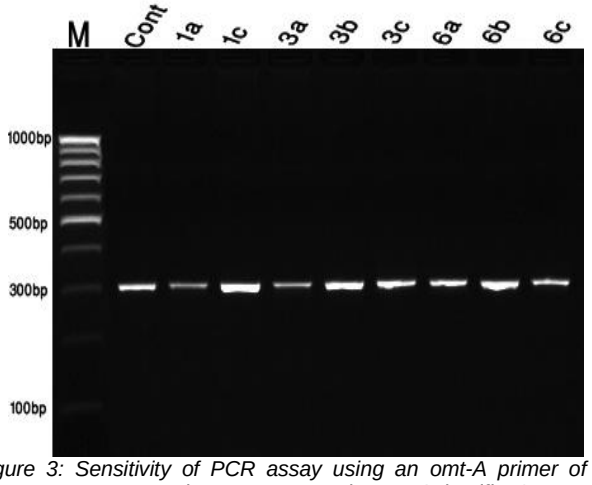

Figure 3: Sensitivity of PCR assay using an omt-A primer of A. flavus. DNA as template Lane1 M, Thermo Scientific GeneRuler 100bp DNA Ladder; lane 2 to lane8 isolates treated with nanoparticles

Moreover, samples 6a (AgNPs HA1N) aflatoxin gene compared with 1C (AgNps HA2N) and 3C (AgNps EH) samples. The RT-PCR experiment elucidated that the AgNpS consolidated expression of aflatoxins pathway *omt-A* gene [35]. On the other hand, Jing et al. [36] found that the treatment of AgNps significantly decreased the secretion of AF from *A. flavus*. Also, they explained the mechanisms of AgNps could cause the depression of AF production by *A. flavus.*

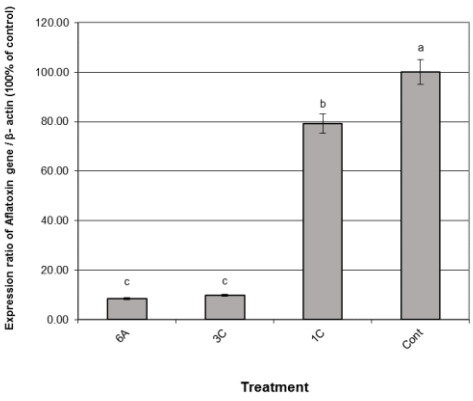

Figure 4: Expression levels of AFB1 gene in A. flavus isolates treated with 1.5 mg /100ml medium AgNPs of 6a AgNps HA1N, 3C AgNps EH and 1C AgNps HA2N. Data are presented as mean ± SEM ^{a,b,c} followed by different superscripts are significantly different ($P \leq 0.05$)

In conclusion, based on the results of the present study, AgNPs inhibit growth and AFB_1 produced by *Aspergillus flavus* ATCC28542. This was confirmed through RT-PCR approach showing the effect of AgNPs on *omt-A* gene involved in aflatoxin biosynthesis.

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Clinical Science

The Spectrum of Histopathological Changes in the Renal Allograft - a 12 Months Protocol Biopsy Study

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Abstract

INTRODUCTION: Renal transplantation became a routine and successful medical treatment for Chronic Kidney Disease in the last 30 years all over the world. Introduction of Luminex based Single Antigen Beads (SAB) and recent BANFF consensus of histopathological phenotypes of different forms of rejection enables more precise diagnosis and changes the therapeutic approach. The graft biopsies, protocol or cause, indicated, remain a golden diagnostic tool for clinical follow up of kidney transplant recipients (KTR).

AIM: The study aimed to analyse the histopathological changes in renal grafts 12 months after the surgery in KTR with satisfactory kidney function.

MATERIAL AND METHODS: A 12-month protocol biopsy study was performed in a cohort of 50 Kidney transplant recipients (42 from living and 8 from deceased donors). Usual work-up for suitable donors and recipients, standard surgical procedure, basic principles of peri and postoperative care and follow up were done in all KTR. Sequential quadruple immunosuppression including induction with Anti-thymocyte globulin (ATG) or Interleukin-2R antagonist (IL-2R), and triple drug maintenance therapy with Calcineurin Inhibitors (CNI), Mycophenolate Mofetil (MMF) and Steroids were prescribed to all pts. Different forms of Glomerulonephritis (16), Hypertension (10), End Stage Renal Disease (13), Hereditary Nephropathies (6), Diabetes (3) and Vesicoureteral Reflux (2) were the underlying diseases. All biopsies were performed under ultrasound guidance. The 16 gauge needles with automated "gun" were used to take 2 cores of tissue. The samples were stained with HE, PAS, Trichrome Masson and Silver and reviewed by the same pathologist. A revised and uploaded BANFF 2013 classification in 6 categories (Cat) was used.

RESULTS: Out of 48 biopsies, 15 (31%) were considered as normal, 4 (8%), Borderline (BL-Cat 3), 5 (10%) as Interstitial Fibrosis/Tubular Atrophy (IF/TA-Cat 5), 5 (10%) were classified as non-immunological (Cat 6), 2 as a pure antibody-mediated rejection (ABMR-Cat 2) and T-cell Mediated Rejection (TCMR-Cat 4). The remaining 17 samples were classified as a "mixed" rejection: 7 (41%) ABMR + IF/TA, 5 (29%) ABMR + BL + IF/TA, 2 (11%) BL + IF/TA, 1 (5%) ABMR + BL, 1 (5%) ABMR + TCMR and 1 (5%) TCMR + IF/TA. The mean serum creatinine at the time of the biopsy was 126.7 ± 23.4 μmol/L, while GFR-MDRD 63.4 ± 20.7 ml/min, which means that the majority of the findings were subclinical. Among the non-immunological histological findings (Cat 6), 3 cases belonged to CNI toxicity, 1 to BK nephropathy and 1 to recurrence of the primary disease.

CONCLUSION: Our 12-month protocol biopsy study revealed the presence of different forms of mixed subclinical rejection. Use of recent BANFF classification and scoring system enables more precise diagnosis and subsequently different approach to the further treatment of the KTR. More correlative long-term studies including Anti HLA antibodies and Endothelial Cell Activation- Associated Transcripts (ENDAT) are needed.

Introduction

Kidney transplantation is an incredible success of modern medicine. The better understanding of the basic immunological mechanism and the introduction of some new molecules in everyday practice enables improved long-term graft and patient's survival, better quality of life and practically uneventful clinical course for many years [1] [2] [3] [4]. According to the recent data, 20 years graft survival could be expected in 60% of kidney transplant recipients [5] [6]. However, there are still unsolved problems and questions that remain to be investigated from the scientific and practical point of view. One of the major causes of graft loss is still a rejection, either cellular or humoral. It has been accepted today that approximately 60% of the long-term graft loss belongs to the acute or chronic antibody-mediated rejection (ABMR) [7] [8] [9] [10]

The introduction of some new diagnostic tools such as Luminex based Single Antigen Beads (SAB), enables to detect a huge amount of anti HLA antibodies, non HLA antibodies and some fraction of activated complement system. The presence of anti HLA antibodies, especially Donor Specific Antibodies (DSA) in the patient's sera leads to chronic allograft nephropathy and long-term graft loss [11] [12] [13].

Thus, overcoming kidney allograft rejection could have a beneficial effect on long-term graft survival. However, other important pathological features rated as CNI toxicity, BK nephropathy, and recurrence of the primary kidney disease have also a substantial impact on the graft survival rate [14]. Despite modern diagnostic procedures implemented in everyday clinical practice, the kidney allograft biopsy remains a gold standard to determine the cause of graft dysfunction. Biopsy findings change the clinical diagnosis in an average of 36% of patients (range 24-76) and immunosuppressive therapy in 59% [15] [16]. But, the allograft biopsy does not contribute only to clinical diagnosis. It could also be used as a prognostic marker and guide to individual therapeutic approach to different patients [17] [18]. The development of so-called Banff scoring system in the last 25 years enables a much better understanding what is happening inside of the grafts [19] [20]. Introduction of the protocol biopsies gave very useful information about relevant histopathological changes in patients without any clinical evidence of graft dysfunction. They created practically new pathological entity named "subclinical acute or chronic rejection" which was very important regarding possible treatment and further clinical course of transplant recipients [21] [22] [23]. It is true that in the new era of potent immunosuppression therapy, the frequency of acute cellular or antibody-mediated rejection falls between 8-12 % and, therefore, the use protocol biopsies became a little bit controversial, but they are still very useful regarding treatment changes or individual approach to different patient circumstances. In any case, either protocol or clinically indicated, allograft biopsy is a condition sine qua non for modern clinical follow up of any organ transplant patient [24] [25] [26].

The study aimed to analyse the histopathological changes in renal grafts 12 months after the surgery in KTR with satisfactory kidney function.

Material and Methods

Forty-eight successful biopsied patients with haploidentical living (40) and 8 deceased donor transplantation were included in a 12-month prospective study. Renal transplantation was performed at the University Clinical Centre Mother Teresa-Skopje, Republic of Macedonia, by the well-known principles from the surgical and nephrological aspect. Hypertension, Glomerulonephritis, Hereditary nephropathies and End Stage Renal Disease (ESRD) were predominant underlying kidney diseases. Standard pre-transplant workup was done to all potential donors and recipients.

Table 1: Updated 2013 Banff classification categories

1. Normal
2. Antibody-mediated
Acute/active ABMR; all three features must be present for a diagnosis ***1. Histologic evidence of acute tissue injury, including one or more of the following:*** • Microvascular inflammation (g > 0 and/or ptc > 0) Intimal or transmural arteritis (v > 0) • Acute thrombotic microangiopathy, in the absence of any other cause ***2. Evidence of current/recent antibody interaction with vascular endothelium, including at least one of the following:*** • Linear C4d staining in peritubular capillaries (C4d2 or C4d3 by IF on frozen sections, or C4d > 0 by IHC on paraffin sections) • At least moderate microvascular inflammation ([g +ptc] > 2) • Increased expression of gene transcripts in the biopsy tissue indicative of endothelial injury if thoroughly validated ***3. Serologic evidence of donor-specific antibodies (DSAs) (HLA or other antigens*)** **Chronic, active ABMR; all three features must be present for a diagnosis** ***1. Morphologic evidence of chronic tissue injury, including one or more of the following:*** • Transplant glomerulopathy (TG) (e.g.> 0), if no evidence of chronic thrombotic microangiopathy Severe peritubular capillary basement membrane multilayering (requires EM) • Arterial intimal fibrosis of new onset, excluding other causes ***2. Evidence of current/recent antibody interaction with vascular endothelium, including at least one of the following:*** • Linear C4d staining in peritubular capillaries (C4d2 or C4d3 by IF on frozen sections, or C4d > 0 by IHC on paraffin sections) • At least moderate microvascular inflammation ([g +ptc] > 2) • Increased expression of gene transcripts in the biopsy tissue indicative of endothelial injury, if thoroughly validated ***3. Serologic evidence of DSAs (HLA or other antigens)*** **C4d staining without evidence of rejection; all three features must be present for a diagnosis** 1. Linear C4d staining in peritubular capillaries (C4d2 or C4d3 by IF on frozen sections, or C4d > 0 by IHC on paraffin sections) 2. g = 0, ptc = 0, eg = 0 (by light microscopy and by EM if available), v = 0; no TMA, no peritubular capillary basement membrane multilayering, no acute tubular injury (in the absence of another apparent cause for this) 3. No acute cell-mediated rejection (Banff 97 type 1A or greater) or borderline changes
3. Borderline changes: 'Suspicious' for acute T-cell mediated rejection (may coincide with categories 2 and 5, and 6)
This category is used when no intimal arteritis is present, but there are foci of tubulitis (t1, t2, or t3) with minor interstitial infiltration (i0, or i1) or interstitial infiltration (i2, i3) with mild (t1) tubulitis
4. T cell-mediated rejection (TCMR, may coincide with categories 2 and 5 and 6) **Acute T-cell mediated rejection (Type/Grade:)** **I A**. Cases with significant interstitial infiltration (>25% of parenchyma affected, i2 or i3) and foci of moderate tubulitis (t2) **I B.** Cases with significant interstitial infiltration (>25% of parenchyma affected, i2 or i3) and foci of severe tubulitis (t3) **II A**. Cases with mild to moderate intimal arteritis (v1) **II B**. Cases with severe intimal arteritis comprising >25% of the luminal area (v2) **III**. Cases with 'transmural' arteritis and/or arterial fibrinoid change and necrosis of medial smooth muscle cells with accompanying lymphocytic inflammation (v3) **Chronic active T-cell mediated rejection** 'chronic allograft arteriopathy' (arterial intimal fibrosis with mononuclear cell infiltration in fibrosis, the formation of neo-intima)
5. Interstitial fibrosis and tubular atrophy, no evidence of any specific aetiology
(may include nonspecific vascular and glomerular sclerosis, but severity graded by tubulointerstitial features) **I**. Mild interstitial fibrosis and tubular atrophy (>25% of cortical area) **II**. Moderate interstitial fibrosis and tubular atrophy (26-50% of cortical area) **III**. Severe interstitial fibrosis and tubular atrophy/loss (>50% of cortical area)
6. Other: Changes not considered to be due to rejection-acute and/or chronic

Cg, Banff chronic glomerulopathy score; EM, electron microscopy; ENDAT, endothelial activation and injury transcript; g, Banff glomerulitis score; GBM, glomerular basement membrane; IF, immunofluorescence; IHC, immunohistochemistry; ptc, peritubular capillary; TCMR. T cell-mediated rejection; v. Banff arteritis score.

According to the centre policy, 50% was a minimum accepted HLA matching in both, living and deceased donor transplantation. A sequential quadruple Immunosuppression including ATG or IL-2R antagonist induction and Prednisolone, MMF and CNI as a triple-drug maintenance therapy was

introduced to all recipients. After the surgery, the patients were followed by the same team according to the KDIGO recommendations. Usual Lab analyses, proteinuria, GFR, trough immunosuppressant levels, graft ultrasound tomography including Doppler were done practically every month on the outpatient basis.

Table 2: Pathological features and Banff score

Feature	Banff term	Banff Score			
		0	1	2	3
Interstitial inflammation (% of nonfibrotic cortex)	i	<10%	10–25%	26–50%	>50%
Total inflammation (% all cortex)	ti	<10%	10–25%	26–50%	>50%
Tubulitis (maximum mononuclear cells/tubule)	t	0	1–4	5–10	>10
Arterial inflammation (% lumen endarteritis)	V	None	<25%	>25%	Transmural or necrosis
Glomerulitis (% glomeruli involved)	g	None	<25%	26–50%	>50%
Capillaritis (cells per cortical PTC, requires >10% of PTC to be affected for scoring)	ptc	<10%	<5/PTC	5–10/PTC	>10/PTC
C4d deposition in PTC (% positive)	C4d	0%	I–9%	10–50%	>50%
Interstitial fibrosis (% of cortex)	ci	<5%	6–25%	26–50%	>50%
Tubular atrophy (% cortex)	ct	0%	<25%	26–50%	>50%
Arterial intimal thickening (% narrowing lumen of most severely affected glomerulus)	cv	0%	<25%	26–50%	>50%
Transplant glomerulopathy (% of capillaries with duplication in most severely affected glomerulus)	cg	0%	<25%	26–50%	>50%
Arteriolar hyalinosis (number with focal or circumferential hyaline)	ah	None	1 focal	>1 focal	1 circumferential >50%
Mesangial matrix increase (% affected glomeruli)	mm	0%	<25%	26–50%	>50%

A total of 48 biopsies (one for each patient included in the study) were done exactly on the 12th month after the transplantation. All the biopsies were performed under ultrasound guidance 16 gauge needle was used with an automated “gun”. Samples routinely comprised 2 cores to get a sufficient amount of glomeruli. The formalin-fixed biopsies were embedded in paraffin, serially sectioned at 3 and 5 μm thickness and stained with hematoxylin-eosin (HE), Periodic Acid-Schiff (PAS), Masson trichrome as well as methenamine silver. Biopsies were considered adequate when they contained ≥ 7 glomeruli and at least one artery.

Renal lesions were reviewed for evidence of chronic and acute changes by the same pathologist using descriptive criteria according to the Banff 2013 scoring schema using a scale from 0-3. At the same time, a frozen section sample was used for Complement (C3) immunofluorescence microscopy. C4d immunohistochemistry was performed on 3 μm thick paraffin sections using “Novolink” Polymer detection system with rabbit anti-human C4d monoclonal antibody [27] [28].

Histological findings were classified into six categories according to BANFF 13 modified and uploaded system: Normal (category 1), Antibody-mediated rejection (ABMR category 2), Borderline (BL -category 3), T – cell-mediated rejection (TCMR – category 4), Interstitial fibrosis and tubular atrophy (IF/TA-category 5) and other non-immunological changes (category 6). The BANFF scoring system (from 0-3) was used for the grading of acute and chronic changes occurring in the interstitium, tubules, glomeruli, arteries and arterioles. For diagnosis of ABMR, a revised BANFF 13 criteria which include “C4d negative ABMR” were used [29] [30], (Table 1 and 2). The research was performed following the tenets of the Declaration of Helsinki. Informed consent was obtained from the patients for the protocol biopsies. The research was approved by the Ethical Committee of the Medical Faculty Skopje.

Descriptive statistic was used, frequencies and percentages for categorical data; average values and standard deviation for continuous data.

Results

The demographic and clinical characteristics are present in Table 3.

Table 3: Clinical and demographic data

Data	Number
Age	34.5 ± 11.7
Gender W/M	14/37
Underlying disease	
Glomerulonephritis	16
Hereditary Nephropathy	6
Hypertension	10
Diabetes	3
VUR	2
ESRD	13
HLA Missmatch	3.1± 0.4
Living/deceased donors	40/8
CIT Living/ deceased donor	3.7 ± 0.3/ 10.4 ± 4.2
Induction therapy-Sim/ATG	19/31
Maintenance immunosuppression	
CNI-Cyclosporin/ Tacrolimus	19/31
MMF / Steroids	50/50
Rejection (clinical)	6 (11%)
Serum creatinine (12 month)	126.7 ± 23.4
GFR-MDRD (12 month)	63.4 ± 20.7

VUR – Vesico-ureteral Reflux, ESRD-End Stage Renal Disease, CIT-Cold ischemia time, CNI-Calcineurin Inhibitors, MMF-Mycophenolat Acid, Sim-Simulect, ATG – Anti -thimocyte Globulin.

A total of 50 biopsies in 50 patients were performed. Forty-eight were successful and available for analysis (> 8 glomeruli). The histopathological findings of the 12th month's protocol biopsies were categorised according to the updated Banff 13 scoring system. (Table 4). We noticed that only 15 (31%) cases belong to the category 1 which means normal biopsy. On the other hand, respecting the Banff 2013 criteria strictly, only 1 as sample could be determined as a pure ABMR and one pure TCMR which represents 4% of the cases. Five samples were classified as IF/TA and other 5 to “others”, which mean non-immunological histological changes including CNI nephrotoxicity, BK nephropathy or recurrence of the primary disease.

Table 4: Categorization of biopsies according to the updated Banff 2013 scoring system (n = 48)

Banff diagnostic category	Number of cases	Percentage
Normal (Category 1)	15	31
Pure ABMR (Category 2)	1	2
Borderline T-cell rejection (Category 3)	4	8
T-cell mediated rejection (Category 4)	1	2
IF/TA (Category 5)	5	10
Other (Category 6)	5	10
Mixed	17	35

The crucial point of every kidney allograft biopsy, protocol or indicated, is the issue of rejection, whether cellular or humoral. For TCMR (Cat 4) and TCMR borderline rejection (Cat 3) we used Banff scoring for interstitial infiltration (i), tubulitis (t) and arterial inflammation (v) whereas for ABMR glomerulitis (g), transplant glomerulopathy (cg), peritubular capillaritis (ptc), arterial inflammation (v), tubulitis (t) and positive C4d [31] [32] [33].

Table 5: Analysis of "mixed" category (n = 17)

Category	Number of cases	Percentage %
ABMR (Cat 2) + IF/TA (Cat 5)	7	41
ABMR(Cat 2) + BL (Cat 3)	1	5
ABMR (Cat 2) + BL (Cat 3) + IF/TA (Cat 5)	5	29
BL (Cat 3) + IF/TA (Cat 5)	2	11
ABMR (Cat 2) + TCMR (Cat 4)	1	5
TCMR (Cat 4) + IF/TA	1	5

The rest of kidney biopsies belonged to the category of so-called "mixed" rejection which is presented in Table 5 and Figure 1.

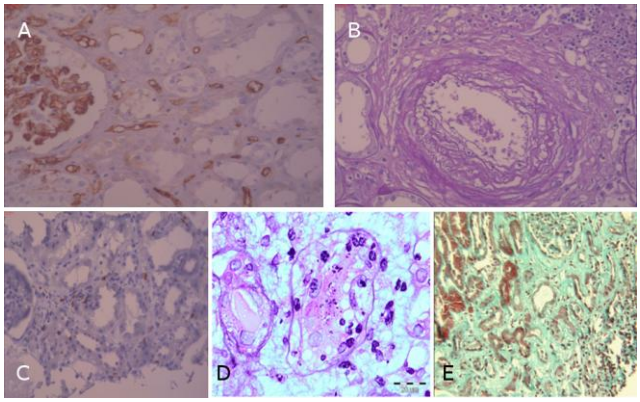

Figure 1: A: C4d positive immunostaining in peritubular capillaries in acute humoral rejection; B: Chronic allograft vasculopathy (arterial blood vessel with fibrointimal thickening); C: CD3 immunostaining for T cell in acute cellular mild rejection. (brown - T lymphocytes); D: Acute cellular rejection – tubulointerstitial – grade 1a: (mild interstitial infiltration and focuses of mild tubulitis > 4 cells cross tubular section/); E: Trichrome Masson histochemical staining: IF/TA (Interstitial fibrosis and tubular atrophy)

Discussion

ABMR

According to literature, the frequencies of ABMR in biopsies 12 months after transplantation differed between patients with/without HLA-DSA (0.2% vs 12%). Our results were closer to the protocol biopsies in patients without HLA-DSA which is 2.2%. Based on the recent BANFF data and a broad spectrum of different opinions about the humoral rejection, we used so-called "expanded criteria" for diagnosis or even "suspicious" for ABMR. It was the reason why we had too much of Category 2 (it means ABMR) in our analysis presented in Table 5. Certainly part of the criteria was also detectable DSA, non-DSA or Non -HLA antibodies. Regarding Cd4 and grading scores, we accept that presence of C4d was not anymore one of the criteria for ABMR. After many controversial findings regarding C4d, a revised Banff 2013 classification included the category of "C4d negative ABMR" [29] [30]. Theoretically, it means that there are alternative non-complement depending reactions of HLA antibodies and graft endothelium. The story of ABMR becomes complicated and this was shown by the fact that some typical histopathological ABMR changes may be seen even if HLA and non HLA antibodies exist in the examined serum samples. In the Banff 2013 scoring system the term borderline or "suspicious" was accepted in the criteria for ABMR. It means that suspicious ABMR may even exist if only C4d staining is present without positive anti HLA antibodies and any other typical changes for definitive ABMR [30]. Additionally, any simple presence of vascular inflammation could be sufficient for the diagnosis of ABMR. Despite the different phenotypes, we unified ABMR as one category including "suspicious", acute/active or chronic/active forms which facilitated further analysis and conclusions [26] [28].

TCMR and Borderline Rejection

The percentage of pure TCMR was less than those in the literature 2% vs 8.2% respectively, but if the number is corrected for the numbers of "borderline" changes and TCMR in a mixed group, the percentage was closer to previous reported. The finding of TCMR which was present in different forms and phenotypes in 13 of the biopsies was a certainly interesting issue: one as a pure TCMR, 4 as a Borderline TCMR, 5 as a Borderline together with ABMR and IF/TA, and 3 with IF/TA. According to the previous Banff session, any classified "borderline" changes" should be understood as a rejection and should be treated appropriately, especially if there is an increase of serum creatinine [29] [30] [42]. Moreover, there is evidence that TCMR or borderline TCMR can provoke real ABMR and shorten the graft survival rate. The presence of some ABMR pattern together with TCMR or 'Borderline" TCMR, also deserves attention. Is it evidence that ABMR is already activated by TCMR, or it is simply two separated parallel findings? We are more inclined to the conclusion that, if there is no anti HLA antibodies and any C4d activity, it could be evident that TCMR had already been initiated ABMR [15] [22] [28].

Mixed Rejection

While analysing the findings and classification presented in Table 5, we can conclude that in 35% of the biopsies there was a broad spectrum of histopathological changes which usually does not correspond with the clinical picture. Wehmeier et al., in their study, reported the percentage of mixed rejection in biopsies from 2.6% in patients without DSA and 14% in patients with DSA, up to 22% in biopsies by indication in DSA patients. Mixed rejection means that both TCMR and ABMR with their different

sub -phenotypes are equally present in the graft biopsy. Potentially it could be quite possible that some interactions among them are happening all the time. Anyway, we don't know who came first in the battle, but it is quite frequent especially in the protocol biopsies. According to the very dynamic issue of BANFF meetings and changes which usually come every other year, we strongly believe that the conclusions are still very far from the real clinical use. Bearing in mind that our study is protocol biopsy based, most of our cases belong to the entity of "subclinical" rejection, or simply, subclinical important histopathological changes [22] [28] [34] [35]. It is obvious that there are many different pathohistological patterns which belong to well-defined forms of rejection as ABMR and TCMR but also, at the same time, to TCMR-borderline or ABMR "suspicious" phenotypes. The recent Banff 2013 recognised so-called "Chronic active TCMR" which is different than the usual acute TCMR with features of chronic allograft arteriopathy [30]. Some of the histological changes are very similar to ABMR. Thus, it is not easy to define "mixed" rejection as an entity not only from diagnosis but much more from the point of further treatment. Hence, the clinical decision "what to do" if there are not sufficient data for definitive diagnosis, could be a very complicated issue. Whether these changes could have a negative impact on the long-term clinical outcome or not, it remains to be seen after several years.

IF/TA

In Table 4 and 5, we noted that interstitial fibrosis and tubular atrophy is more frequent finding compared with others studies. Regarding the severity of the IF/TA pathohistological changes in 4 biopsies, they were present in more than 50% of active cortical tissue, in 13 between 25-50% and only one less than 25%, which is close to normal. As a pure form, IFTA is confirmed in 5 cases, but it was present much more in so-called mixed rejection, usually with ABMR (7 cases), Borderline and ABMR (5 cases), Borderline and TCMR (2 cases). IF/TA, especially without histological signs of rejection is considered as a chronic process [18] [28] [36]. But, the fact that IF/TA is the most frequent finding in our 12 months protocol biopsy study means that probably it is part of a permanent process of rejection, either humoral or cellular. The recent gene expression studies confirmed that even without histological evidence of inflammation IF/TA showed a molecular profile of immune-mediated inflammation [37] [38]. In our study, we noticed that among the cases in group "mixed", 7 (41%) belong to ABMR (Cat 2) and IF/TA (Cat 5). Therefore, the finding of any signs of microvascular inflammation, and/or active glomerular lesions together with IF/TA mean that ABMR was directly involved in the interstitial fibrosis. Equally important was a possible role of TCMR and Borderline changes together with IF/TA [39] [40]. Therefore, interstitial fibrosis may be part of broad immunological events, especially if DSA or Non-DSA are detectable in the patients' sera. However, the presence of inflammation in IF/TA became an unresolved issue even among the experts of the Banff group [15] [18] [41].

Others

In the final 6th group of biopsies, CNI toxicity was a predominant finding, usually as an arterial hyalinosis grade 2 or 3. According to the recent discussion about the nature of chronic CNI toxicity in the era of antibody-mediated rejection, it seems that we should strictly divide what CNI toxicity is and what ABMR is on the other hand [14]. Especially if the anti HLA antibodies are present in the patient's sera. Therefore, the chronic CNI toxicity could be simply replaced by some of the ABMR phenotypes shortly [14]. Until the consensus of that issue is reached, we remain on the actual CNI toxicity pattern among our biopsies despite the fact that we did not observe any potentially toxic CNI level during the 12 months follow up. In the last two biopsies of this group, a BK nephropathy and FSGS recurrence were diagnosed which was also clinically confirmed.

ENDAT

After all that was presented in our analysis, it is evident that additional tests are required to increase the prognostic power of pathohistological assessment in renal transplant patients. In the last two Banff reports, 2013 and 2015, the criteria of "increased expression of gene transcripts indicative of endothelial injury, if thoroughly validated" was included in the whole complicated picture of ABMR. "Thoroughly validated" practically means that it should be confirmed only in a single centre (University of Alberta). Searching for new molecular markers for active endothelial injuries the term "ENDAT" (Endothelial Cell Activation-Associated Transcripts) was broadly introduced [30]. Despite the primary confusion in the definition of rejection as a whole process, the recent data fully justified gene transcripts as a relevant diagnostic tool which should facilitate the diagnosis and clinical use of the biopsy as a golden standard for kidney graft recipients [37] [39].

In conclusion, histologic assessment of kidney transplant recipients by use of 12 months protocol biopsy revealed a huge amount of different histopathological phenotypes and sub-phenotypes of ABMR, TCMR and mixed rejection. Most of them were clinically silent which was very important for further treatment and follow up of kidney transplant recipients. Therefore, 12 months protocol biopsies together with a strict follow up of anti HLA antibodies and clinical picture of kidney transplant recipients is necessary for a successful and long-term graft and patients survival.

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Clinical Science

Serum Apelin: A New Marker of Early Atherosclerosis in Children with Type 1 Diabetes Mellitus

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Keywords: Type 1 diabetes mellitus; carotid intima-media thickness; Serum apelin; cholesterol; TG; LDL; case-control study

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Abstract

INTRODUCTION: Type 1 diabetes mellitus (T1DM) is one of the most common chronic diseases in children that may be complicated by micro or macrovascular complications. Measurement of the carotid intima-media thickness (CIMT) allows the early detection of atherosclerotic alterations of blood vessels that may complicate T1DM.

SUBJECTS AND METHODS: This study is a case-control study. Participants were classified into two groups. The first group included 40 children with T1DM and the second group included 30 matched healthy controls. The studied cases were recruited from Endocrinology and Diabetology Unit, Pediatric Hospital, Ain Shams University. Serum apelin, cholesterol, TG, LDL were measured for every case. Also, albumin level was analyzed in urine. Measurement of the carotid intima-media thickness (CIMT) was done for all cases.

RESULTS: Comparison between T1DM patients and controls revealed that serum apelin, cholesterol, TG, LDL and albuminuria were significantly increased in cases compared to controls. Significant positive correlations were detected between HbA1C, albuminuria and lipid profile with apelin in the diabetic group ($p < 0.05$). CIMT has significant positive correlation with serum apelin levels ($r = 0.36$, $p = 0.05$). Also, this study found positive correlations between CIMT and some variables as LDL, SBP z-score and duration of the illness.

CONCLUSION: Increased levels of serum apelin in T1DM patients may be considered as predicting factor for the ongoing development of vascular sequels. This study highlighted the possible validity of apelin assay as an early predictor of atherosclerosis in T1DM children. Evaluating CIMT in these patients is of at most important for early detection of subclinical atherosclerosis.

Introduction

Type 1 diabetes mellitus (T1DM) is a multifactorial health problem worldwide. It is one of the most common chronic diseases in children that may be complicated by micro or macrovascular complications [1]. Endothelial dysfunction and subclinical organ damage indices, such as greater Carotid Intima-Media Thickness (CIMT) and arterial atherosclerotic changes, reflecting higher cardiovascular diseases (CVD) risk in adulthood, are increased with hyperglycemia, hypertension, high LDL and TG concentrations, insulin resistance, increased BMI, proinflammatory status and disturbances in adipocytokines [2]. The direct impedance of adipokines on the endothelium needs further investigations. Endothelial dysfunction is considered the early stage of atherosclerotic changes that may affect blood vessels in T1DM [3]. It is characterised by abnormalities in the lumen and endothelium of the blood vessels, resulting in vasodilatation response. These changes in vascular endothelium result in increased secretion of inflammatory cytokines that augment adhesion of cellular molecules and other biologically active substances and finally lead to a proinflammatory and prothrombotic state [4]. These mechanisms represent an important step in the development of the initial atherosclerotic changes and subsequent complications in diabetic children [5]. Measurement of the carotid intima-media thickness (CIMT) allows the early detection of atherosclerotic alterations of blood vessels. It is considered a strong predictor of vascular abnormalities in high-risk

individuals, such as those with T1DM [6]. Measurement of the intima-media thickness of the large arteries, especially the carotids, has considered as the method of choice for detecting the anatomical extent of arterial wall deterioration and for assessing cardiovascular risk [7]. Several investigators have recommended the clinical use of this technique for detecting subclinical (asymptomatic) atherosclerosis and for identifying subjects at high-risk.

Apelin is one of the adipokines that is secreted from white adipose tissue and has various functions as insulin sensitivity. It has an important role in diabetes mellitus. Furthermore, its concentration is changed according to insulin resistance. It has been shown that apelin has a crucial role in energy metabolism and pathogenesis of diabetes mellitus because white adipose tissue that secretes apelin acts as an endocrine organ [8].

Apelin had been discovered by Tatemoto et al., in 1998 [9]. They suggested that it is the endogenous ligand for angiotensin II protein J (APJ) receptor [10]. Apelin and its receptor APJ are expressed in several tissues like heart, lung, stomach and skeletal muscles. In the past few years, it has been found the possible roles played by apelin in human physiology, It is considered as regulating peptide of cardiovascular, hypothalamus-hypophysis, gastrointestinal and immune systems [11]. Scientists stated that apelin might be a risk indicator in young children prone to atherosclerosis and type 2 diabetes [12]. Moreover, some previous studies found apelin as a novel biomarker for predicting diabetes especially T1DM [13]. In healthy individuals, the level of apelin depends on the nutritional state. It was reduced in fasting and increased by feeding, while in diabetic patients and impaired glucose tolerance it may increase due to insulin resistance [14]. Insulin increased the expression of apelin in adipocytes [15].

This study aims to analyse the role of the adipokine apelin in the pathogenesis and complications of type I diabetes mellitus in children. Also, we aimed to clarify the relation between serum apelin and lipid concentrations and to verify the presence of CIMT and initial structural atherosclerotic changes in children with Type 1 diabetes mellitus (T1DM).

Subjects and Methods

This study is a case-control study. Participants were classified into two groups. The first group included 40 children with T1DM, their mean age was 9.15 ± 3.64 years, and the mean duration of disease was 4.5 years. The second group included 30 matched healthy controls, of same age group. Cases with T1DM were classified into two subgroups according to HbA1c, group I with HbA1C of 8% or less (well-controlled T1DM) and group II with HbA1c greater than 8% (poorly controlled T1DM).

The studied cases were recruited from Endocrinology and Diabetology Unit, Pediatric Hospital, Ain Shams University. Healthy children were peers of diabetic patients and from local schools. Cases with the chronic or inflammatory disease, cases which were on medications or hormones (other than insulin), cases with known renal disease and systemic disease and acute infection at the time of testing were excluded from the study. The study was approved by the local ethics committee of National Research centre, and written informed consent was obtained from both parents of every participant.

All participants were subjected to:

- Full history with special emphasis on the onset of diabetes.

- Clinical examination and anthropometric measurements including Height (in centimetres) using Harpenden stadiometer and Weight (in kilograms) using an electronic weight scale.

- calculation of BMI: weight in kg/(height in meters)^2.

- Weight for age, height for age and BMI Z - score were determined using the new WHO reference [16].

- Measuring blood pressure (BP) by a sphygmomanometer.

Venous blood samples (3 ml) were taken from each child participating in the study and divided into two parts: the first part was added to a tube containing EDTA for glycosylated haemoglobin determination by cation-exchange resin and the second part was put in a serum separator tube. The separated serum was stored at – 20^{0}C for determination of Apelin, fasting blood sugar, total cholesterol, and triglyceride (TG), HDL and LDL. Fasting blood glucose level was performed on automated clinical chemistry analyser (Olympus AU400). Total cholesterol, triglyceride and HDL were determined using colourimetric techniques on Synchron Cx7 (Beckman Instruments Inc., California, USA). LDL cholesterol was measured by Friedwald formula [17]. For random urinary albumin measurement, an early morning mid-stream specimen was used. The cloudy samples were centrifuged before use, and the clear supernatant was stored at -20°C until analysis. Albumin concentrations were measured in urine using a Minineph microalbumin kit based on nephelometry method on Mininephnephlometer (AD200) (The Binding Site, Birmingham, UK) [18]. We compared albumin in the sample against its creatinine concentration (measured by Jaffe reaction) on a Synchron Cx7 autoanalyser, and the albumin/creatinine ratio was calculated [19]. Serum Apelin was measured by quantitative commercial enzyme-linked immunosorbent assay

ELISA kit supplied from Elabscience Biotechnology Co., Ltd, Wu Han, China-Catalog No: E – EL-H0456 (www.elabscience.com), detection range was between 62.5-400 pg/ml.

Measurement of carotid intima-media thickness (CIMT) was performed using high-resolution B mode ultrasound to detect the thickness of Common Carotid Arteries. All of the carotid scans were done using carotid Doppler ultrasound scanner (Toshiba Ultrasonography machine [Xario], Tokyo, Japan) with a 10.0-MHz linear array transducer following a predetermined standardised scanning protocol [20].

The data were coded, entered and processed by computer using Statistical Program for Social Science version 22 (SPSS Inc., Chicago, IL, USA). Quantitative variables were described in the form of mean and SD concerning age, BMI Z-score, systolic and diastolic BP Z-score, level of HbA1C, cholesterol, TG, and level of Apelin and qualitative variables were described as number and percent concerning sex distribution and presence of albuminuria. To compare quantitative parametric variables between two groups, Student t-test was used. Pearson correlation coefficient was employed to measure the strength and direction of the linear relationship between two variables. A p-value < 0.05 was considered the cut-off value for significance in all analyses.

Results

Subjects in this study were classified into two groups. The first group is cases which involve 40 children with type I diabetes mellitus, of them 17 were males and 23 were females. The second group controls which involve 30 healthy children, of them 11 were males, and 19 were females. No significant difference was found between cases and controls in relation to age.

Table 1 shows a comparison of anthropometric and clinical parameters between cases and controls; there was highly significant difference between the two groups regarding SBP and DBP z-score (p < 0.01). No significant difference was found between the two groups as regards weight, height and BMI z-score.

Table 1: Comparison of anthropometric and clinical parameters between cases and controls

Variable	Cases (n = 40) Mean ± sd	Control (n = 30) Mean ± sd	P
Age (years)	9.15 ± 3.64	9.70 ± 4.38	0.63
Weight z-score	0.61 ± 0.47	-0.42 ± 0.67	0.12
Height z-score	-0.48 ± 1.21	-0.27 ± 1.14	0.77
Bmi z- score	0.67 ± 1.28	0.41 ± 0.83	0.52
Sbp z-score	0.76 ± 0.44	0.16 ± 0.36	0.001*
Dbp z-score	1.1 ± 0.49	0.41 ± 0.38	0.000*

Table 2 shows a comparison of laboratory markers between cases and controls. Serum apelin, cholesterol, TG, LDL and albuminuria were significantly increased while HDL was significantly decreased in cases compared to controls.

Table 2: Comparison of laboratory markers between cases and controls

Variable	Cases (n = 40) Mean ± sd	Control (n = 30) Mean ± sd	P
Apelin (pg/ml)	1040 ± 576	741.8 ± 231.6	0.015*
Albuminuria (mg/gm creatinine)	46.46 ± 32.08	9.83 ± 5.07	0.02*
Cholesterol (mg/dl)	217.6 ± 44.30	139.27 ± 19.3	0.000*
TG (mg/dl)	156.8 ± 18.94	76.53 ± 7.12	0.000*
LDL (mg/dl)	183.32 ± 46.71	58.16 ± 13.45	0.000*
HDL (mg/dl)	23.27 ± 5.43	46.14 ± 10.2	0.000*

Table 3 shows correlations between Apelin and anthropometric, clinical and laboratory data in the Diabetic group. DBP z-score has significant positive correlation with apelin in diabetic group (p < 0.05). HbA1C, albuminuria and lipid profile of diabetic children had a highly significant positive correlation with apelin (p < 0.001). Mean CIMT in diabetic children was 0.5 ± 0.1 mm.

Table 3: Correlation between Apelin and anthropometric, clinical and laboratory data in the Diabetic group

variable	Diabetic (N = 40) r	p
DBP z-score	0.292	0.02*
HbA1C	0.441	0.001*
Cholesterol	0.404	0.001*
TG	0.401	0.001*
LDL	0.402	0.001*
CIMT	0.362	0.05*
Albuminuria	0.887	0.000*

CIMT had significant positive correlation with serum apelin levels as shown in figure 1 (r = 0.36, p = 0.05). Also, this study found a positive correlation between CIMT and some variables as LDL, SBP z - score and duration of the illness (p < 0.05). Also, Duration of diabetes was positively correlated with LDL (p = 0.03). HbA1C was positively correlated with DBP z-score and albuminuria (p < 0.05).

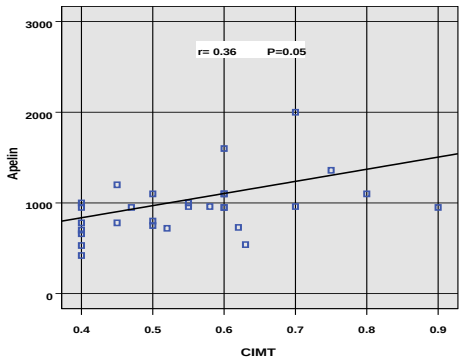

Figure 1: Correlation between serum apelin levels and CIMT

We also investigated the influence of different clinical and laboratory variables on diabetic

nephropathy occurrence. It revealed that serum apelin and Hb1C had a significant influence on the appearance of diabetic nephropathy. This is discovered by detecting a positive correlation between albuminuria and serum apelin, HbAc ($p < 0.05$).

Discussion

Apelin is an adipokine that is secreted from adipocytes in different organs. It has been shown that serum apelin was involved in glucose homeostasis and also it regulates insulin secretion. In this study, we found that serum apelin levels are significantly increased in children with T1DM in comparison to healthy subjects, this is in agreement with previous studies [21] [22]. This is maybe explained by that apelin action on glucose metabolism is additive to insulin as it increases glucose uptake and transport in tissues [15], also it increases intestinal absorption of glucose [23]. All reported previous actions of apelin make it an anti-diabetic agent. Thus, our study hypothesised that increased levels of apelin in T1DM is a result of compensatory mechanism devoted to decreased insulin levels and to overcome insulin resistance in these patients. This study also found a highly significant positive correlation between serum apelin and HbA1c in diabetic subjects; this is in contrary with a previous study [22], who stated that serum apelin levels were negatively correlated to HbA1c in type 2 diabetic patients, suggesting that circulating apelin is associated with the better glycemic control. This difference may be explained by the fact that patients with T1DM in our study were treated with insulin which plays an important role in apelin secretion and expression.

In this study, we found a significant influence of serum apelin on diastolic blood pressure, it increases with increased serum apelin levels, but it does not affect systolic blood pressure. This is in disagreement with a previous study [12], which discovered that there was not a significant influence of serum apelin on SBP, but there was a tendency to lower DBP with higher levels of serum apelin, this discrepancy is due to that they studied elderly subjects with an increased risk to develop T2DM. To check the further influence of serum apelin on vascular integrity, we studied the CIMT as a parameter for early atherosclerotic changes in blood vessels in T1DM patients. CIMT has been described as a mirror of the atherosclerotic burden and a predictor for the subsequent sequel as myocardial infarction and stroke [24]. From the previous advantages of this technique, we decided to use it as a gold standard for early detection of subclinical atherosclerosis in T1DM patients. We discovered positive correlations between CIMT and LDL; this ensures our recommendation that CIMT should be done for every T1DM patient for early detection of atherosclerosis. We found that serum apelin levels have a significant positive correlation with CIMT in T1DM. Thus, we can consider increased levels of serum apelin in T1DM as predicting factor for the further development of vascular complications of DM in these patients. In our study, HbA1C was positively correlated with DBP z-score. This may be explained by that increased glycemic state is associated with increased vasoconstriction and hypertension due to inflammatory state that induces oxidative stress that alters nitric oxide secretion and degradation and finally has a deleterious effect on vascular endothelial cells. Our results concerning hyperglycemia and increased blood pressure are in agreement with a previous study [25]. Furthermore, this study also clarifies the role of apelin in the occurrence of diabetic nephropathy. We found that serum apelin and Hb1C have a significant influence on the appearance of these complications. We discovered that serum apelin was associated with increased diabetic nephropathy which correlated with microalbuminuria. This is ensured by another study [26], which stated that apelin induce glomerular endothelial cells proliferation and then nephropathy so we can state that increased serum apelin may worsen the condition in T1DM through its influence on diabetic nephropathy.

In our study, we reported that serum cholesterol; TG and LDL were significantly increased in T1DM patients than healthy subjects, but serum HDL was significantly decreased in T1DM patients than healthy subjects. From the previous results, we can consider children with T1DM at higher risk of developing premature atherosclerosis because of hyperlipidemia and thus, should be screened well for this serious complication. Also, we found significant positive correlations between serum apelin and serum cholesterol, LDL and TG in diabetic patients. In support of these results, other study found statistical differences between diabetic and non-diabetic groups as regards serum levels of TG and cholesterol [25]. Also, other study reported the same results as regards serum TG [13]. Few studies describe the effects of apelin on lipid metabolism; one of them stated that apelin was shown to inhibit lipolysis [27]. This was ensured by another study [28] that found that apelin increases the stability of lipid vacuoles making them more resistant to lipases. All these findings support our results that apelin is associated with increased serum lipids and thus can be used as a predictor of premature atherosclerosis in T1DM patients.

In conclusion, increased levels of serum apelin in T1DM patients may be considered as predicting factor for the ongoing development of vascular sequels so measuring serum apelin in these patients is of benefit for early detection of disease complications. Premature subclinical atherosclerosis was documented among T1DM patients due to hyperlipidemia detected in these patients, so we recommend evaluating their CIMT for early detection of subclinical atherosclerosis. CIMT was correlated

well with dyslipidemia and serum apelin, a finding that highlighted the possible validity of apelin assay as an early predictor of atherosclerosis in T1DM children.

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Practical Approach to Lung Health – Experience from the Republic of Macedonia

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Abstract

BACKGROUND: Among the adults and children aged 5 yrs who attend PHC settings, 20-30% seeks to care for respiratory symptoms. Over 80-90% of the respiratory patients suffer from acute respiratory infections (ARI), followed by chronic obstructive pulmonary diseases (COPD), asthma, and less frequently with pneumonia and tuberculosis (TB). To improve the quality of care in patients who seek assistance for respiratory symptoms in PHC settings and the efficiency of respiratory service delivery within healthcare systems, WHO has designated several initiatives among which one is PAL (Practical Approach to Lung Health). PAL is an integrated and symptom-based approach focused on all priority respiratory illnesses encountered in PHC, including TB. Its patient-centred syndromic approach aims to improve the quality of diagnosis and treatment of respiratory illnesses in a PHC setting.

AIM: To evaluate the short-term impact of PAL approach in improving the management of patients with the most frequent respiratory diseases by the GPs from PHC settings in the Republic of Macedonia.

MATERIAL AND METHODS: A total of 588 GPs were educated for the most frequent respiratory diseases during the PAL training from 2013-2016. To evaluate the efficiency of GPs education from PHC settings, GPs fill in a form out of 69 questions for the patients enrolled before (baseline survey) and after PAL training (impact survey), and the results of the two surveys were compared. This analysis aimed to assess if the theoretical and practical skills obtained during the PAL training have been used in the routine practice of the GPs who attended the training and to what degree.

RESULTS: Our results showed that in the impact study more patients with ARI ($P < 0.000001$) and more patients with COPD exacerbations were treated in the PHC settings ($P < 0.000008$). More patients suspected of asthma were referred to upper health level for diagnosis ($P < 0.037$). The comparison of the findings between the baseline and impact surveys suggest that training on PAL had an impact in decreasing drug prescription through a reduction in a prescription for antibiotics for ARI, COPD and asthma. Our study indicates that training on PAL is likely to increase the prescription of inhaled corticosteroids and tends to decrease the prescription of other formulation of these drugs ($P < 0.0000001$). The impact surveys showed that the patients with COPD and pneumonia in the PHC settings were better managed due to the more frequent use of CAT questionnaires and CURB test. And regarding TB cases, our study indicates that the GPs were more aware of this disease and were more willing to take part in the patient treatment follow-up ($P < 0.000001$).

CONCLUSION: The results from this study showed that implementation of PAL approach for GPs from the PHC setting in our country have positive results (effect) in the management of patients with respiratory symptoms: it is likely to reduce prescribing for antibiotics, to increase the use of inhalation medication which is highly recommended in the management of asthma and COPD, and to decrease the referral of patients with chronic diseases to the upper health level. However, more experience is needed for long-term influence on the effects over the cost-effectiveness of respiratory care services and on strengthening the health care system.

Introduction

Among the adults and children aged 5 yrs who attend PHC settings, 20-30% seeks to care for respiratory symptoms [1]. Underlying causes are wide-ranging, spanning from the common cold to pulmonary tumours and their frequencies of occurrence differ widely [2]. Over 80-90% of respiratory patients suffer from acute respiratory infections (ARI), with a majority suffering from upper respiratory infections [3]. Among these respiratory infections, pneumonia and tuberculosis (TB) are usually infrequent (1-2%). Chronic respiratory diseases account chronic obstructive pulmonary disease (COPD), and asthma is encountered more often than TB and less frequently than ARI. However, worldwide asthma prevalence has been on the

increase in several settings during the past few decades [4]. The common point is that all respiratory diseases, if not diagnosed, treated and managed timely and correctly, are problematic for individuals and public health alike. In PHC settings, clinical symptoms presented by pulmonary TB patients are, in general, very similar to those symptoms displayed by non-tuberculous respiratory patients, particularly in those with persistent symptoms. Clinicians in primary health care (PHC) facilities, who are at the centre of all this, often feel challenged, as finding the right path in a vast jungle of similar respiratory symptoms can be a tedious and misleading endeavour. The complex path constitutes making the correct diagnostic, treatment and management choices for all respiratory patients [5].

The World Health Organization (WHO) estimates that approximately 50% of people with TB are never diagnosed as having the disease and so cannot benefit from treatment, leaving the epidemic unchecked despite increasing global coverage by treatment programs [6]. Improved passive case detection is fundamental for the control of the TB epidemic and depends on alert clinicians identifying TB in patients seeking primary care for respiratory symptoms. To remedy this challenging situation, the Stop TB Department of WHO has designated several initiatives to improve global TB control, among which one is PAL (Practical Approach to Lung Health), which was initiated in 1998. PAL is an integrated and symptom-based approach focusing on all priority respiratory illnesses encountered in PHC, including TB. PAL tries to shine a guiding light in the vast respiratory jungle. Its patient-centered syndromic approach aims to improve the quality of diagnosis and treatment of respiratory illnesses in a PHC setting. It seeks to standardise service delivery through development and implementation of clinical guidelines and managerial support within district health system. It is intended to coordinate among different levels of health care and between tuberculosis (TB) control and general health service [5].

PAL has the following specific goals: 1) to improve the quality of management of patients with respiratory symptoms in the setting of PHC. 2) To improve the efficiency of the delivery of respiratory services within the overall health care system, with a focus on coordination and integration of respiratory case management within the district health care system of low-and-middle-income countries, particularly those with already successful TB control programs or a high prevalence of HIV infection [5]. PAL is aimed at improving the management of major respiratory disorders, and in the process increases the identification of TB patients among all those with compatible symptoms who seek care in PHC settings [6] [7] [8].

In October 2013, the national tuberculosis program (NTP) of the Republic of Macedonia initiated the process of PAL approach for PHC settings as part of the activities from the Global Fund Project for HIV/AIDS, malaria and TB. PAL project was conducted with the aim to educate the general practitioners (GPs) from PHC settings how to improve the quality of care in patients who seek assistance for respiratory symptoms in PHC settings and the efficiency of respiratory service delivery within health care systems, focusing on the district health. PAL concentrates on the most prevalent respiratory diseases at first-level health facilities-pneumonia, acute bronchitis and other acute respiratory infections, TB, chronic respiratory conditions including chronic bronchitis, asthma and COPD.

It aims at improving the identification and management of TB concerning the other respiratory illnesses, as well as the identification and management of non-tuberculous respiratory conditions concerning TB. It also aims to improve coordination between the different components and programs of the health care system, including the National TB Program, as many components are involved in the management of patients with respiratory symptoms [6].

The study aimed to evaluate the short-term impact of PAL approach in improving the management of patients with the most frequent respiratory diseases by the GPs from PHC settings in the Republic of Macedonia.

Material and Methods

PAL implementation followed 9 standardized steps: 1) enlist the national working group (NWG) who will support the PAL strategy; 2) estimate the burden of respiratory diseases; 3) assess the capabilities of the health infrastructure in implementing the PAL strategy; 4) develop clinical guidelines; 5) formulate an information system to monitor and evaluate the implementation; 6) develop training materials; 7) test the implementation of the clinical guidelines and the information system in a pilot area; 8) develop a national implementation plan; and 9) organize systematic supervision and evaluation of the PAL strategy.

The NWG for PAL implementation has worked within the National TB Program in collaboration with the Ministry of Health. Training material was developed by the NWG, and the implementation was targeted with appropriate utilisation of PAL guidelines by the health care workers in their daily tasks. At the PHC level, respiratory patients have been managed by their symptoms, but this is not carried out in a systematic and standardised manner [11]. So, it was very important to prepare guidelines adapted for primary care. The guidelines were designed to improve the

case management of respiratory diseases at PHC outpatient services and first referral hospitals. Guidelines took into account the context of the country, the health policy and the existing national guidelines. They have also to be evidence-based. Healthcare providers use the guidelines to perform step-by-step patient evaluations, to determine the specification of the most suitable form for disease management. It was decided to provide all GPs from PHC settings with peak flow meters.

The first phase of PAL education started at the end of 2013 and engaged 84 GPs from PHC settings. In the second phase, PAL education was enhanced to nearly the whole territory of the country, and the total number of 588 GPs was engaged until the end of 2016. PAL educations were organised as workshops within two days. The maximum number of the participants was 30 divided into two working groups.

Any patients aged 5+ years that sought care for at least 1 respiratory symptom in any of the selected PHC settings during the study period were eligible to be enrolled in the surveys. The GPs enrolled in the study registered specific information on every enrolled patient in a registry form. To evaluate the efficiency of GPs education from PHC settings, GPs filled in a form out of 69 questions for the patients enrolled before (baseline survey) and after PAL training (impact survey), and the results of the two surveys were compared. Both surveys were carried out in the same season and the same PHC settings and involved the same GPs. This analysis included the number of examined patients, especially those with respiratory symptoms as well as the manner the GPs treated them and how many patients were suspected for TB and were admitted to the higher level, then the subscribed therapy for the patients, particularly the number of antibiotics and inhalation therapy. The analyzed period in the baseline survey was one month before the educational training and the period in the impact survey was one month after the educational training. This analysis aimed to assess if the theoretical and practical skills obtained during the PAL training have been applied in the routine practice of the GPs who attended the trainings and to what degree.

Chi-squared test was used to for comparison of the proportions between the data in the baseline and impact study. A statistical difference was considered significant when the p-value was < 0.05.

Results

Among the total number of 80746 patients who admitted to the PHC settings due to different symptoms before the PAL education, 21 762 or 26.9% were patients who sought care for respiratory symptoms. One month after the PAL education, 84 449 patients visited PHC settings, and 24 152 or 28.6 % sought care for respiratory symptoms. So the baseline survey registered 21 762 patients and the impact survey 24 152 patients with respiratory symptoms (Table 1).

Table1: Total number and % of patients admitted to the PHC settings and patients with respiratory symptoms

Patients admitted to PHC settings	Baseline survey		Impact survey	
	Amount	%	Amount	%
Total patients	80 746	100	84 449	100
Total patients with respiratory symptoms	21 762	26.9	24 152	28.6

Among the patients with respiratory symptoms in the PHC settings, the most frequent were patients with ARI-72%, then the patients with COPD, asthma, pneumonia and TB. The patients with different respiratory diseases had very similar distribution before and after PAL education (Table 2).

Table 2: Distribution of patients with different respiratory diseases

Respiratory diseases	Baseline survey		Impact survey	
	Amount	%	Amount	%
ARI	15647	72,0	17075	70,6
COPD	3916	17,9	4257	17,6
Asthma	1721	7,9	2077	8,6
Pneumonia	435	2,0	531	2,2
TB	43	0,2	212	0,9
Total	21762	100	24152	100

In Table 3 were showed that more patients with ARI were treated in the PHC setting in the impact than in the baseline survey and it was statistically significant (P < 0.000001). Regarding the prescribed antibiotics, in the impact survey, a smaller number of patients (46%) received antibiotics in comparison with the baseline survey.

Table 3: Management of patients with ARI

	Baseline survey		Impact survey	
	Amount	%	Amount	%
Treated in PHC settings	12703	81.2	16682	97.7
Referral to the upper health level	2944	18.8	393	2.3
Total	15647	100	17075	100
	Statistical significant P < 0.000001			
Prescribed antibiotics	8590	54.9	7854	46.0

The results showed that statistically significantly smaller number of patients with exacerbation of COPD was referred to the upper health level in the impact survey (P < 0.000008) (Table 4). There was a decrease in the number of prescribed antibiotics for COPD patients in the impact survey. Another positive result was that the CAT questionnaire (for the assessment of the patient's symptoms) was used more frequently (18.1%) from the GPs in the PHC settings in the impact survey than in the baseline (13.2%).

Table 4: Management of patients with COPD

	Baseline survey		Impact survey	
	Amount	%	Amount	%
Treated in PHC settings	2910	74.3	3342	78.5
Referral to the upper health level because of exacerbation	1006	25.7	915	21.5
Total	3916	100	4257	100
Statistical significant		P<0.000008		
Prescribed antibiotics	2102	53.7	1230	28.9
Referral to the upper health level for diagnosis	3739	95.5	2132	50.1

Regarding the patients with asthma, there were statistically significant differences in the number of patients treated in the PHC settings and the referral to the upper health level (Table 5) in the baseline and impact surveys. It was obvious that more patients for diagnosis were referred to the upper health level after the PAL training.

Table 5: Management of patients with asthma

	Baseline survey		Impact survey	
	Amount	%	Amount	%
Referral to the upper health level for treatment	235	13.7	334	16.1
Treated in PHC settings	1486	86.3	1743	83.8
Total	**1721**	**100**	**2077**	**100**
Statistical significant		P<0.037		
Referral to the upper health level for diagnosis	270	15.7	615	29.7

Regarding the prescribed therapy for the patients with asthma, in the baseline survey inhaled corticosteroids (CS), oral CS and antibiotics were prescribed in 70.9%, 11.6% and 20.5 % of patients, and in the impact study in 75.8%, 9.1% and 17.4% separately. It was statistically significant that the GPs prescribed more inhaled that oral CS, and fewer antibiotics in the impact survey (Table 6). Also, more clinicians use to measured peak expiratory flow (PEF) for monitoring the patients with asthma after PAL education (47.2%) in comparison with the period before PAL education (21.6%).

Table 6: Prescribed therapy for the patients with asthma

	Baseline survey		Impact survey	
	Amount	%	Amount	%
Prescribed inhaled CS	1220	70.9	1574	75.8
Prescribed oral CS	199	11.6	189	9.1
Prescribed antibiotics	352	20.5	361	17.4
Pef	371	21.6	980	47.2
Total	1721	100	2077	100
Statistical significant P < 0.0000001				

There were no statistically significant differences in the treatment of patients with pneumonia before and after PAL education. But it is obvious that GPs used the CURB test most frequently for assessment of the degree of severity of pneumonia and the appropriate management of patients (Table 7).

Table7: Management of patients with pneumonia

	Baseline survey		Impact survey	
	Amount	%	Amount	%
Referral to the upper health level for treatment	61	13.8	72	13.6
Treated in PHC settings	374	86.2	459	86.4
Total	435	100	531	100
Statistical significant		P = 0.83		
CURB test	97	22.4	199	37.6

The number of TB cases in the PHC setting was very low: among the total number of patients with respiratory diseases, there were only 21 and 24 suspect cases for TB (0.1%), in the baseline and the impact survey. It is important to notice that in Macedonia, diagnosis, treatment and follow-up of TB cases are in the responsibility of the clinicians from dispensaries and hospitals for lung diseases and TB and the GPs from the PHC settings are in very small proportion included in the management of these patients. But with the PAL approach, there were a statistically significantly bigger number of TB cases for whom GPs from PHC settings were aware and had followed their treatment during the continuous phase of the treatment regime. In the impact survey, there were 43 patients with diagnosing for TB and in the baseline survey 212. GPs in the PHC setting were aware for the adverse reactions due to the TB drugs in 7.1 before and 12.5 % out of the TB cases after the PAL education (Table 8) (P < 0.000001).

Table 8: Management of patients with tuberculosis

	Baseline survey		Impact survey	
	Amount	%	Amount	%
Suspect for TB	21	0.1	24	0.1
TB cases	43	0.2	212	0.9
Adverse reaction of TB drugs	3	7.1	26	12.5
		P < 0.000001		

Discussion

The Practical Approach to Lung Health (PAL) is one of the strategies intended to overcome the challenge posed by weak health systems. This initiative is aimed at managing respiratory patients in primary health care settings while expanding TB detection and good-quality of TB services. Thus, it provides clear orientation on the coordination of healthcare between different levels and within relevant structures of general health services, incorporating well-defined country adapted criteria for patient referral.

To meet the requirements established by WHO for PAL testing in this work the same study protocol was used both, in the baseline and impact surveys [7]. Both surveys took place in the same seasons, in the same PHC settings and with the same GPs to ensure comparability between the data sets of the 2 surveys. As the 588 GPs were the same in both surveys, it is expected that the changes in their work with respiratory patients are due to the PAL education.

Our results showed that in the impact study more patients with ARI (P < 0.000001) and more patients with COPD exacerbations were treated in the PHC settings (P < 0.000008). More patients suspected of asthma were referred to upper health level for diagnosis (P < 0.037). The comparison of the findings between the baseline and impact surveys

suggest that training on PAL had an impact in decreasing drug prescription through a reduction in a prescription for antibiotics for ARI, COPD and asthma. Our study indicates that training on PAL is likely to increase the prescription of inhaled corticosteroids and tends to decrease the prescription of other formulation of these drugs ($P < 0.0000001$). The impact surveys showed that the patients with COPD and pneumonia in the PHC settings were better managed due to the more frequent use of CAT questionnaires and the CURB test. And regarding TB cases, our study indicates that the GPs were more aware of this disease and were more willing to take part in the patient treatment follow-up ($P < 0.000001$).

Similar results were reported in other countries where PAL methods have been initiated. The proportion of diagnosed cases with chronic respiratory diseases increased in the Jordan impact study [9]. In the same way, asthma diagnosis increased in Algeria after the PAL training (15.3% versus 10.3%) [10], whereas in Syria, the proportion of patients with asthma remained at 4.5% in both surveys, contrasting with the increase in COPD diagnosis (1.5% versus 0.7%) [11]. In Kyrgyzstan, significant achievements were reported within the PAL implementation period as mortality rates from respiratory diseases were reduced by 23% [12].

The implementation of PAL guidelines promotes rational use of drugs for respiratory diseases [13]. Inappropriate reliance upon antibiotics and underuse of inhaled corticosteroids in asthma are very frequent in PHC facilities [14]. Overuse of antibiotics due to underdiagnoses and misdiagnosis of respiratory conditions is a major concern [14]. Preventing and managing antimicrobial resistance is imperative as the presence of multidrug-resistant organisms has generated substantial apprehension among clinicians and public health experts [15]. In Nepal, the implementation of PAL guidelines implementation resulted in a reduction in multiple drug prescription and increased the prescription of generic drugs, as well as prescriptions from the essential drug list [16]. Use of PAL guidelines increased TB suspicion and TB detection in the majority of the countries [10] [11] [14]. Detection was improved whatever the country prevalence of TB was. The advantages were not only increased in the sputum smear-positive case TB detection, but also in increased detection of extrapulmonary TB [10]. Results from some study showed improvement in the quality of care for TB patients, and improvement of the successful treatment completion rates among TB retreatment cases [17]. In Kyrgyzstan, however, no improvement was observed concerning TB [3].

GPs in the PHC setting have the following advantages from the PAL implementation: to correctly interpret the key signs and symptoms; assess diagnosis; determine the degree of severity (e.g.in asthma patients); suggest adequate treatment; and, if necessary, identify referral options.

It is expected that long-term application of PAL will further underscore possible PAL impacts. The improved integration of respiratory care is expected to increase the proportion of respiratory patients managed in PHC and to decrease the proportion of hospitalised cases. Improved diagnostic quality of pulmonary TB among respiratory patients and reduced cost of respiratory case management are also among the long-term advantages that PAL will ensure.

The results from this study showed that the implementation of the PAL approach for GPs from the PHC setting in our country had had a positive effect in the management of a patient with respiratory symptoms. The findings suggest that implementation of PAL approach in the PAL settings is likely to reduce the prescribing of antibiotics, to increase the use of inhalation medication which is highly recommended in the management of asthma and COPD, and to decrease the referral of patients with chronic diseases to the upper health level. The PAL approach identified syndromes using symptoms and signs that best predict each disease. These results are in line with the findings of studies on PAL carried out in other country settings. However, more experience is needed for long-term influence on the effects on cost-effectiveness over the respiratory care services and over strengthening the healthcare system.

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Clinical Science

The Alteration of Plasma Matrix Metalloproteinase-9 Level after the Addition of Bromelin 500 mg to Standard Therapy of Acute Ischemic Stroke and Its Correlation with Outcome

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Abstract

BACKGROUND: Matrix metalloproteinase-9 (MMP9) expression due to ischemic cause spreading of brain damage. Previous studies have reported that Bromelin was beneficial as anti-inflammation and prevent brain tissue damage.

AIM: This study aimed to determine the alteration of plasma MMP9 level after addition of Bromelin 500 mg to Standard therapy and its correlation with outcome in acute ischemic stroke.

METHODS: This was a preliminary report of a prospective randomised, double-blind study with pre and post-test design, forty-six acute ischemic stroke patients were randomly allocated with Bromelin and Standard groups. Measurement of MMP9 and outcome were performed before and after 14-days treatment.

RESULT: The Bromelin group showed a significant decrement of MMP9 level, from 6.02 ± 0.32 ng/ml before treatment to 5.50 ± 0.94 ng/ml after treatment (p = 0.028). There was a negative correlation between MMP9 level and mRS (r= -0.03; p = 0.905) and a positive correlation toward BI (r = 0.039; p = 0.859), while the Standard group showed increased MMP9 level from 5.82 ± 0.71 ng/ml to 5.91 ± 0.83 ng/ml (p = 0.616) which was correlated insignificantly to outcome.

CONCLUSION: We concluded that the addition of 500 mg Bromelin to standard ischemic stroke therapy reduced MMP9 level significantly and correlated to outcome improvement. However, there is a tight statistical correlation.

Introduction

Ischemic cascade is a complex event and not yet understood entirely, but it can be concluded as bioenergy failure due to focal brain hypoperfusion followed by excitotoxicity, oxidative stress, disruption of blood-brain barrier, microvascular injury, hemostatic activation, post-ischemic inflammation results in cell death, and irreversible dysfunction of neuron cells, glial cells, and endothelial cells [1] [2] [3].

Up until now, the only FDA (The Food and Drug Administration) approved treatment for acute ischemic stroke is a thrombolytic therapy using rt-PA for reperfusion and save the brain tissue from ischemia. This treatment is effective if performed within 3 hours after stroke onset (in the USA), or in Europe within 4.5 hours. Data from the National Institute of Neurological Disorders and Stroke (NINDS) rt-PA Stroke Study revealed that rt-PA treatment given 3 hours after onset could improve clinical outcome and quality of life after 3 months of treatment. But until now, the number of patients who arrived at the hospital under 3 hour after onset is still very low. Also, there are many requirements for this therapy can be performed. In the USA, only 10 % of the patients receive this facility. In the other hand, reperfusion using rt-PA can sometimes cause hemorrhagic transformation which can be dangerous [4] [5] [6] [7].

Inflammation is an important aspect of the pathophysiology of stroke. Recent studies have proved that inflammation and immune response play an important role in a person's vulnerability of having stroke and degree of prognosis, this is due to the extent of brain tissue damage caused by them. The ischemic condition will trigger activation of microglia, acting as a sensor and is a resident immune cell in the central nervous system. But over activation of microglial can be neurotoxic by the release of Reactive Oxygen Species (ROS), Nicotinamide Dinucleotide Phosphate (NaDPH) oxidase, proinflammatory cytokine and induction also activation neurovascular proteinase such as matrix metalloproteinase (MMP), particularly MMP9. After stroke onset, MMP expression becomes uncontrolled, as proteolysis that disrupts the integrity of blood-brain barrier, causing increased permeability of blood-brain barrier leading to brain oedema, neuronal injury, apoptosis/cell death. Other than that, the MMP9 increment will trigger inflammation response through resident cells activation and greater leucocyte infiltration that can cause oedema to worsen. The extent of blood-brain barrier disruption is correlated to type, severity and duration of ischemia [2] [5] [8] [9] [10] [11] [12] [13] [14] [15] [16] [17].

Matrix Metalloproteinase-9 is the most common MMP related to stroke event, and many interventional studies have been performed with the objective to inhibit MMP9 showed better clinical outcome. In normal condition, MMP9 expression in brain tissue is minimal to undetected [5] [7] [12] [14].

Naturally, there is inhibition of MMPs by TIMPs. The work of MMPs and their inhibitors are the backgrounds of developing a new therapy for acute ischemic stroke to suppress the ischemic cascade that can spread brain damage so that morbidity and mortality from a stroke can be reduced [18].

A study by Zhao et al. 2006 (cit. Zlokovic 2006) on rats reported that rats that had increased MMP9 showed worse neurological clinical outcome with hemorrhagic complication [18]. The study by Yamashita and Abe, 2011 revealed that edaravone suppress MMP9 expression which protects brain microvascular integrity showed survival improvement and neurological clinical outcome [19]. Lo et al. (cit. Cui et al. 2012) reported that minocycline, a broad spectrum MMP inhibitor can reduce neuronal cell death after ischemia and adding time window for thrombolytic therapy [14].

Bromelin is a proteolytic enzyme (protease) fall under hydrolase, which can break peptide bond, separating proteins and amino acids. In 2006, FDA included Bromelin as a food additive and was a safe substance. Its proteolytic activity makes Bromelin widely used, for example in the food industry, as a supplement, and the substance that prevents browning in apple juice, meat tenderiser, an additive in the cosmetic industry for peeling effect, leather industry for smoothing and washing, and in textile industries [22]. It is accepted as phytotherapy agent and has been accepted as a therapeutic drug for its safety and efficacy. It was first introduced in 1957 (Kelly 1996) for acute inflammation and sports injury. This enzyme has broad-spectrum therapeutic efficacy proven in vitro and in vivo [20] [21] [23] [24] [25] [26] [27].

This enzyme has broad-spectrum therapeutic efficacy, proved in vitro and in vivo, has anti-oedema property, anti-inflammation, antithrombotic, fibrinolytic and malignancy. It's very low toxicity makes this drug save to be used in controlling chronic inflammation diseases [20] [21] [22] [23] [24] [25] [27] [28] [29] [30].

The objective of this study was to determine alteration of plasma MMP9 level in acute ischemic stroke patients after addition of Bromelin 500 mg administered twice a day to standard therapy (aspirin 300 mg once a day) for 14 days, and its correlation to stroke clinical outcome evaluated with modified Rankin Scale (mRS) and Barthel Index (BI).

Material and Methods

This was a double-blind clinical trial with pre and post-test design, performed at Adam Malik General Hospital in Medan, Indonesia, within April 2016 to April 2017, toward acute ischemic stroke patients, diagnosed by Head CT-scan. Inclusion criteria were acute ischemic stroke patients above 18 years old, the first attack and giving consent to be enrolled in this trial. Exclusion criteria were a cardioembolic stroke, brain stem lesion, not having upper gastrointestinal bleeding, and not having history on using anticoagulant drugs, antiplatelet aggregation drugs or anti-inflammation drugs. There were 46 participants who met the criteria, randomised into 2 treatment groups: Standard and Bromelin group, with 23 subjects in each group. All subjects were given acetylsalicylic acid (Aptor) 300 mg once a day as standard therapy for ischemic stroke. In Bromelin group, subjects were given capsule containing Bromelin 500 mg by a dose of 2 times daily for 14 days, while in standard group were given capsule containing dextrin 500 mg (Placebo), also twice daily for 14 days. Dextrin was chosen as placebo due to not having active substance. To meet double-blind criteria, Bromelin and placebo were packaged in the same colour and weight capsules and administered in the same manner also.

All subjects agreed to participate and signed informed consent voluntarily after receiving a detailed description of the study procedures and purposes. The study was approved by the Health Research Ethical Committee of North Sumatera/Adam Malik

General Hospital, Medan, c/o Medical School, University of Sumatera Utara.

Blood samples were collected in vacutainer tubes. Serum separation was isolated by centrifugation at 1000 g over 15 minutes. The serum samples should always be pre-diluted 1:5 (100 µl serum + 400 µl water) and stored at 2-8^{o}C until the time of analysis.

Matrix metalloproteinase-9 concentrations in plasma samples were analysed using MMP9 human ELISA Assay Kit (EA 100106-Origene) according to manufacturers' instructions and Chemwell 2910 analyser. Limit of detection of MMP9 human ELISA Assay Kit (EA 100106-Origene) is 31 pg/ml or 0.3 µg/ml.

The plasma MMP9 level and outcome measurement were performed twice, before and after treatment. The outcome was determined using modified Rankin Scale (mRS) and Barthel Index (BI). To evaluate alteration of the MMP9 level before and after treatment, paired t-test was used and to determine the correlation between plasma MMP9 level and mRS and BI score, Pearson correlation test was used. All data were expressed as mean ± S.D. P < 0.05 was considered to be significant.

Results

The total subject was 46 participants. Bromelin group was consisted of 23 subjects, with 17 male (73.9 %), while the standard group was also comprised of 23 subjects, with 14 male (60.9 %). Mean of age in the Bromelin group was 56.04 ± 10.13 year, while in standard was 60 ± 12.6 year, and there was no significant difference between group (p = 0.583). Subject's demographic characteristics are presented in Table 1.

Table 1: Demographic Characteristics of Studied Groups

Characteristics	Total	Bromelin 500 mg	Standard Drug
N (%)	46 (100%)	23 (50%)	23 (50%)
Mean Age ± SD	58.20 ± 11.48	56.04 ± 10.13	60 ± 12.60
Gender			
Male (%)	31 (67.4%)	17 (73.91%)	14 (60.87%)
Female (%)	15 (32.6%)	6 (26.09%)	9(39.13%)
Marital Status			
Married (%)	46 (100%)	23 (50%)	23 (50%)
Single (%)	0 (0%)	0 (0%)	0(%)
Ethnicity			
Bataknese	21 (45.7%)	9 (39.13%)	12 (52.17%)
Karonese	5 (10.9%)	4 (17.39%)	1 (4.35%)
Javanese	15 (32.6%)	8 (34.78%)	7 (30.43%)
Melayunese	2 (4.3%)	0 (0.00%)	2 (8.70%)
Acehnese	1 (2.2%)	1 (4.35%)	0 (0.00%)
Padangnese	2 (4.3%)	1 (4.35%)	1 (4.35%)
Hypertension			
Yes	37 (80.4%)	18 (78.26%)	19 (82.61%)
No	9 (19.6%)	5 (21.74%)	4 (17.39%)
Diabetes Mellitus			
Yes	8 (17.4%)	4 (17.39%)	4 (17.39%)
No	38 (82.6%)	19 (82.61%)	19 (82.61%)
Hypercholesterolemia			
Yes	37 (80.4%)	21 (91.30%)	16 (69.57%)
No	9 (19.6%)	2 (8.70%)	7 (30.43%)
Smoking			
Yes	19 (41.3%)	10 (43.48%)	9 (39.13%)
No	27 (58.7%)	13 (56.52%)	14 (60.87%)

N= number of patients; S.D= Standard Deviation.

There were 23 subjects on the Bromelin 500 mg group, measured their MMP9 level, mRS score and BI score, before and after treatment. Since the test of normality showed normal distribution data, paired t-test was performed to determine the difference of MMP9 level, mRS, and BI score before and after treatment, with the level of significance p < 0.05.

The result of this study on MMP9 after Bromelin 500 mg administration showed significant decrement of the MMP9 level. The mean of MMP9 before treatment was 6.02 ± 0.32 ng/ml and after treatment was 5.50 ± 0.4 ng/ml, where the decrement was statistically significant (p = 0.028) (Table 2).

Table 2: Plasma MMP9 Level (ng/ml) and Outcome

	MMP9		P	mRS		P	BI		p
Drugs	Pre	Post		Pre	Post		Pre	Post	
Bromelin 500mg	6.02± 0.32	5.50± 0.94	0.028*	3± 1.107	2± 1.340	0.604*	50± 27.83	60± 30.60	0.002*
Standard Drugs	5.82± 0.71	5.91± 0.83	0.616*	3± 0.949	3± 1.033	0.002*	60± 23.45	75± 22.55	0.001*

* = Paired T-test

The result of mRS after 500 mg Bromelin administration showed unsignificant decrement, where mRS score before treatment was 3 ± 1.107 and after treatment was 2 ± 1.34 (p = 0.604) (Table 2).

The study result on BI score after Bromelin 500 mg administration showed a significant increment, where before administration the score was 50 ± 27.83 and after administration was 60 ± 30.6 with p = 0.002 (Table 2).

The result on the MMP9 level in the standard group showed unsignificant increment. The mean of MMP9 before treatment was 5.82 ± 0.71 ng/ml and after treatment was 5.91 ± 0.83 ng/ml, this was not statistically significant (p = 0.616) (Table 2).

This study result on mRS score in standard group revealed changes, where before treatment, the mRS score was 3 ± 0.949 and after treatment was 3 ± 1.033 (p = 0.002) (Table 2).

The result of this study on BI score after standard therapy showed significant increment, where BI score before treatment was 60 ± 23.45 and after treatment was 75 ± 22.55, with p = 0.001 (Table 2).

Using Pearson correlation test between decrement of MMP9 and mRS after Bromelin 500 mg administration, there was a negative correlation but not statistically significant (r =-0.03; p = 0.905) (Table 3)

Table 3: Correlation of MMP9 Decrement and Outcome

	mRS		BI	
	r	p	r	P
MMP9 Decrement Level	-0.03	0.905**	0.039	0.859**

Pearson correlation test between decrement of MMP9 level and increment of BI score showed positive unsignificant correlation (r = 0.039; p = 0.859) (Table 3)

Discussion

Matrix metalloproteinase-9 (MMP9) is a family of proteolytic enzyme, involved in the breakdown of extracellular matrix during tissue remodelling [7]. The action of the MMPs on the basal lamina and tight junction proteins (TJPs) in endothelial cells is the final common pathway for the opening of the BBB, which allows cells to enter the central nervous system and attack invading organisms [31]. During a stroke, it attacks the extracellular matrix around the blood vessels and neurons, facilitating neural cell death. MMP-9 disrupts the blood-brain barrier in the early phase following cerebral ischemia, leading to leakage, leukocyte infiltration, brain oedema, and haemorrhage [7].

This study found that plasma MMP-9 level to be high in the two groups of patients. In Bromelin groups mean of the MMP-9 level were 6.02 ± 0.32 and the Standard group were 5.82 ± 0.71. Expression of MMPs in the adult brain is very low to undetectable [32]. This study proved that expression of MMP-9 is upregulated in the brain in response to ischemic stroke. This is by the study by Heo *et al*. 2003 (*cit.* Abdelnaseera *et al*. 2015), found that MMP -9 serum level on admission was significantly higher in stroke patients compared with the control group [33].

In this study, the addition of Bromelin 500 mg to standard therapy showed significant decrement of plasma MMP9 level (p 0.028), the increment of mean BI score (p = 0.002) and unsignificant decrement of mRS score (p = 0.604), proved that clinically there was outcome improvement. This concluded that MMP9 decrement could improve clinical outcome. Study of Lindsell *et al*. 2005 (*cit.* Abdelnaseera *et al*. 2015) showed there was an association between higher serum levels of MMP-9 and more severe stroke, supported the role of MMP-9 as an independent predictor of clinical severity of ischemic stroke in the acute stage [33]. Previous several studies reported that treatment with MMP inhibitors or MMP neutralising antibodies decreases infarct size and prevents BBB breakdown after focal ischemic stroke [31].

We concluded that the addition of 500 mg Bromelin to standard ischemic stroke therapy reduced MMP9 level significantly and correlated to outcome improvement. However, there is a tight statistical correlation.

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Association between Myeloperoxidase Levels and Risk of Insulin Resistance in Egyptian Obese Women

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Abstract

BACKGROUND: Myeloperoxidase (MPO) is an enzyme involved in the pathogenesis of several diseases.

AIM: The current study aimed to investigate serum MPO levels in obese Egyptian women and assess its relation with insulin resistance (IR) and other biochemical risk parameters.

METHODS: The study included 80 obese women and 50 age-and-sex-matched healthy controls. Insulin resistance (IR) was evaluated by the Homeostasis Model Assessment-Insulin Resistance (HOMA-IR). Serum MPO, fasting glucose, insulin and blood lipids and anthropometry were measured. Obese cases were divided into three groups based on MPO tertiles. ROC analysis was performed to obtain the optimal cut-off values of MPO to predicate IR in obese women.

RESULTS: The mean serum MPO was significantly higher in obese cases than controls. Cases in the highest MPO tertile had higher HOMA-IR, blood lipids and pressure levels compared with those in the lower tertile. The cutoff point of MPO was > 87.8 (ng/mL) and area under curves was 0.82 ($p < 0.01$) for diagnosis of IR. MPO levels were higher in obese Egyptian women than healthy controls.

CONCLUSION: Elevation of MPO was associated with abnormal metabolic parameters. MPO might be used as an earlier biomarker for IR and metabolic disturbance in obese women.

Introduction

MPO is a heme peroxidase that is highly expressed in leukocytes and considered as a principal enzyme in the innate immune response. It is substantially stored in cytoplasmic granules and might be discharged into the extracellular compartment following phagocyte activation [1]. As MPO can commence lipid peroxidation process, it acts as an earlier marker of oxidative damage [2]. In this regard, lots of evidence indicate the association between the oxidative stress and metabolic syndrome (MS) parameters [3] [4], where oxidative stress affects the pathophysiology of MS and its components [5]. Although MPO plays an important role in the innate immune system, it has a very deleterious effect on a large number of inflammatory-mediated diseases. It has been found in cases with an acute coronary syndrome that the serum level of MPO is highly correlated with elevated risk of subsequent cardiovascular diseases.

Central obesity, dyslipidemia, insulin resistance, and hypertension constitute the main components of the metabolic syndrome and metabolically linked to cardiovascular risk factors [6]. Levels of antioxidants in metabolic syndrome cases varied in different races [7]. Plasma levels of MPO are found to be high in patients with stable coronary artery disease. Furthermore, it has been demonstrated that MPO is a powerful prognosticator of the undesirable clinical outcomes in cases with chronic heart failure, acute coronary syndrome, and of future coronary artery disease in a healthy population. Moreover, the

main risk factor for cardiovascular diseases and mortality, type 2 diabetes, is positively correlated with MPO levels [8]. It seems that the MPO plays a role as a mediator in the vascular inflammation and in the production of oxidant species that are important in the pathophysiology of the inflammatory diseases. Therefore, this study aimed to evaluate the association between serum levels of MPO with metabolic and biochemical parameters in obese women.

Methods

The study sample included 80 obese women and 50 age-and-sex-matched healthy controls. Obese cases were divided into three groups according to MPO tertiles. This research has been approved by the Ethical Committee of National Research Centre, Egypt (number = 16361), by the World Medical Association's Declaration of Helsinki.

All patients and controls were subjected to full medical history and clinical examination. All anthropometric measurements were taken 3 times on the left side of the body, and the mean of the 3 values was used. Body weight was measured to the nearest 0.1 kg and height was measured to the nearest 0.1 cm. Height was measured with the patients standing with their backs leaning against a stadiometer scale. BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Waist circumference (WC) and hip circumference (HC) were measured in cm using a plastic, non-stretchable tailor's tape. WC was measured with light clothing at a level midway between the lower rib margin and the iliac crest standing and breathing normally. HC was measured at the level at the widest circumference over the buttocks (at the greater trochanter). Subsequently, the waist-hip ratio (WHR) was calculated as WC divided by HC. Skin-fold thickness was measured to the nearest mm, except for low values (usually 5 mm or less) where it was taken to the nearest 0.5 mm. These readings were done at the biceps, triceps, subscapular, supra-iliac and abdominal areas using Holtain calliper. The biceps skin-fold thickness was measured at the level of the mid-point between the acromion (lateral edge of the acromion process) and the radius (proximal and lateral border of the radius bone) on the mid-line of the anterior surface of the arm. Triceps skin-fold thickness was measured vertically at the midway between acromion and olecranon processes on the posterior surface of the arm. The subscapular skin-fold was measured below the lower angle of the left scapula at a diagonal in the natural cleavage of the skin. The position of the suprailiac skinfold was the diagonal fold just above the iliac crest even with the anterior axillary line. Abdominal skin-fold was at 5 cm adjacent to the umbilicus to the right side. Subsequently, the sum of skinfolds was calculated. Anthropometric measurements were obtained according to standardised equipment and following the recommendations of the International Biological Program [9]. Body fat % was assessed by Tanita Body Composition Analyzer (SC -330).

Presence of IR was defined by HOMA-IR > 2.47 [10].

Systolic and diastolic blood pressures (SBP and DBP) have been measured twice in the right arm in a sitting position after a 10-min rest period, and the average of the two measurements was used for analysis. Blood pressure was measured according to a standardised operating procedure using a calibrated sphygmomanometer and brachial inflation cuff (HEM - 7200 M3, Omron Healthcare, Kyoto, Japan).

Venous blood samples were collected by direct venipuncture after an overnight fast (minimum 12 h). Fasting plasma glucose and serum lipids (total cholesterol, high-density lipoprotein cholesterol (HDL-C) triglycerides (TG) were measured by enzymatic colourimetric methods using a Hitachi autoanalyser 704 (Roche Diagnostics. Switzerland). Low-density lipoprotein cholesterol (LDL-C) was calculated according to certain equation (LDL – C = Total cholesterol-Triglycerides/5 + HDL-C). Serum insulin concentration was analysed by chemiluminescent immunoassay (Immulite2000, Siemens, Germany. Insulin resistance was determined by the Homeostasis Model Insulin Resistance (HOMA-IR) is calculated as the product of the fasting plasma insulin level (IU/mL) and the fasting plasma glucose level (mmol/L), divided by 22.5 [11]. Clinical history and physical examination were performed for each subject.

Serum myeloperoxidase (MPO) was estimated using Quantikine ELIZA kit of R&D systems catalogue number DMYE00B for the quantitative determination of human MPO concentration in serum according to the manufacturer's instructions.

All statistical analyses were performed using SPSS16.0 for Windows (SPSS Inc). The Kolmogorov-Smirnov test of normality was used to verify whether the distribution of variables followed a Gaussian pattern. Normally distributed data in groups were expressed as means ± SDs. A receiver operating characteristic (ROC) curve analysis was performed to obtain the optimal cutoff values of MPO to diagnosis IR. The optimal cutoff values were obtained both from the point on the ROC curve. The area under the curve (AUC) and the 95% confidence interval (CI) were used for diagnostic validity. The Youden index, calculated as (sensitivity + specificity-1) was estimated to determine optimal cut-off.

Differences in clinical and biochemical characteristics between groups were tested using

one-way analysis of variance (ANOVA) and post hoc tests for differences between groups.

Results

Table 1 shows the clinical and biochemical characteristics of obese cases and controls. Mean age of obese women participated in the study was 31.5 ± 4.8 years and was 32.7 ± 4.7 in controls. Obese women showed significantly higher values of BMI, MPO, waist circumference, SBP, DBP, FBG, total cholesterol, TG, LDL-C and lower HDL-C than controls.

Table 1: Clinical and biochemical characteristics of obese cases and controls

Characteristics	Obese	Controls
Age (years)	31.5 ± 4.8	32.7 ± 4.7
BMI (kg/m^2)	32.21 ± 5.4*	22.24 ± 4.2
WC (cm)	96.2 ± 6.12*	81.6 ± 4.3
WHR	0.82 ± 0.8	0.79 ± 0.6
Sum SF	149. 9 ± 23. 9	143.9 ± 21.9
Body fat%	29.9 ± 9.9	27.5 ± 8.4
SBP (mmHg)	135.55 ± 9.8*	100. 3 ± 9.4
DBP (mmHg)	88.8 ± 6.9*	65.24 ± 8.8
HOMA-IR	6.8 ± 1.2*	2.2 ± 1.2
FBG (mg/dL)	111.4 ± 24.7*	81. 0 ± 21.1
TC (mg/dL)	189.9 ± 36.2*	118.9 ± 22.2
TG (mg/dL)	146.8 (120-148.8) *	47.55 ± 10.45
HDL-C (mg/dL)	35.6 ± 4.9*	47.77 ± 10.8
LDL-C (mg/dL)	163 (88-162)*	165 (88-164)*
MPO (ng/mL)	86 (66 – 92)**	22 ± 12 (20–30)

BMI: body mass index; WC: waist circumference; WHR: waist to hip ratio; Sum SF: sum of skin folds; SBP: systolic blood pressure; DBP: diastolic blood pressure; HOMA-IR: homeostasis model assessment-insulin resistance; FBG: fasting glucose; TC: total cholesterol; TG: triglycerides; HDL -C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; MPO: myeloperoxidase; *p < 0.05, ** p < 0.001.

Biochemical and metabolic characteristics in obese women according to the different levels of MPO tertiles are summarised in Table 2. Compared with participants in the first tertile of MPO, those in the third tertile had higher levels of HOMA-IR, total cholesterol, TG, LDL-C, BP and lower levels of HDL-C. Fig 1 shows ROC curves for MPO to identify IR in obese women, the cutoff points for MPO was > 87.8 (ng/mL) and area under curves was 0.82 (sensitivity 93.7%, specificity 71.4%) as a most sensitive/specific cut off.

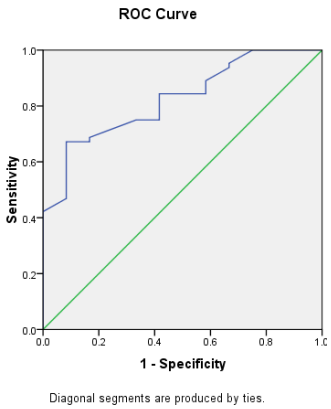

Figure 1: the Receiver-operating characteristic curve of sensitivity plotted against1-specificity of serum MPO to identify IR among obese women

Table 2: Biochemical and metabolic characteristics in obese women according to the Myeloperoxidase (MPO) tertiles

Characteristic	I Lower tertile ≤ 61.7	II Intermediate tertile 61.8-82.6	III Higher tertile > 82.7
Age (years)	31.2±4.1	33.5±3.5	31.1 ±4.8
BMI(kg/m2)	31.8 ± 5.4	33.4 ± 6.6	34.9 ± 6.23
WC (cm)	86.0 ± 10.9	87.9 ± 11.1	96.9 ± 10.9*
WHR	0.7 ± .8	0.8 ± .7	0.9 ± .9
Sum SF	131.6 ±10.5	134.2 ± 11.0	138.4 ± 14.9
Body fat%	29.9 ± 9.8	32.6 ± 10.9	33.5 ± 12.8
SBP (mmHg)	111.1±10.5	125.4 ± 9.4	151.3 ± 11.4*
DBP (mmHg)	71.5 ± 8.6	76.3±6.8	89.9±8.5*
HOMA-IR	3.5 ± 1.1	3.7 ± .7	5.6 ± 1.2*
FBG (mg/dL)	83.1 ± 20.8	86.3 ± 25.7	121. 8 ± 22.9*
TC (mg/dL)	136.7 ± 20.7	149.9 ± 21.9	193 ± 23.5*
TG (mg/dL)	139.9 ± 20.2	149.8 ± 22.5	195.8 ± 21.5*
HDL-C (mg/dL)	46.4 ± 10.5	41. 6 ± 11.4	35.4±12.2*
LDL-C (mg/dL)	124.7 ± 21.3	129.3 ± 20.9	156.6 ± 21.9*
MPO (µg/L)	54.1 (33.4-57.9)	69 (60.7-66.9)	91.3 (83.4- 96.9) *

BMI: body mass index; WC: waist circumference; WHR: waist to hip ratio; Sum SF: sum of skin folds; SBP: systolic blood pressure; DBP: diastolic blood pressure; HOMA-IR: homeostasis model assessment-insulin resistance; FBG: fasting glucose; TC: total cholesterol; TG: triglycerides; HDL -C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; MPO: myeloperoxidase; Data are mean ± SD, median (intertertile range); Tertile I vs Tertile III *p < 0.05.

Discussion

This study showed that MPO is positively associated with IR in the obese women. As it is linked to obesity, IR is kept rising and consequently Type 2 Diabetes affects a large number of young individuals [8]. It has been demonstrated that elevated concentrations of glucose and free fatty acids lead to oxidative stress and start IR in genetically predisposed individuals with diabetes [12]. Previous studies have found that MPO might be used as a predictor of both early [13] [14] and late unfavourable cardiac events in cases suffered from chest pain and other clinical features of the acute coronary syndrome. MPO plays an important role in the oxidation of HDL in vivo [14] which result in functional inactivation. The subsequent decrease in plasma HDL causes a reduction in the reverse cholesterol transport which stimulates atherosclerosis [15]. Moreover, HDL is a very important anti-inflammatory, antioxidant, and antithrombotic factor. In patients with metabolic syndrome, the activated leukocytes might be responsible for the increased activity of MPO. Furthermore, insulin resistance causes higher concentrations of proinflammatory mediators [16]. Also, the inflammatory cytokines are produced by adipocytes, especially in obese individuals, to compromise the insulin signalling [17]. In MS patients, both insulin resistance and central obesity are demonstrated, however, the association between the augmented MPO expression and chronic inflammation is also evident [18]. The connection between proinflammatory regulators and pathogenesis of the MS has been previously demonstrated, where the impairment in the inflammatory response could be noticed during the disease [19]. MPO acts as a physiological mechanism that connects the matrix proteins degradation by metalloproteinases.

Our results found a higher concentration of MPO in obese group than controls. Also, we found

that cases in the higher tertile of MPO had higher levels of HOMA-IR, total cholesterol, TG, LDL-C, SBP, DBP and lower levels of HDL cholesterol than those in the lower tertile. The present analysis showed that the cutoff point of MPO was > 87.8 (ng/mL) for diagnosis of IR in obese Egyptian women ($p < 0.01$). In agreement with our findings, it has been previously reported that MPO is associated with IR [20]. It is evident that MPO is an effective mediator of endothelial dysfunction where a strong connection has been observed between serum MPO levels and endothelial dysfunction in overweight subjects [21]. Obesity is the principal cause for low HDL, and in order to enhance the HDL level, an optimizing healthy lifestyle is required including moderate weight loss along with exercise and smoking cessation. High oxidative stress and systemic inflammation are correlated with increased plasma triglyceride-rich lipoproteins and oxidized lipoprotein (a) phospholipids which lead to cardiovascular risks [22] [23]. Losing of the anti-oxidative, anti-inflammatory and athero-protective properties of HDL and its apolipoproteins could even increase those biochemical disturbances. Metabolic disorders such as insulin resistance, oxidative stress, inflammation, and hyperlipidemia represent connected disturbances in women with metabolic syndrome [24]. Obesity has certain anthropometric, biochemical and physiological anomalies that lead to IR and cardiovascular disease (CVD). Therefore, numerous studies have indicated the associations between obesity, inflammation, CVD and IR in both adults and children [25]. MPO activity was used as an adjuvant marker for the inflammatory and oxidative status in obese women [26] [27] [28]. Also, there is increasing evidence that MPO contributes to cardiovascular disease. It has been reported that the presence of obesity in childhood stage in association with the risk factors hurt the vascular health status in adulthood [29]. Furthermore, in apparently healthy adults, augmented serum MPO levels were found to be associated with higher CVD risk [30].

In conclusion, MPO might be used as an earlier biomarker for IR in obese women and might be useful in determining patients who are at high risk for a biochemical disturbance.

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Cigarette Smoking and Hyperglycaemia in Diabetic Patients

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Abstract

BACKGROUND: The incidence rate of diabetes mellitus has increased throughout the year. Various studies indicate that smoking may affect glucose metabolism and cause hyperglycemia in diabetes mellitus. This study aimed to compare the blood glucose and HbA1c level in diabetic smoking patients and non-smoking diabetic patients.

METHODS: This study used the cross-sectional approach. The study population consisted of 30 diabetic smoking patients and 30 non-smoking diabetic patients. The diabetes history and the smoking status of the study population obtained by questionnaire-based interview, the blood glucose and HbA1c level were measured by hexokinase and immunoturbidimetry method using cobas 6000 analyser module c501 (Roche Diagnostics, Switzerland).

RESULTS: The result in this study showed the fasting blood glucose, postprandial blood glucose, and HbA1c were higher by 23.64 mg/dl ($p = 0.325$), 58.00 mg/dl ($p = 0.016$), 0.39% ($p = 0.412$) in smoking diabetic patients compared to non-smoking diabetic patients. After statistical analysis, there was a significant difference ($p < 0.05$) of postprandial glucose level between smokers group and non-smokers group, but the non-significant difference of fasting blood glucose and HbA1c

CONCLUSIONS: This study concluded that there was a significant difference in postprandial glucose level between smokers group and non-smokers group but the non-significant difference of fasting blood glucose and HbA1c.

Introduction

The incidence rate of Diabetes Mellitus (DM) has been increasing in every year in the worldwide. International Diabetes Federation estimates in 2015 that 8.5% (equivalent to 78.3 million people) of the adult population in South-East Asia suffers from diabetes. In Indonesia, an increase in diabetes incidence has been noted, from 1.1% in 2007 to 2.1% in 2013 [1] [2]. Diabetes is a metabolic disorder characterised by the presence of chronic hyperglycemia. Hyperglycemia may be caused by insulin resistance syndrome, insulin deficiency, or both [3]. Diabetes can be diagnosed by biomarker of hyperglycemia, i.e. the random blood glucose test, fasting blood glucose test, or postprandial glucose test. Many factors are known to affect blood glucose in the diabetic patient, including lifestyle factors such as smoking [4].

Cigarette smoking is independently associated with the incidence of diabetes mellitus [5][6]. Smoking-induced oxidative stress might have some effect on blood glucose as well as directly alter blood glucose homeostasis and cause insulin resistance. The exact biological pathway of this theory has not been fully elucidated, but it is suspected that high concentration of circulating epinephrine and norepinephrine due to smoking may contribute to hyperglycemia by increasing the rate of hepatic gluconeogenesis and glycogenolysis [5] [6] [7].

Another method used to assess blood

glucose over a longer period is by analysing the concentration of glycated haemoglobin (HbA1c). HbA1c is a result of non-enzymatic attachment of a hexose molecule to the haemoglobin molecule. This process occurs continually over the entire lifespan of the erythrocyte and is dependent on blood glucose concentration and the duration of erythrocyte exposure to blood glucose [8]. Therefore, the HbA1c reflects the mean glucose concentration over the previous period. The international expert committee stated that HbA1c could be used as a diagnostic test for diabetes [9]. Smoking might alter glucose homeostasis; it might be as well affecting HbA1c concentration [10].

This experiment was conducted to assess the difference of blood glucose concentration and HbA1c among diabetic smoking patient and non-smoking diabetic patients.

Methods

The protocol of the study was approved by the Research Ethics Committee of Faculty of Medicine of University of Sumatera Utara (NO: 198/TGL/KEPK FK USU-RSUP HAM/2017). This study used the cross-sectional approach. The study population included diabetic patients attending Endocrine Clinic University of Sumatera Utara Hospital from June to September 2017. The study population consisted of thirty diabetic smoking patients and thirty non-smoking diabetic patients. To avoid confounding factors, the diabetic patients included in this study was in 50-60 years age range and has been diagnosed with diabetes for the past 5 to 10 years.The exclusion criteria were: (1) history of using any antioxidant supplement; (2) history of current acute or chronic infection; (3) history of malignancy; (3) History of red blood cell membrane disorder or anaemia.

To obtain data on age, duration of diabetes, and history of smoking (duration of smoking and cigarette per day), a questionnaire-based interview was used. The biomarker of hyperglycemia is, i.e. fasting blood glucose, postprandial blood glucose, and the HbA1c level was measured by hexokinase and immunoturbidimetry met hod using Cobas 6000 analyser module c501 (Roche Diagnostics, Switzerland). Diabetes patients with HbA1c higher than 6.5% were categorised as uncontrolled diabetes. All data were processed using the statistical package for social science (SPSS). The differences among groups were tested by using Mann-Whitney, and p-values of < 0.05 were considered significant.

Results

This study was carried out among 60 diabetic patients attending the endocrine clinic in North Sumatera University Hospital from June to September 2017. The characteristic of the study population is shown in Table 1 below.

Table 1: Characteristics of the study population

	Smoking Status		
	Non-Smokers	Smokers	Total
Sex			
Male	13 (43.33%)	26 (86.67%)	39 (65%)
Female	17 (56.67%)	4 (13.33%	21 (35%)
Age	57.7 (± 6)	57 (± 9.9)	-
Duration of Diabetes	7.76 (± 9.26)	7.03(± 6.76)	-
Diabetes Criteria			
Controlled Diabetes	6 (20%)	2 (6.67%)	8 (13.33%)
Uncontrolled Diabetes	24 (± 80%)	28 (93.33%)	52 (86.67%)
Duration of Smoking	-	29.7(± 10.92)	
Cigarettes Per Day	-	15.93 (± 10.24)	

Table 1 shows that the study population consists of 39 male diabetic patients and 21 female diabetic patients. Among 39 male diabetic patients, 26 were smokers while the other 13 were not. As for female patients, 4 were smokers, and 17 were non-smokers. The mean age of the participant in this study is 57.7 for the control group and 57 for the study group. Among 60 diabetic patients in this study, 36 of them were diagnosed with diabetes during the past 5 years. Diabetic patients with HbA1c higher than 6.5% were categorised as uncontrolled diabetes. Table 1 showed 52 diabetic patients have uncontrolled diabetes. Among the 52 diabetic patients, 24 were a non-smoker, and the other 28 were smokers. For the smokers group, the average duration of smoking was 29.7 (± 10.92) years, with average cigarettes per day were 15.93 or equivalent to almost 1 pack of cigarettes per day.

The comparison between fasting blood glucose, postprandial blood glucose, and HbA1c in each study group is shown in Table 2.

Table 2: Blood glucose level and HbA1c in the studied group

		Non-smokers	Smokers	*p*
FBG	Min.	94	93	
	Max.	307	486	
	Median	155	177	
	Mean ± *SD*	170.36 (± 54.74)	194 (± 83.95)	0.325
PBG	Min.	117	130	
	Max.	407	611	
	Median	242	294	
	Mean ± *SD*	249.67 (± 76.07)	307.67 (± 97.22)	0.016
HbA1c	Min.	5	7	
	Max.	13	12	
	Median	9	9	
	Mean ± *SD*	8.65 (± 1.91)	9.04 (± 1.53)	0.412

A Higher level of fasting blood glucose was observed in the study group compared to the control group. The mean difference between groups was 23.64 mg/dl. The Mann-Whitney test showed *p*-value 0.325. There was 58.0 mg/dl difference in postprandial glucose between study and control group. The Mann-Whitney test showed *p*-value 0.016. HbA1c was 0.39 mmol/mol higher in the study group

compared to the control group with *p*-value 0.412.

Discussion

All three chemical biomarkers were higher in the smokers group compared to the non-smokers group after adjustment for a possible confounding variables such as age, diet, physical activity, and types of medication used. There was no significant difference in fasting blood glucose levels between study and control groups. There was also no significant difference of HbA1c among the group as the independent t-test shows the p-value > 0.05. For postprandial glucose, the independent t-test showed p-value < 0.05 which means there was a significant difference in postprandial glucose between both groups.

The postprandial blood glucose test is used to evaluate the ability to regulate glucose metabolism. The postprandial glucose test also provides an insight on insulin sensitivity. Compared with fasting blood glucose and HbA1c cut points, the postprandial glucose test value diagnoses more people with diabetes [9]. Various studies have demonstrated that insulin resistance is dose-dependently related to smoking. The level of basal insulin secretion and insulin resistance (evaluated by HOMA-IR protocol) were higher in smoking compared to non-smoking patients [5] [6] [11] [12].

The prevalence of type 2 diabetes increases with age, and it is also well documented that ageing is associated with a decline in insulin action as well as pancreas function. Normally, pancreatic beta cells have a long lifespan with low proliferation rate; however, during increased metabolic demand or after injury, adult pancreas could be able to produce new cells. As we age, this proliferative capability of pancreas declines [13] [14]. Smoking causes oxidative stress due to an increase in ROS that found in smokers body12] [15]. This condition causes oxidative damage such as lipid peroxidation, protein oxidation, and DNA damage. The island of the pancreas is particularly vulnerable to damage caused by ROS accompanied by a lack of antioxidant enzymes in the cells. ROS will induce the activation of Poly-ADP-Ribose-Polymerase (PARP) that causes NAD depletion. This will result in the apoptosis of insulin-producing cells [12].

There is a clear, dose-dependent relation between diabetes or glucose intolerance and both active and passive cigarette exposure. Adiponectin concentration seems to partially mediate the effect of smoking on glucose homeostasis [5] [17] [18].

High level of ROS generated by smoking will inhibit phosphatidylinositol–3-kinase activity, thus decreasing the secretion of adiponectin from adipose tissue. This lower concentration of adiponectin is a common finding in obese or diabetic patients [18]. Adiponectin stimulates the phosphorylation and activation of 5′*-adenosine monophosphate-activated protein kinase* in the liver and skeletal muscles, thereby directly affecting glucose homeostasis and insulin sensitivity [5].

Another experiment showed how nicotine alters glucose metabolism in animal models. The dose of nicotine used in this experiment was chosen to mirror the average cigarette smokers peak blood level of cotinine. Acute nicotine treatment for 30 minutes, caused hyperglycemia and glucose intolerance. The hyperglycemic effect of acute nicotine treatment was mediated by the activation of certain nAchR subunit because the hyperglycemia was abolished by CSM (nAchR inhibitor). This acute nicotine treatment also increases both basal insulin secretion, glucose-stimulated insulin secretion, and decreases insulin sensitivity [7]. Furthermore, smokers have been shown to have higher fasting plasma cortisol concentrations than non-smokers. Higher cortisol concentrations may be a consequence of the stimulation of sympathetic nervous system activity that is induced by smoking, and higher cortisol may lead to hyperinsulinemia [11] [17].

For HbA1c variable, the result in this study was in contrast with a previous study that shows HbA1c was higher by 0.08% in smokers compared to non–smokers [19]. Glycated haemoglobin provides a better indication of long-term glycemic control than blood glucose levels. During the lifespan of erythrocyte, they are constantly exposed to glucose and result in non-enzymatic attachment of glucose to the haemoglobin molecule within the erythrocyte. Due to the longer lifespan of erythrocyte, haemoglobin reflects the mean blood glucose over previous periods (approximately 3 months, as the average lifespan of the erythrocyte is 120 days). The rate of HbA1c formation is directly proportional to the mean of blood glucose during the lifespan of the erythrocyte. Due to its properties, HbA1c is often used to monitor blood glucose in the diabetic patient and also used to monitor patient response toward diabetes therapy [9] [10]. Many studies have reported the unfavorable effects of smoking for diabetes mellitus. Smoking increases the risk of developing diabetes, and aggravates the micro-and macro-vascular complications of diabetes mellitus [6] [9]. Diabetic patients are also more likely to develop various oral health problems that may be aggravated by smoking [20].

The result in this study did not represent the entire smoking and non-smoking diabetic patients due to the cross-sectional design and relatively very small study population. Future studies are needed to analyse the exact biological effect of various factors

such as types of medication used, physical activity and lifestyle that can affect blood glucose and HbA1c, since a substantial portion of HbA1c and blood glucose concentration may be determined by these non-glycemic factors [10] [15] [21].

This study concluded that postprandial glucose levels were different in smoking compared to non-smoking diabetic patients. Smoking may contribute to the development of insulin resistance as there were higher postprandial glucose levels in smoking diabetic patients compared to the non-smoking diabetic patients. Although further studies are needed for this specific population regarding the impact of smoking on glucose metabolism and insulin resistance, smoking cessation programs should be offered to the diabetic population.

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The Comparison of Olanzapine and Risperidone Treatment in Male Schizophrenic Patients using Positive and Negative Syndromes Scale (PANSS)

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Abstract

INTRODUCTION: The most common method to compare olanzapine and risperidone is by calculating the score of positive and negative syndromes scale (PANSS). However, there were some conflicting results mentioned from previous studies.

AIM: This study aimed to compare the treatment of olanzapine and risperidone using PANSS, limited to only male patients receiving inpatient treatment to obtain more significant results.

METHODS: The subjects of this study were male schizophrenic patients in the acute phase of treatment (N = 68) who were hospitalised at the Mental Hospital Prof. Dr M. Ildrem, Indonesia. These participants were divided into two groups and treated with different atypical antipsychotics (olanzapine, 20 mg/day [n = 34]; risperidone, 6 mg/day [n = 34]). The scores of PANSS from both groups were collected from pre-test and post-test after 6 -week treatment.

RESULTS: The improvement of the schizophrenia symptoms measured by PANSS after 6 weeks showed significant differences in the scores of PANSS between the male patient groups treated with olanzapine and risperidone (p-value < 0.001).

CONCLUSION: The group of olanzapine shows a higher improvement of the scores of PANSS than that the group of risperidone.

Introduction

Schizophrenia is a psychotic disorder characterised by a disorder of mind, mood, and behaviour. Schizophrenia accounts for around 1% of the population. This disorder usually starts before the age of 25, survives throughout life, and may affect people from all social classes. Schizophrenia consists of positive symptoms, negative symptoms, cognitive symptoms, aggressive symptoms, and affective symptoms. The positive symptoms include delusions, hallucinations, disorganised speech or behaviour, catatonic behaviour, and agitation. The negative symptoms include blunted or flat affect, emotional withdrawal, poor rapport, indifference, withdrawal from social life, abstract-thought disorder, alogia, avolition, anhedonia, and concentration disorder [1].

Among atypical antipsychotics, risperidone and olanzapine have been shown to reduce both the positive and the negative symptoms significantly of schizophrenia [2], and are also more effective than conventional antipsychotic drugs [3]. Risperidone and olanzapine exploit multiple actions on serotonin and dopamine which resulted in improved adherence by minimising the side effects of extrapyramidal [4] [5].

One way of measuring the positive symptoms, the negative symptoms, and the general symptoms in schizophrenic patients is by using Positive and Negative Syndrome Scale (PANSS). The measurement of the scores of PANSS has been proven to be sensitive and specific about the use of pharmacology from the assessment of the positive symptoms and the negative symptoms in schizophrenic patients [6]. Several studies have been

conducted to compare the effectiveness of olanzapine and risperidone using the scores of PANSS. Conley et al. (2001) found that olanzapine leads to better improvement in both positive and negative symptoms, better overall response rates, and less severe side effects than risperidone. In addition, patients treated with olanzapine appear to maintain a better response than patients treated with risperidone [7]. This contrasts with previous findings by Tran et al. (1997) which showed differences only in the negative scale, in which the group treated with olanzapine had a higher score than the group treated with risperidone. On the other hand, the positive scale and the total score between the two groups were found to be comparable [8]. Similarly, Shah et al. (2011) and Kumar et al. (2016) reported that although the improvement in the negative scale was significantly higher in the group treated with olanzapine, there were no significant differences for the positive scale and the total score of PANSS in both groups [9] [10].

Some factors which might influence different results in previous studies mentioned above are the methods of the patient's treatment (inpatient or outpatient care), the dose of drugs used by the patient during the assessment (flexible dose or fixed dose), and gender of the patient (male or female). To our best knowledge, there is no previous study which limits participants only to patients with a specific gender. Therefore, this study aimed to measure differences in the scores of PANSS on the use of olanzapine and risperidone limited to male with schizophrenia who were hospitalised with a fixed dose. The inpatient method was chosen because this method could easily control the physical activity and lifestyle of the participants so that the results would have a more valid value. The reason for selecting male participants was because male schizophrenic patients tend to have negative symptoms, a worse pre-morbid history, and a greater likelihood of having deficit syndromes and worse prognosis than female schizophrenic patients [11].

Methods

The research design was a quasi-experiment with pre-test and post-test, between the olanzapine group and the risperidone group in the period of June-September 2017. The drop-out criteria used was an intention to treat analysis in which if there was a drop - out subject, the subject would still be included for the analysis in the last week with the data used was the data recorded in the last week before the subject dropped-out [12].

The subject used was determined by non - probability sampling known as the consecutive sampling [13]. Subjects who met the criteria were required to sign an informed consent after being briefed about the study. According to that, this study recruited subjects of 68 male patients with schizophrenia who were hospitalised in the acute phase of treatment at the Mental Hospital Prof. dr. M. Ildrem, North Sumatra, Indonesia. These participants were divided into two groups based on simple randomisation consisting of 34 patients treated with olanzapine treatment and 34 patients treated with risperidone treatment. If the subject encountered the side effects of extrapyramidal symptoms during the treatment, the subject would be given anticholinergic (i.e. triheksilpenidil) with a dose range of 2-5 mg for 2-4 times of administration during 1-2 weeks [14] [15].

The inclusion criteria were including male patients with schizophrenia which corresponded to the Guidelines of Diagnostic Classification for Mental Disorders IIIprevailed in Indonesia with semi-structured interviews mini ICD – 10 [11], in the acute phase of treatment (total score of PANSS 80-150) [16], between the age of 20 and 45 years, duration of illness ≤ 5 years, normal body mass index (BMI = 18.5-24.99), PANSS excited component (PANSS-EC) < 20. The PANSS-EC consisted of 5 items: excitement, tension, hostility, uncooperativeness, and poor impulse control. It was rated from 1 (not present) to 7 (extremely severe) with the score ranged from 5 to 35 [17]. The exclusion criteria consisted of having general medical disorders or other comorbidities, history of substance use (except caffeine and nicotine), and hypersensitivity or unresponsive to olanzapine dan risperidone.

The administration of olanzapine was carried out with an initial dose of 10 mg/day. Then, this dose was increased to 15 mg/day on the 7^{th} day while the dose at night was increased to 20 mg/day on the 14^{th} day. The last dose was used for 6 weeks. The initial dose of risperidone was 2 mg/day; then this dose was increased to 4 mg/day on the 4^{th} day. Finally, the dose was increased to 6 mg/day at the 8^{th} day. The last dose was used in the morning and at night for 6 weeks. The measurement of PANSS began in the first week with the fixed dose. At the end of the research or the 6^{th} week, the total score of PANSS was measured.

The results measured in each symptom were the change in the mean value from the baseline to the end-point in the score of PANSS. Before the intervention, the baseline was assessed through interviews using a questionnaire of PANSS which consisted of 30 items with 3 subscales: 7 items were the positive symptoms scale (P1-P7), 7 items were the negative symptoms scale (N1-N7), and 16 items were general psychopathology symptoms scale (G1-G16). Each item was scored on a 7-point Likert scale ranging from 1 to 7. Thus, the range of the positive and the negative sub-scales were 7 to 49 while the range of the general psychopathology scale was 16 to 112 [18]. The agreement test was obtained with a numerical agreement comparative test (Bland Altman) performed by an independent, professional, and trained psychiatrist. Furthermore, the scores of

PANSS of subjects were evaluated on a weekly basis using the same questionnaire with the previous questionnaire until the endpoint, the 6th week.

The data processing and the statistical analysis were performed with the use of IBM SPSS statistical software version 22. The baseline comparison for the demographic characteristics of the subjects was performed using an independent t-test (normal distribution) or Mann-Whitney U test (non - normal distribution), and Chi-Square test. The mean score in each group before and after the intervention was obtained using paired t-test (normal distribution) or Wilcoxon test (non-normal distribution). The mean score at the end-point of the 6th week was obtained using an independent t-test or Mann-Whitney U test [19].

Results

Table 1 shows the demographic characteristics of the olanzapine group (n = 34) and the risperidone group (n = 34). Most participants were from the primary education level (64.7% for both groups) and unmarried status (76.5% for the olanzapine group and 85.3% for the risperidone group). The mean age of the olanzapine group was 30.29 years (SD = 6.89), and the mean age of the risperidone group was 30.24 years (SD = 6.40).

Table 1: Demographic characteristics of research subjects

Variable	Group of Olanzapine Treatment (n = 34)		Group of Risperidone Treatment (n = 34)		
	Mean	SD	Mean	SD	*p*
Age (years)	30.29	6.89	30.24	6.40	0.971*
Body mass index	22.08	1.45	21.78	1.31	0.386*
Duration of illness (years)	2.67	1.09	2.50	1.08	0.397**
Education level	N	%	N	%	
Primary	22	64.7	22	64.7	1.000**
Middle	12	35.3	12	35.3	
Marriage status					
Married	8	23.5	5	14.7	0.537**
Unmarried	26	76.5	29	85.3	

*Independent T-test, **Chi-square Test.

The mean duration of illness experienced by the subjects was 2.67 years (SD = 1.09) for the olanzapine group and 2.50 years (SD = 1.08) for the risperidone group. As shown in Table 1, the two groups had insignificant differences for all variables indicated by the *p*-value > 0.05. These results indicate that the overall results of this study are unbiased.

Table 2: Baseline of the scores of PANSS (0-week)

PANSS	Group of Olanzapine Treatment (n = 34)		Group of Risperidone Treatment (n = 34)		P*
	Mean	SD	Mean	SD	
Positive scale	32.26	2.16	32.62	2.23	0.510
Negative scale	24.41	2.45	24.35	2.42	0.892
General psychopathology scale	38.65	3.60	37.91	3.46	0.394
Total Score	95.26	4.57	94.94	4.33	0.765

*Independent T-test.

Table 2 shows the baseline for the scores of PANSS at the 0-week. According to the table, there was no significant difference between the two groups.

Table 3: The differences in the PANSS scores before and after 6 weeks of treatment with olanzapine

PANSS	Before treatment		After 6 weeks of treatment		
	Mean	SD	Mean	SD	*P**
Positive scale	32.62	2.23	12.00	1.02	<0.001
Negative scale	24.41	2.45	9.03	1.34	<0.001
General psychopathology scale	37.91	3.46	14.00	1.39	<0.001
Total Score	94.94	4.33	34.97	2.98	<0.001

*Paired T-test.

Table 3 and Table 4 show the mean score of PANSS in the olanzapine group and the risperidone group before and after the treatment. As shown in the table, there was a significant change in the positive scale, the negative scale, the general psychopathology scale, and the total score of PANSS of the subjects before and after the treatment in both groups.

Table 4: The differences in the PANSS scores before and after 6 weeks of treatment with risperidone

PANSS	Before treatment		After 6 weeks of treatment		
	Mean	SD	Mean	SD	*P**
Positive scale	32.26	2.16	16.18	1.40	<0.001
Negative scale	24.35	2.42	12.38	1.30	<0.001
General psychopathology scale	38.65	3.60	19.59	1.39	<0.001
Total Score	95.26	4.57	47.71	2.69	<0.001

*Paired T-test.

Table 5 shows the differences in the score of PANSS after 6 weeks of treatment between the olanzapine group and the risperidone group. According to the table, the mean of the PANSS score in a male with schizophrenia at the end-point of the 6th week for the positive scale in the olanzapine group was 12.00 (SD = 1.01) whereas the risperidone group showed the mean score of 16.18 (SD = 1.40). The mean of the negative scale for the olanzapine group was 9.03 (SD = 1.34) while the mean of the negative scale for the risperidone group was 12.38 (SD = 1.30). The mean of the general psychopathology scale for the olanzapine group was 14.1 (SD = 1.39) while the mean of the general psychopathology scale for the risperidone group was 19.59 (SD = 2.19).

Table 5: The differences in the PANSS scores after 6 weeks of treatment between the olanzapine group and the risperidone group

PANSS	Group of Olanzapine Treatment (n = 34)		Group of Risperidone Treatment (n = 34)		
	Mean	SD	Mean	SD	P
Positive scale	12.00	1.01	16.18	1.40	<0.001
Negative scale	9.03	1.34	12.38	1.30	<0.001
General psychopathology scale	14.00	1.39	19.59	2.19	<0.001
Total Score	34.97	2.98	47.71	2.69	<0.001

*Independent T-test.

Thus, the mean of the total score of PANSS obtained was 34.97 (SD = 2.98) for the olanzapine group and 47.71 (SD = 2.69) for the risperidone group. Based on these results, there was a very

significant difference between the two groups at the end-point of the 6th week (*p*-value < 0.001). The scores of PANSS obtained from olanzapine group showed a higher improvement than that of risperidone group.

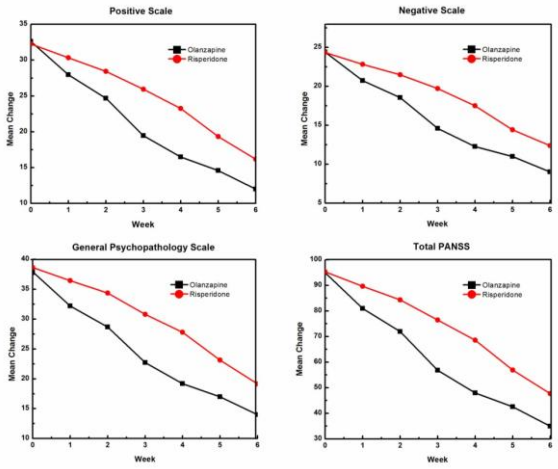

Figure 1: Mean change from baseline in weekly for positive scale, negative scale, general psychopathology scale, and a total score of PANSS of male schizophrenic patients treated with olanzapine or risperidone (p-value < 0.001)

Figure 1 shows the mean change from baseline in weekly scores of PANSS. The decreases in the scores of PANSS from baseline to each visit were significantly greater in the olanzapine group than that in the risperidone group from week 1 through week 6.

Discussion

This study has provided very clear boundaries in recruiting participants as the research subjects. The selected participants were male patients aged 20-45 years. As previously mentioned, the male participants were recruited because male patients tend to have worse symptoms than female patients. The age selection is also because the most frequent onset of schizophrenia is the age of 15-30 years whereas the peak of the attack in men is between the ages of 10-25 years. Also, 90% of patients receiving schizophrenic treatment are between the ages of 15-55 years [1]. Patients under the age of 18 were excluded from this study as a consideration of their long-term safety, including cardiometabolic effects and effects on their growth, maturity, and behavioural development. In addition, geriatric patients were also excluded because these patients experience the decreased function of kidney, liver, and heart, and increased tendency of postural hypotension [20].

As previously explained, the use of risperidone and olanzapine in this study was because both have been shown to significantly reduce the positive symptoms and the negative symptoms of schizophrenia [21]. The determination of dose for both was based on several studies on olanzapine and risperidone in people with schizophrenia. Based on monograph products, the daily dose target for people with schizophrenia is 10 mg for olanzapine and 6 mg for risperidone. However, in clinical practice, the average dose of olanzapine and risperidone is recommended to be higher than the target dose recommended in the monograph [22]. Marder et al. (2017) [23] found that olanzapine was effective if given in the dose of 10-20 mg/day in the acute phase of schizophrenia [23] although a higher dose up to 60 mg/day was reportedly used for people with schizophrenia resistant to treatment. Also, the administration of olanzapine at night can improve general sedation tolerance in early treatment [24]. On the other hand, risperidone has a target dose of 3-6 mg in a day. Risperidone at a dose of 6 mg is as effective as the higher dose, but risperidone at a medium dose (4-6 mg/day) suggests a lower risk of extrapyramidal symptoms (EPS) than the first generation of antipsychotics [21]. Based on the above description, it has been determined that the dose of olanzapine administered was 20 mg/day and the dose of risperidone administered was 6 mg/day because this dose would be well tolerated without adverse events.

The calculation results of the scores of PANSS in this study showed significant differences in the scores of PANSS for the positive scale, the negative scale, general psychopathology scale, and the total score of PANSS in male schizophrenic patients. This is similar to a study conducted by Conley et al. (2001) in Los Angeles which compared the treatments of olanzapine and risperidone to 134 patients who were hospitalised. The patients were treated with risperidone and olanzapine with flexible dose for 8 weeks. In both groups, there was also a significant difference in the scores of PANSS for the positive scale, the negative scale, general psychopathology scale, and the total score of PANSS at the end of the 6th week ($p < 0.01$) [7]. In contrast, a study by Shah et al. (2011) [11] in Nepal found that both groups showed significant improvement at the end of the 6th week. More specifically, the negative scale was higher in the group treated with olanzapine than risperidone while the positive scale and the total score of PANSS had improvement, but there was no significant difference in the two groups ($p = 0.498$) [8]. This difference was because Shah et al. (2011) used participants with the outpatient method, so the physical activity and the dose of drugs could not be fully controlled while the study by Conley et al. (2001) used participants who were hospitalised. Nevertheless, Tran et al. (1997) [9] and Kumar et al. (2016) [10] who recruited participants who were hospitalised also found similar results to the results of Shah et al. (2011) [8].

The results of this study were not biased because the comparative test results between the variables on the demographic characteristics indicated that there was no significant difference in terms of age, education level, marital status, duration of illness, body mass index, positive scale, negative scale, general psychopathology scale, and total score of PANSS in both groups.

The limitations of this study were that this study did not analyse changes in people's lifestyle and other behaviour patterns, such as smoking.

In conclusion, the results of this study showed that there were significant differences in the scores of PANSS including the positive scale, the negative scale, general psychopathology scale, and the total score of PANSS in male schizophrenic patients between the group of olanzapine treatment with the fixed dose of 20 mg/day and the group of risperidone treatment with the fixed dose of 6 mg/day at the end of the 6^{th} week with the *p*-value < 0.001. This is in accordance with the results obtained by Conley et al. (2001) who studied men and women as subjects using a flexible dose. Based on these different scores, it can also be concluded that olanzapine provides a higher improvement in the positive symptoms and the negative symptoms than risperidone.

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Correlation between Lymphocyte CD4 Count, Treatment Duration, Opportunistic Infection and Cognitive Function in Human Immunodeficiency Virus-Acquired Immunodeficiency Syndrome (HIV-AIDS) Patients

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Keywords: CD4; HIV – AIDS; Cognitive function

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Abstract

BACKGROUND: Human immunodeficiency virus (HIV) infection is an epidemic worldwide, despite the marked benefits of antiretroviral therapy (ARV) in reducing severe HIV-associated dementia. A milder form of neurocognitive disorders are still prevalent and remain a challenge.

AIM: This study aimed to determine the correlation between plasma cluster of differentiation 4 (CD4) lymphocyte, duration of ARV treatment, opportunistic infections, and cognitive function in HIV-AIDS patients.

METHODS: A cross-sectional study involving 85 HIV-AIDS patients was conducted at Adam Malik General Hospital Medan, Indonesia. All subjects were subjected to physical, neurologic examination and Montreal Cognitive Assessment-Indonesian Version (MoCA-INA) to assess cognitive function and measurement of lymphocyte CD4 counts.

RESULTS: Out of the 85 subjects evaluated, the proportion concerning sexes include 52 males (61.2 %) and 33 females (38.8%). The mean age was 38.53 ± 9.77 years old. There was a significant correlation between CD4 lymphocyte counts and MoCA-INA score ($r = 0.271$, $p = 0.012$), but there was no significant correlation between duration of ARV treatment and MoCA-INA score. There was also no difference in MoCA-INA score based on the presence of opportunistic infection.

CONCLUSION: Lymphocyte CD4 count was independently correlated with cognitive function in HIV-AIDS patients.

Introduction

Human immunodeficiency virus (HIV) infection is a global epidemic. According to estimates from the 2017 UNAIDS report on the global Acquired Immunodeficiency Syndrome (AIDS) epidemic, there were approximately 36.7 million people living with HIV worldwide [1]. There were 15,534 people with HIV infection and 1700 AIDS cases at the end of 2014 in Indonesia, with a cumulative number from April 1987 to 2014 stands at 142,950 HIV cases and 55,623 AIDS cases. The cumulative number of HIV-AIDS cases in Sumatera Utara province ranked tenth in Indonesia, with 8,794 HIV cases and 1,573 AIDS cases at the end of 2014, with a prevalence of 12.12 per 100.000 population [2].

Neurologic disorders in HIV infection can be divided into primary and secondary disorders. The secondary disorders are caused by opportunistic infections resulting from immunosuppression and have become less frequently found since the establishment of antiretroviral (ARV) treatment. The primary disorders, including HIV-associated neurocognitive disorders (HAND) representing the deleterious effects of HIV on brain [3] [4] [5] There are three severity categories for HAND: HIV-associated asymptomatic neurocognitive impairment (ANI), HIV - associated mild neurocognitive disorder (MND), and HIV-associated dementia (HAD). Although the use of ARV has made severe forms of cognitive impairment less common, mild to moderate degrees of cognitive disturbances have become more prevalent and remain a challenge [6] [7] [8]. ARV has improved survival substantially and may also reverse neurocognitive deficits in many cases. The impact of recognising ANI in clinical practice and research would be to encourage more frequent neuropsychologic assessment for individuals with ANI,

to detect early cognitive changes, functional impairments and potential transition to MND or HAD. It might also promote the initiation of ARV independent of CD4 count or plasma HIV RNA levels, especially in the developing countries where measurement of plasma viral load are not readily available, or in a limited resource setting [8].

This study aimed to determine the correlation between plasma CD4 lymphocyte count, ARV duration, opportunistic infection and cognitive function in HIV-AIDS patients.

Methods

This was a cross-sectional study conducted in Adam Malik General Hospital Medan North Sumatera Indonesia, between June and November 2017. HIV - AIDS patients 18 yrs and above were included in this study, with the following profiles: compos mentis, speak Bahasa Indonesia fluently, ability to read and write, and gave written consent. We excluded patients who had psychiatric disorders, aphasia, dementia, traumatic brain injury and central nervous system opportunistic infection. The evaluation encompassed standard physical and neurologic examination, Montreal Cognitive Assessment-Indonesian Version (MoCA-INA) and CD4 lymphocyte measurement.

All statistical procedures were performed with SPSS version 20. We used Pearson's or Spearman correlation test to evaluate the correlation between CD4 count, age and cognitive function (MoCA-INA score), ANOVA or Kruskal Wallis to evaluate the difference on MoCA INA score based on level of education and t-test or Mann Whitney to evaluate the differences of CD4 count and ARV duration based on MoCA-INA score.

The study was performed with approval obtained from the Health Research Ethical Committee Medical Faculty of Universitas Sumatera Utara/H. Adam Malik General Hospital with number 325/TGL/KEPK FK USU-RSUP HAM/2017.

Results

Out of the 85 subjects evaluated, the proportion concerning sexes includes 52 males (61.2 %) and 33 females (38.8%). The mean age was 38.53 ± 9.77 years old, ranging from 19 to 65 years old. The mean MoCA INA score was 22.94 ± 3.38, ranging from 15 to 28. The mean CD4 counts were 432.58 ± 332.24 cells/μL. Table 1 shows characteristics of subjects.

Table 1: Characteristics of Subjects

Characteristic	Number (n = 85)	Percentage
Age (years), mean ± SD	38.53 ± 9.77	
Sex		
Male	52	61.2
Female	33	38.8
Level of education		
Elementary	7	8.2
Junior High School	43	50.6
High School	25	29.4
College/University	10	11.8
Occupation		
Entrepreneur	37	43.5
Employee	5	5.9
Housewife	23	27.1
Farmer	9	10.6
Student	4	4.7
Unemployed	7	8.2
Ethnic		
Batak	38	44.7
Melayu	6	7.1
Javanese	18	21.2
Tionghoa	6	7.1
Karo	17	20.0
MoCA-INA Score		
≥ 26	21	24.7
< 26	64	75.3
Opportunistic Infection		
Yes	60	70.6
No	25	29.4
ARV duration (months), mean±SD,	37.5 ± 25.91	
MoCA-INA Score, mean± SD	22.94 ± 3.38	
Absolute CD4 count (cell/μL), mean ± SD	432.58 ± 332.24	
CD4 percentage (%), mean±SD	18.80 ± 9.93	

There was no significant difference on MoCA-INA score based on the level of education (Table 2). There was also no significant correlation between age and MoCA-INA score (r = 0.034, p = 0.76).

Table 2: Comparison of MoCA-INA Scores based on the level of education

Level of education	Mean ± SD	p
Elementary school	23.14 ± 3.80	0.849*
Junior high school	22.74 ± 3.37	
High school	23.40 ± 3.58	
College	22.50 ± 2.95	

SD = standar deviation; *Kruskal-Wallis Test.

The absolute lymphocyte CD4 count and lymphocyte CD4 percentage were significantly different based on MoCA INA score, but there was no significant difference in ARV duration based on MoCA-INA score.

Table 3: Differences in the CD4 absolute count, CD 4 percentage and ARV duration based on MoCA-INA Score

MoCA-INA Score	Lymphocyte CD4 absolute count (cell/μL)		Lymphocyte CD4 percentage (%)		ARV Duration (months)	
	Mean ± SD	p	Mean ± SD	p	Mean ± SD	p
Normal (n = 21)	591.35 ± 369.33	0.003	22.94 ± 9.35	0.010	45.12 ± 38.54	0.914
Abnormal (n = 64)	283.00 ± 258.18		14.83 ± 9.29		38.39 ± 24.3	

Mann Whitney.

There was a significant correlation between absolute CD4 lymphocyte count and MoCA-INA score (r = 0.271, p = 0.015) and between percentage CD4 count and MoCA-INA Score (r = 0.227, p = 0.037). Figure 1 shows the MoCA-INA Score plotted against absolute CD4 lymphocyte counts. The normal MoCA INA score should be above or equal to 26. In this study, most of the subjects had scored lower than 26 (n = 64), and from that 64 subjects, most of them had scored between 21-25, but the overall mean score was 22.94 ± 3.38.

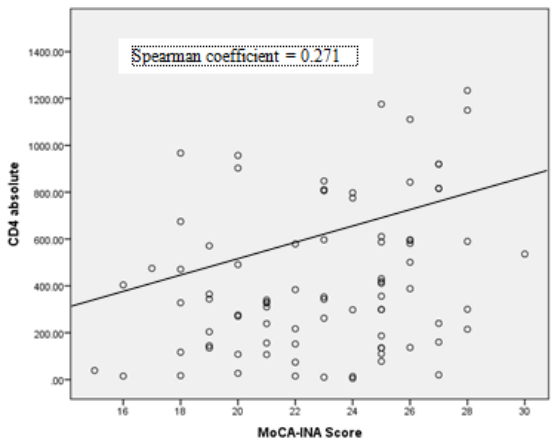

Figure 1: MoCA-INA Score plotted against CD4 lymphocyte counts

The duration of ARV treatment was 37.5 ± 25.91 months. There was no significant correlation between duration of ARV treatment with MoCA-INA score (r = 0.091, p = 0.407). Most of the subjects (60 patients, 70.6%) had an opportunistic infection which was lung tuberculosis. There was no significant difference on MoCA-INA score based on the presence of opportunistic infection (Table 4).

Table 4: Comparison of MoCA-INA Scores based on the presence of opportunistic infection

Opportunistic Infection	Mean ± SD	p
Yes	23.02 ± 3.29	0.625
No	22.76 ± 3.64	

Mann Whitney Test.

Discussion

Our data show that there was a significant correlation between CD4 lymphocyte count with cognitive function in HIV-AIDS patients as measured by MoCA-INA score. This correlation was independent of age and level of education. This is in line with several previous studies [9] [10] [11] [12]. Valcour et al. (2006) [9] found that after adjusting for age and duration of HIV, lowest historical CD4 was related to a current diagnosis of HAD (odds ratio 1.395 (1.106-1.761) Patients with CD4 lymphocyte below 200 cells/mm^3 were considered highly vulnerable to neurological complications associated with infection, including cognitive impairment and the risk increased with further reductions in CD4 lymphocyte count [9]. Ellis et al. (2011) [10] found that lowest historical CD4 was a predictor of HIV neurocognitive impairment in the era of a combination of antiretroviral therapy [10]. A study by Childs et al. (1999) [11] found that levels of plasma CD4 counts and HIV RNA were predictive of HAD and it develops in parallel with immunodeficiency and with more advanced stages of HIV [11]. This finding could partially reflect the effect of marked immunodeficiency in HIV patients to cognitive function. The correlation between CD4 count and cognitive function was also independent of the duration of ARV treatment and the presence of opportunistic infection. A study by Reger et al. (2005) [12]. Suggested that plasma HIV RNA levels may not predict the degree of neurobehavioral disturbances in HIV infection among patients receiving ARV treatment [12]. HIV causes damage in the central nervous system through two separate but linked processes: the first is a host-derived cascade of inflammatory molecules released by activated or infected microglia, macrophages and astrocytes and the second process involves the direct neurotoxic effects of HIV-encoded proteins such as gp120, Vpr, and Tat, which also modulate transcription of HIV-1 long terminal repeat (LTR) promoter activity and affects several cellular functions [5]. The neuronal damage that occurs is likely caused by shedding viral proteins such as gp120 and Tat or indirectly through the elevated production of neurotoxic molecules released by activated astrocytes macrophages and microglia [6]. Although neurons are not directly infected by HIV, secondary neuronal damage caused by other infected cells is probably required to cause cognitive impairment [4]. HIV infection causes damage to the elaborate network of connections between neurons that take place at dendrites and synapses. This synaptodendritic injury disrupts the highly integrated functioning of neural systems that are required to process information, leading to HIV -associated neurocognitive disorders (HAND), which consist of asymptomatic neurocognitive impairment (ANI), HIV-associated mild neurocognitive disorder (MND), and HIV-associated dementia (HAD) [8]. ANI describes individuals with usually mild impairment in two or more cognitive areas, demonstrated by neuropsychological testing, without a clear effect on everyday functioning. MND refers to the presence of mild to moderate deficits in two or more cognitive areas which create at least mild interference in everyday functioning. Finally, HAD describes individuals with documented moderate to severe deficits in two or more cognitive areas, with substantial impairment in everyday functioning making the person incapable of employment and often unable to live independently [6] [8].

Our study did not classify the cognitive impairment further based on the cognitive domains. MoCA-INA was only used to assess cognitive function. It can assess several domains, including executive function, visuospatial function, attention and concentration, memory, language, calculation and orientation [13]. The Indonesian version of MoCA, namely MoCA-INA has been developed and validated in Indonesia, and so it can be used as a cognitive tool [14]. HIV infection characteristically generates a “sub-cortical” pattern of neurocognitive dysfunction with deficits predominantly affecting executive functions, speed of information processing, attention/working memory, motor speed, new learning and retrieval of

new information, while long-term (semantic) memory, many language skills, and visuo -spatial abilities may remain intact [6]. The pattern of neurocognitive dysfunction, however, is not consistent across individuals and may be even less consistent across individuals from markedly different backgrounds [5]. MND is often an antecedent syndrome that can precede the onset of frank dementia but presents with the clinical hallmarks of HAD albeit with less-severe signs and symptoms and at higher CD4þ T-cell levels. Symptoms of HAD typically begin once an individual's lymphocyte CD4 count drops below 200 cells/mL. Clinical risk factors for HAD include low CD4 T-cell levels, high viral load in cerebrospinal fluid (CSF) or plasma, anaemia, extremes of age, injection drug use, and several host genetic polymorphisms [6]. This study did not analyse the factors that could associate with CD4 counts. A study by Li et al (20110 [15] found several important modifiable risk factors were associated with lower CD4 counts, including older age (> 50 years), lower CD4 counts at the time of HAART initiation, the number of switches in HAART regimen in the first 5 years and hepatitis B and C virus infection [15].

An interesting finding from this study was the fact that the number of patients with abnormal cognitive function (MoCA-INA score < 26) was higher than the number of patients with normal cognitive function (MoCA-INA score ≥ 26), although most of the patients did not have any subjective cognitive complaint. Although this finding could be attributed to the fact that most of the patients had a level of education lower than 12 years, there were no significant differences in MoCA-INA score based on the level of education, also there was no significant correlation between age and cognitive function. This could emphasise the importance of cognitive assessment in HIV-AIDS patients to evaluate HIV effects on the CNS, to detect early cognitive changes, functional impairments and potential transition to MND or HAD.

This study concludes that CD4 lymphocyte count correlates with cognitive function in HIV-AIDS patients. This finding could reflect that immune status as marked partly by CD4 count might contribute to the development of CNS complications, including cognitive dysfunction.

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Clinical Science

"Bladder Effect" - An Urodynamic Parameter to Distinguish Subtypes of Urinary Incontinence in Women

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Abstract

BACKGROUND: Urinary incontinence (UI) is defined by the International Continence Society (ICS) as the involuntary loss of urine that represents a hygienic or social problem to the individual. The aetiology is multifactorial. The diagnosis of UI is important because it can result in the application of appropriate therapy. Urodynamics is a golden standard, without which every UI diagnosis is insufficient.

AIM: The goal of this study was, based on urodynamic results, to prove the existence of evident differences between the subtypes of UI.

METHODS: Eighty patients with UI were evaluated (50 with urinary stress incontinence-USI and 30 with detrusor instability-DI) according to a standard evaluation protocol. Exclusion criteria were: mixed UI and diseases that simulated UI. All patients were 36-65 years of age (mean 56). The following parameters were measured: maximal and average flow, maximal and average voiding pressure. These parameters were compared between both groups, to determine the diagnostic significance of the parameter "Bladder Effect" (BE). It is a product of the urine flow and the pressure during voiding.

RESULTS: The results showed a significant difference with a high confidence interval. Mean BEmax was 577 units in the patient group with USI, and 1014 in the DI group. Similarly, BEav was 313 units in the USI group, and 499 units in the DI group, with a significant difference and a high interval of confidence.

CONCLUSION: In conclusion, the results of the study suggested that BE could be a useful diagnostic parameter to distinguish between USI and DI.

Introduction

Urinary incontinence (UI) is defined by the International Continence Society (ICS) as the involuntary loss of urine that represents a hygienic or social problem to the individual [1]. Urinary incontinence can be checked as one of the patient's symptoms, a sign which can objectively be demonstrated as a disorder. There is no specific aetiology for the appearance of this phenomenon. Most of the individual cases verify multifactorial nature of this entity. The aetiology of UI is still not completely understood. Each patient suffering from urinary incontinence must go through basic clinical evaluation which includes: anamnesis, gynaecological status and urine analysis. Additional investigations such as voiding chart, pad-test, Marshall stress test, and measurement of residual urine, cystoscopy, urodynamics and methods of visualisation (ultrasonic or radiologic) can be performed to help define the type of UI. Video urodynamic studies are reserved for complex cases of urinary stress incontinence (USI). They combine radiologic results and multichannel urodynamics.

Of great interest are the two entities of transurethral urinary incontinence: Urinary stress incontinence (USI) associated with involuntary leakage of urine when the intra-abdominal pressure is suddenly increased, and Urgent (imperative) urinary incontinence (UUI) associated with the detrusor instability (DI). Some authors mention mixed urinary incontinence as a separate entity, but it is the same combination of USI and UUI. The success of the

applied therapy practically depends on the kind of therapeutic approach, which needs to be adequate for each UI, since different type of UI requires a different type of therapeutic approach.

Urinary incontinence, or involuntary micturition, is another disorder or deviation of the physiology of voiding [2]. The art of diagnosing a UI is to determine at what level a disorder of the physiology of voiding has occurred. This leads to proper therapy, and if adequate, it is indeed successful. The prognosis of UI is quite good in more advanced healthcare systems.

The term urodynamics dates from 1954, when David Davis used it while presenting his work in measuring the pressure of the upper urinary tract and renal injuries [3]. Not long after, Hodgkinson [4] [5] declared the urodynamic standards and differentiated USI from an unstable detrusor. Since then, our understanding of the urinary tract, the equipment used, even the width and definition of urodynamics has greatly been expanded. Today, the term "urodynamics" refers to a group of tests used to gather information about the function of the lower urinary tract, as well as the time needed for the bladder to be filled and emptied [6]. Urodynamics is used as a golden standard in the detection of a precise cause for occurrence of UI, in the absence of which, each diagnosis would be insufficient. It has been discovered that the symptoms of USI tend to have a weak correlation with specific urodynamic findings and a strong correlation with the unstable detrusor, which makes the urodynamic evaluation a superb diagnostic tool for identification of the signs of UI. Urodynamic studies in patients suffering from UI enable acquisition of a large amount of data of the lower urinary tract function. They can guide us toward determination of the adequate treatment. They could also point to the possible reasons of the previous treatment failure or help in foretelling the outcome of the recommended treatment [7].

The ICS recommends the urodynamic studies to be carried out in the investigation of UI in women. The urodynamic evaluation, as a necessary step, should include bladder pressure tests (cystometry), flow measurement of the urine (flowmetry), urethral pressure profile, and pressure measurement during micturition (voiding cystometry). The part of urodynamics that analyses the evacuation of the urine from the bladder is known as voiding cystometry. Voiding cystometry can objectively be achieved when the measurements of voiding parameters are taken with the bladder at its full capacity. Western authors interpret this analysis as pressure/flow studies.

During this examination, pressures are measured during voiding. Micturition is examined with the same catheters used to fill the bladder. This phase helps in grading two critical parameters related to bladder voiding: detrusor contractility (normal versus damaged) and urinary flow (uninterrupted versus interrupted) [8].

The most widespread use of voiding cystometry is determining the presence of a urethral obstruction, commonly found in men. With the beginning of 1960 [9], nomograms were developed to standardise the definitions of obstruction and contractility of the bladder [2] [10] [11]. These nomograms are well established and widely accepted in diagnosing urinary obstruction in benign hyperplasia of the prostate in men. However, the nomograms did not achieve the expected results in women, and as such, never gained widespread use in clinical practice [8] [12] [13] [14] [15].

The goal of this study was to identify, based on urodynamic measurements, differences between subtypes of UI. The fact that part of the UI results (i.e. voiding cystometry) provide an abundance of unclassified information was a motive to find a correlation between them. It was perceived that particular segments of the findings, which were routinely done in UI patient investigation, could increase their capacity and their usage, especially the parameter Pressure x Flow = BE (bladder effect) and to assess its diagnostic value.

Material and Methods

This study included a total of 80 female patients, divided into two groups: a group of 50 patients with USI and a group of 30 patients with DI.

Inclusion criteria of the study were: female individuals with diagnosed USI (according to the UI investigation protocol); female individuals with diagnosed DI (according to the UI investigation protocol).

Exclusion criteria were the presence of urinary tract infection; diabetes; conditions that lead to polyuria; neurologic disease (cerebrovascular insult, spinal cord injury, multiple sclerosis, peripheral neuropathy); urogenital fistula; and other states that could influence voiding and its parameters (i.e. urolithiasis).

All patients were between 36-65 years of age, with an average age of 56 years for both groups. All of them underwent the standard diagnostic procedure during their examination, according to the UI investigation protocol. Each patient went through: an interview, examination of the urine sediment and urine culture, urogynecology exam, USI verifying tests and urodynamic tests which included: urethral pressure profile (UPP), cystometry, flowmetry and voiding cystometry.

In order to execute voiding cystometry (after measuring the UPP, flowmetry and cystometry) the patients were placed on a voiding collector in a sitting

position, with a pressure measuring catheter in the bladder. Then the patient was asked to void the previously filled fluid in the bladder to its maximum capacity. During the act of voiding, urine flow and pressure parameters were noted. From the flow curve, using interpolation, the pressure was plotted on the pressure curve at any moment of interest during the voiding. All patients signed informed consent, and the study was approved by the Ethics committee.

The average and maximum flows were measured (which the urodynamic system presented automatically) and according to them, the pressure during maximal voiding flow (calculated by interpolating it at the moment of maximal flow), as well as the average voiding pressure (the average of maximal and minimal voiding pressures). These parameters were compared between both groups, to determine the diagnostic significance of their nominal values. Special attention was given to the BE parameter, which was a product of urine flow and pressure during voiding. The data were processed using statistical methods and procedures for comparing numeric values. (The SPSS Statistics package version 23).

Results

The obtained results of each patient for the maximal and average "bladder effect" (BEmax and BEav) were the product of maximal urine flow (Fmax) and the pressure at that time point (Ppf) and the average urine flow (Fav) with the average voiding pressure (Pav) respectively, according to the formula BEmax=Ppf x Fmax and BEav=Pav x Fav.

Table 1: BEmax values in USI and DI

	N	Mean ± Std. Deviation	p-value
BEmax DI	30	1014.04 ± 673.34	P < 0.0001
BEmax USI	50	576.80 ± 174.73	

BEmax-maximal bladder effect; USI – urinary stress incontinence; DI-detrusor instability.

The mean values of the parameters above, for the two different patient groups, were presented in Table 1 and Table 2.

Table 2 – BEav values in USI and DI

	N	Mean ± Std. Deviation	p-value
BEav DI	30	498.94 ± 333.47	P < 0.0001
BEav USI	50	313.27 ± 118.16	

BEav– average bladder effect; USI – urinary stress incontinence; DI-detrusor instability.

The results presented in Table 1 and Table 2 indicated a significant difference between the USI and DI groups. The mean value for the BEmax parameter in the USI group was 577 units, and for the DI group, it was 1014 units. Statistical analysis showed that the difference was significant, with a high interval of confidence. The values of the BE parameter (also in a variant of maximal values and average values), were lower in the USI group compared to the DI group.

Both groups showed a statistically significant difference of the average values of both parameters, with $p < 0.001$ and a high 95% Confidence interval.

Discussion

Voiding cystometry is a rich source of bladder function data, which is processed by a wide group of medical and scientific staff, but until today without visible, tangible evidence that can be transformed into parameters that could distinguish UI subtypes or indicate prognosis or success in therapeutical procedures. Given how patients are closed about their difficulties, urodynamics check-ups are valuable during female patient screening, especially in patients that hide their symptoms, have masked UI or simulate certain symptoms in order to receive some formerly suggested treatment, which they perceive as the solution to their problem.

Several pressures and flows exist in voiding cystometry, and statistical analysis by many authors regarding these values was performed in the past. Several authors found significant differences between certain pressures or flows or both, while others disputed those findings. The situation is similar in estimating the correlation between pressures and flows at a certain time during voiding. Due to the diversity that emerged near the end of the 20th century, a fundamental analysis of all the data was made, which showed that the significant parts of the studies were mostly based on a subjective estimate of the results. Because of that, in 1998 the International Continence Society (ICS) recommended ending the pressure-flow studies with the goal of receiving a diagnostic or scientific interpretation of incontinence. About 20 years later, the observations that DI has a larger flow compared to USI and voiding pressures are larger in DI compared to USI, still, pertain. Although this cannot be correlated to something of significance, the opinion that voiding cystometry can be a good diagnostic tool persists. The analysis of pressure and urine flow at the same time, as their mathematical product, leads to a mathematical result which shows the effect of voiding urine, out of the bladder, in the physical sense. Hence, the bladder effect parameter imposed itself as the imperative in studying and its possible use in diagnosing UI subtypes.

The study showed a difference in the values of the given parameters in each group. The statistical sample was small, and it could be the reason for the

high standard deviation. It deviated by 30.3 % from the mean value in the USI group, and 62.8 % in the DI group for the BEmax values, and 37.7 % from the mean in the USI group and 66.8 % in the DI group for the BEav values. Despite this, the unpaired t-test analysis showed a significant difference. This is of great importance due to the partial overlapping of the curves of distribution between the two groups, thanks to the standard deviation, especially in the DI examined group. It is unclear how the BE values would change in patients with mixed UI (USI + DI), i.e. whether they would incline towards a certain group, or remain somewhere in between, and given that, become unable to be classified in either group. The lack of research by which this parameter could be compared complicates analysis further. Most of the published papers covering this area included men and the closest parameter to the BE would be the contraction index parameter, which was based on a formula for voiding flow [10].

Based on the results of this study, we believe that the BE parameter is a new promising tool for differentiation of the subtypes of UI, not previously described by other authors. Statistical analysis showed that this parameter is worth exploring further, on a larger female patient population.

In conclusion: 1) the BEmax parameter in USI patients was significantly lower about DI patients; 2) the BEav parameter in USI patients was significantly lower when compared to DI patients, and the results of our study suggested that BE could become a valuable diagnostic parameter to distinguish USI from DI.

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Clinical Science

Latent Tuberculosis Infection - Diagnosis and Treatment

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Abstract

INTRODUCTION: Latent tuberculosis infection (LTBI) is defined as a state of persistent immune response to stimulation by Mycobacterium tuberculosis antigens without evidence of clinically manifested active tuberculosis (TB). Diagnosis and treatment for LTBI are important for TB, especially in high-risk populations. Tuberculin skin test (TST) and interferon-gamma release assays (IGRAs) are used to diagnose LTBI.

AIM: The study aims to present the first results with IGRA test compared with TST in the screening of LTBI and the treatment results in the cases with LTBI in Macedonia.

MATERIAL AND METHODS: In this study 73 cases diagnosed and treated with LTBI in 2016 were included. For diagnosis of LTBI, we used TST RT -23 5T.U. and commercial IGRA test such as QuantiFERON-TB Gold In-Tube (QFT-IT).

RESULTS: Out of 73 cases with LTBI, 61.64% were men, and 38.36% were women. Among all age groups, the most frequent were cases between 5 and 14 years old (54.79%). Among the evaluated risk groups for LTBI, the most frequent were children household contacts with pulmonary TB cases (61-83.65%), followed by people living with HIV (9-12.33%) and only 3 cases with other medical reasons. Positive TST had 34 cases (46.57%) and positive IGRA test 25 cases (34.25%). Regarding the treatment regimes, we use two regimes: 50 cases (68.44%) received 6 months daily regime with Isoniazid, and 23 cases (31.51%) received 3 months daily regime with Isoniazid and Rifampicin. Treatment outcomes showed that the most patients completed treatment regimes: 55 (75.34%) and only 10 (13.09%) interrupted the treatment.

CONCLUSION: Despite the progress made in the last few years, several challenges remain to be addressed for better management of LTBI which will contribute to strength TB control in the country.

Introduction

Tuberculosis is one of the most prevalent infections of human beings and a formidable public health challenge that shows little sign of abating [1]. Primary infection with *M. tuberculosis* leads to clinical disease in only ~ 10% of individuals. In the remaining cases, the ensuing immune response arrests the further growth of *M. tuberculosis*. However, the pathogen is completely eradicated in only ~ 10% people, while the immune response in the remaining ~ 90% individuals only succeeds in the containment of infection as some bacilli escape killing by blunting the microbicide mechanisms of immune cells and remain in no replicating (dormant or latent) state in old lesions. The process is termed as latent tuberculosis infection (LTBI) and is defined as a state of persistent immune response to stimulation by Mycobacterium tuberculosis antigens without evidence of clinically manifested active TB [2].

Persons with LTBI do not have active tuberculosis and do not feel sick but may develop it in the near or remote future, a process called TB reactivation [3]. The lifetime risk of reactivation TB for a person with documented LTBI is estimated to be 5 - 10%, with the majority developing TB disease within the first five years after initial infection and lifetime risk, is ~ 50% in HIV coinfected individuals [4][5]. However, the risk of developing TB disease following infection depends on several factors, the most important one being the immunological status of the

host. Finding LTBI provides an opportunity to treat and prevent reactivation of the latent infection that leads to active disease, especially in people with compromised immune systems.

Systematic diagnosis and treatment of LTBI is part of the new End TB strategy by World Health Organization (WHO) and achieving ≥ 90% LTBI treatment coverage among people living with HIV (PLHIV), and child contacts of TB cases are one of the global priority targets [6].

Available tests to demonstrate prior tuberculosis infection include the tuberculin skin test (TST) and interferon-gamma release assays (IGRAs) [7].

In the Republic of Macedonia, the systematic screening for LTBI and treating people who have risk factors for developing active TB is part of the National tuberculosis program (NTP). The Macedonian national guideline for LTBI updated in 2016, recommends three main groups with risk factors for management of LTBI: children as household contacts of pulmonary TB cases, PLHIV and patients initiating anti-TNF treatment or receiving immunosuppressive therapy.

The study aims to present our first results with IGRA test compared with TST in the screening of LTBI and the treatment results in the cases with LTBI.

Material and Methods

A retrospective cohort study was undertaken, based on data systematically collected on all persons undergoing the TST-based and the IGRA-based LTBI screening programme and treatment programme during 2016. All data were obtained from the Central unit for registration of TB and LTBI in the Republic of Macedonia, at the Institute for Lung Diseases and TB. In this study 73 cases diagnosed and treated with LTBI were included. For diagnosis of LTBI, we used TST RT-23 5T.U. and induration ≥ 5 mm was considered as positive results. The other test was commercial IGRA such as QuantiFERON-TB Gold In-Tube (QFT-IT), and it was performed by measuring interferon-ɣ (IFN-ɣ) with Enzyme-Linked Immunosorbent Assay (ELISA). In the most cases, diagnosis of LTBI was obtained with both tests: with the exception that TST and IGRA test was not performed in 11 (15.07%) and 36 (49.1%) cases respectively. According to our national guidelines for treatment of LTBI, there are two used therapeutic regimes: the daily regime of Isoniazid (H) for 6 months or daily regime of Isoniazid and Rifampicin (R) for 3 months. All cases with LTBI were followed during the treatment period, and the treatment outcomes were presented.

The differences of category variables were present with the distribution of the frequencies. For comparison of the differences of the data Fischer-exact test was used. A statistical difference was considered to be significant when the p-value was < 0.05.

Results

Of 73 cases with LTBI, 61.64% were men, and 38.36% were women. According to the age, 61 (83.56%) were children, and 12 (16.43) were adults. Among all age groups, the most frequent were cases between 5and 14 years old (54.79%), Table 1.

Table 1: Distribution of cases with LTBI according to the gender and age groups

Gender	n (%)
Men	45 (61.64)
Women	28 (38.36)
Total	73 (100.00)
Age groups	n (%)
0 – 4	21 (28.77)
5 – 14	40 (54.79)
15 – 24	2 (2.74)
25 – 34	4 (5.48)
35 – 44	0 (0,%)
45 – 54	5 (6.85)
55 – 64	1 (1.37)
Total	73 (100.00)

In this study three groups of cases with risk factors were screened for LTBI and the most frequent were children household contacts with pulmonary TB cases (61-83.65%), followed by PLHIV (9-12.33%) and only 3 cases with other medical reasons: 1 case that initiated anti TNF therapy and 2 that received immunosuppressive therapy, named as others (Table 2). According to the data from our NTP, in 2016 among 360 TB contacts examined, 15 were TB cases (4.16%), and 61 were with LTBI (16.9%).

Table 2: Different groups with risk factors for the screening of LTBI

Risk groups	n (%)
Contact with TB cases	61 (83.56)
PLHIV	9 (12.33)
Others	3 (4.11)
Total	73 (100.00)

In Table 3 and Table 4 the results obtain with TST, and IGRA test was separately presented. In Table 5 the combination of both tests was presented, and it is obvious that in all cases included in the study one or both tests were performed.

Table 3: Results of TST at the time of screening for LTBI

TST test	n (%)
Negative	28 (38.36)
Positive :5 – 14mm	11 (15.07)
Positive :15 – 20mm	18 (24.66)
Positive : 20 – 34mm	5 (6.85)
Not done	11 (15.07)
Total	73 (100.00)

Even we used to perform both tests in each case; there were 11 cases without TST and 36 without IGRA test. TST confirmed 34 positive cases (46.57%), IGRA test 25 positive cases (34.25%) and both tests were positive in 11 cases (15.06%) and both negative in 6 cases (8.21%).

Table 4: Results of IGRA test at the time of screening of LTBI

IGRA test	n (%)
Negative	12 (16.44)
Positive	25 (34.25)
Not done	36 (49.31)
Total	73 (100.00)

There were 17 cases in which only TST was performed and 1case in which only IGRA was performed, and the results were negative. Those 18 and those 6 cases were both tests confirmed to be negative, were without laboratory confirmation for LTBI (Total 24).

Table 5: Combination of TST and IGRA

Test	IGRA pos	IGRA neg	IGRA not done	Total
TST pos	10	5	19	34
TST neg	5	6	17	28
TST not done	10	1	/	11
Total	25	12	36	73

In Macedonia, there is obligatory Bacillus Calmette–Guerin (BCG) vaccine for all newborn. Besides this, the results showed that nearly one-third of evaluated cases were with the negative status of BCG vaccination (Table 6).

Table 6: BCG status of cases with LTBI

BCG status	n (%)
Positive	51 (69.86)
Negative	21 (28.77)
Unknown	1 (1.37)
Total	73 (100.00)

Regarding different treatment regime for LTBI, 50 cases received daily regime with H for 6 months and 23 cases received daily regime of H/R for 3 months (Table 7).

Table 7: Different treatment regime for LTBI

Treatment regimes	n (%)
6 months Isoniazid	50 (68.49)
3 months Isoniazid and Rifampicin	23 (31.51)
Total	73 (100.00)

Treatment outcomes showed that 75.34% of treated cases with LTBI completed treatment, and only 13.69% did not finish the whole regime (table 8).

Table 8: Treatment outcomes in 73 cases with LTBI

Treatment outcomes	n (%)
Completed treatment	55 (75.34)
Interrupted treatment	10 (13.69)
Interrupted treatment due to negative control TST	8 (10.96)
Total	73 (100.00)

In an aim to evaluate if there were some factors that influenced the treatment outcome, the correlation test was performed between the gender, age groups, different risk groups for detecting LTBI, different therapeutic regimes and the treatment outcomes, and we did not find any statistically significant correlation (Table 9).

Table 9: Correlation of gender, age groups, risk groups and treatment regimes of cases with LTBI and treatment outcomes

		Treatment outcome		
	N	Interrupted	Completed	Interrupted due to negative control TST
Gender				
Men	45 (61.64)	7 (70)	33 (60)	5 (62.5)
Women	28 (38.36)	3 (30)	22 (40)	3 (37.5)
	Fisher exact, two-tailed p = 0.9			
Age groups				
0 – 4	21 (28.77)	2 (20)	16 (29.09)	3 (37.5)
5 – 14	40 (54.79)	6 (60)	29 (52.73)	5 (62.5)
15 – 24	2 (2.74)	1 (10)	1 (1.82)	0
25 – 34	4 (5.48)	0	4 (7.27)	0
45 – 54	5 (6.85)	1 (10)	4 (7.27)	0
55 – 64	1 (1.37)	0	1 (1.82)	0
	Fisher exact, two-tailed p = 0.875			
Risk groups				
Contact with TB	61 (83.56)	8 (80)	45 (81.81)	8 (100.0)
PLHIV	9 (12.33	2 (20)	7 (12.73)	0
Others	3 (4.11)	0	3 (5.45)	0
	Fisher exact, two-tailed p = 0.54			
Treatment regime				
6 months H	50 (68.49)	7 (70)	38 (69.09)	5 (62.5)
3 months H/R	23 (31.51)	3 (30)	17 (30.91)	3 (37.5)
	Fisher exact, two-tailed p = 0.54			

Discussion

The presented study shows our results of using two tests (TST and IGRA) for detecting the LTBI in the Republic of Macedonia and treatment outcomes: in 2016 there were 73 cases diagnosed with LTBI that received preventive therapy. In Macedonia, for many years ago, only TST was used for screening the LTBI mostly among children household contacts of pulmonary TB cases; we did not use this test for screening LTBI among the adults even they were contacts with TB cases. All these adult contacts according to our NTP were screened only for active TB. Because BCG vaccination is obligatory for all newborns in Macedonia, it was difficult to interpret TST positive results: whether they are due to the cross-reaction with BCG vaccination, or to the infection with Mycobacterium tuberculosis. In 2013, IGRA test with high specificity was introduced for screening LTBI in our NTP which meant to reduce false positivity due to BCG vaccination or nontuberculous mycobacteria (NTM) infection. In the beginning its indication was limited only to children contacts with TB cases and PLHIV, and after this first period we expanded the indication according to the recommendation from Guidelines on the management of latent tuberculosis infection [5] to the patients initiating anti-tumor necrosis factor (TNF) treatment, patients receiving dialysis, patients preparing for organ or hematologic transplantation, and patients

with silicosis, but its implementation was going very slowly. There is a modification in the Macedonian Guidelines for LTBI in 2016, from the 2008 Guidelines. In 2016 there were only 9 cases with PLHIV and 3 cases with other medical risk factors screened and treated of LTBI.

All cases with LTBI in this study were not screened with both tests: according to our practice for many years ago, TST was performed in all children - 61 and only in1 adults (62-84.93%). IGRA was performed in half of the cases (37-50.68%): 12 adults and 25 children.

According to the data from the literature, there is no ideal test for detecting Mycobacterium tuberculosis infection even both tests are moderately sensitive and highly specific [7]. The disadvantages of TST include the need for two visits (to place the TST and to read it 48-72 hours later), inter-reader variability in measuring millimetres of induration, diminished response caused by immunosuppression, boosting on repeat testing, and potential cross-reaction with nontuberculous mycobacteria and *M. bovis* BCG vaccine [8]. The introduction of the IGRA was an advance in diagnostic technology. IGRA detect LTBI by measuring IFN-gamma release in response to antigens present in Mycobacterium tuberculosis, but not BCG vaccine and most nontuberculous mycobacteria.

The evidence base for these tests has expanded rapidly and now, the result of some systematic reviews indicate that in comparison to TST, IGRAs can detect LTBI with a higher specificity, negative (NPV) and positive (PPV) predictive values, as they are not confounded by previous BCG vaccination [9] [10] [11].

Regarding the treatment regimes we use two regimes: 50 cases (68.44%) received 6months daily regime with H, and 23 cases (31.51%) received 3months daily regime with HR. We have to comment that according to our results there were 24 cases without laboratory confirmation of LTBI, but besides that, they were treated with preventive therapy for LTBI. This was because they were children at 5 or under the age of 5 with very close household contact with pulmonary TB. It is according recommendations by WHO and other association that people living with HIV and children under the age of 5 years who are close household or close contact with people with TB and who have negative TST or IGRA results (with normal chest radiograph) should be treated for LTBI and another TST or IGRA performed 8-10 weeks after contact has ended. If a repeat TST or IGRA result is positive, treatment should be continued. If it is negative, treatment can usually be discontinued [5] [12] [13].

According to the Guidelines from WHO and the Centers for Disease Control and Prevention (CDC) there are four treatment regimes for LTBI: 9 or 6 months of daily self-administered H, 4 months of daily self-administered R, 3 months of daily self - administered H/R, and the newest 3 months of once-weekly directly observed isoniazid-rifapentine [5][14]. WHO recommends either 6 or 9 months of daily 9H as the standard for treatment of LTBI [5]. For people living with HIV infection, 9 months of therapy is recommended. The benefits of preventive therapy to individuals with LTBI have been demonstrated in some randomised clinical trials [15] [16].

The results from this study showed that most patients completed treatment regimes: 55 (75.34%) and only 10 (13.09%) interrupted the treatment. 8 (10.96%) of them interrupted treatment because of negative control TST after 8 weeks of the window period. We did not find that some factors such as gender, age groups, different risk groups or treatment regimes correlated with the treatment outcomes. In our study cases, we did not register some serious adverse effects from therapy. In the study of Denholm et al. even adverse effects were frequently identified among the cases with LTBI, there were also, high levels of treatment adherence and completion [17]. The analysis from some studies identified the following determinants as detrimental to treatment completion: adverse drug reactions, longer duration of treatment, immigrant status, long distance from a health facility, the presence of stigma, alcohol and drug use, unemployment and time lag between diagnosis and treatment [5]. One randomised trial showed a significant increase in completion rate in the 3-month weekly rifapentine plus isoniazid regimen compared to the 9-month isoniazid regimen [18].

Analysis of this study presented the program for diagnosis and treatment of LTBI in Macedonia and compared the findings with the actual recommendations. When the IGRA's has been introduced in our program, big progress was made in the widening the indication for detecting LTBI. Detecting and treating cases who are at risk to develop active TB, will contribute to better control of TB in Macedonia. Using IGRA tests among household contacts with pulmonary TB will help for better selection of cases that need preventive therapy. Despite the progress, several challenges remain to be addressed: further strengthening in the optimum use of both tests, to assess the ability of these tests to predict tuberculosis disease, their reproducibility over serial tests, and discordance between tests. Regarding the treatment as soon as possible we should provide the use of 3 months of once-weekly directly observed isoniazid-rifapentine regime.

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Clinical Science

Computed Tomography Scan Findings in Children from a Tropical Region

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Abstract

BACKGROUND: Computed Tomography in the diagnosis of pathologies in children is becoming increasingly popular.

AIMS: To document indications and findings of referrals for CT scan in children in a private Radio - diagnostic Center in a developing environment.

METHOD: Children aged 6 months to 13 years referred to a private Radio - diagnostic Center, between June 2015 to June 2016 were studied. Patients were examined using GE CT Brivo 385 machine.

RESULT: Forty - seven children were referred for CT scan during the period. Of these 45 (95.47%) were referred from the Teaching Hospital located in the same city. A brain scan was most commonly performed (93.6%) while the frequency of abdominal CT was (6.4%). The main indications for the referrals were delayed milestones (61.7%) and seizures (17.0%). The major findings were cerebral atrophy 26 (55.32%), and cerebral infarction 3 (6.38%).

CONCLUSION: This study shows a predominance of brain CT scan request in children, delayed milestones and seizures being the most common indications, while cerebral atrophy and cerebral infarction were the common brain findings. In the absence of MRI, CT scan provides a suitable alternative for imaging of the brain and abdominal pathologies in a developing environment like ours.

Introduction

Neurological and abdominal disorders in children are usually sources of great apprehension in both parents and pediatric surgeons. The use of Computed Tomography (CT) scan in the diagnosis of these disorders has increased rapidly [1]. CT involves much higher radiation than plain radiographs and in developing countries is further limited by availability and cost [2].

Consequently, there is need to ensure that appropriate protocols are employed to avoid misuse [3] [4]. This study was aimed at documenting the sources, indications and findings of referrals for CT scans in children in a Radio - diagnostic Center in Calabar, Nigeria.

This study was aimed at documenting the number of referrals, sources, indications and findings of such referrals for CT scan in children in a private Radio - diagnostic Center.

Methods

This was a retrospective descriptive study involving children referred to Asi Ukpo Radio - diagnostic Centre, Calabar, for CT scan. The Centre is a private diagnostic facility situated in Calabar Municipality. The study was carried out between June 2015 and June 2016. Information concerning the age, gender, indication and type of CT scan conducted and findings were documented. The patients' request cards, stored images and radiology reports were reviewed by the authors. The indications for the scan were documented. The protocol for paediatric CT scan was used in all the patients recruited for this study in keeping with the ALARA (As Low As Reasonably Achievable) principle. Pre and post – contrast images were acquired using GE CT Brivo 385 machine and assessed by the Radiologist. Lead covers were used to shield parts of the body that was not under examination to reduce the radiation risk.

All data were entered into the Microsoft word excel spreadsheet and analysed by the use of simple proportions and percentages. Frequency tables were used to demonstrate the results.

Results

Forty-seven children were seen during the study period giving a rate of 3.92 children per month. Twenty-six (55.31%) of these were males while 21 (44.68%) were females. The ages ranged from 6 months to 13 years with a median of 2.0 years. The majority of children (36.2%) were aged one year and below. Forty - five (95.74%) of the children were referred from the Teaching Hospital located in the same city while 2 (4.26%) were from the private clinics.

A brain scan was the most commonly performed (93.6%) while the frequency of abdominal CT was 6.4%. The main indications for the CT examination were delayed milestones and seizures as shown in table 1. Brain CT scan was the most commonly performed investigation 44 (93.6%) while abdominal Ct was done in three (6.4%).

Table 1: Indications for Referral for CT scan (n = 47)

	Frequency	Percent	Valid percent	Cumulative percent
Delayed milestones	29	61.7	61.7	61.7
Seizures	8	17.0	17.0	78.7
Hemiparesis	1	2.1	2.1	80.9
Hydrocephalus	2	4.3	4.3	85.1
Headache	2	4.3	4.3	89.4
Congenital	2	4.3	4.3	93.6
Head trauma	2	4.3	4.3	97.9
Intra-abdominal malignancy	1	2.1	2.1	100.0
Total	47	100.0	100.0	

The major brain CT findings were cerebral atrophy 26 (55.32%), normal scan 8 (17.0%) and cerebral infarction 3 (6.38%) see Figure 1 to 3.

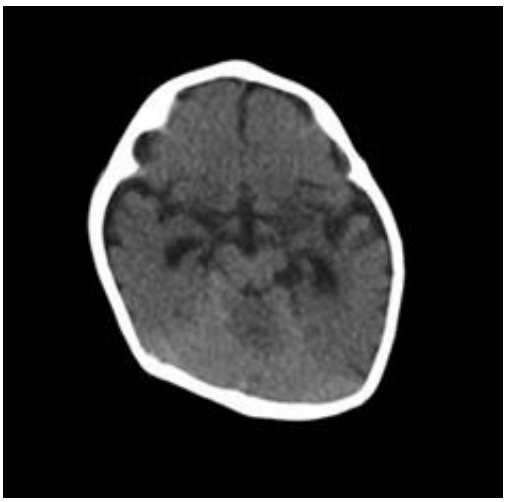

Figure 1: Cerebral atrophy with craniosynostosis

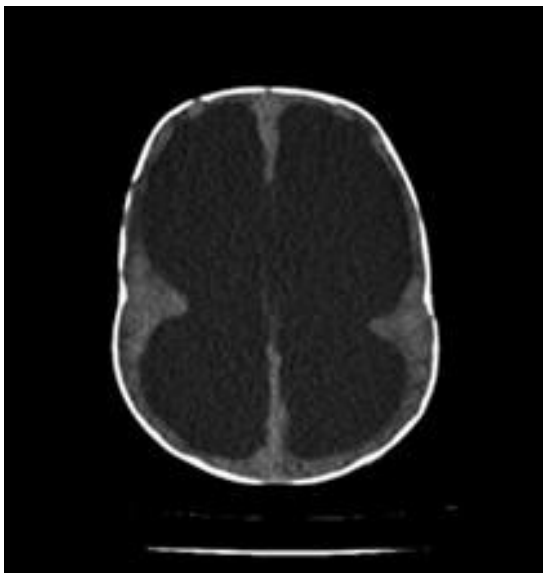

Figure 2: Communicating hydrocephalus with cortical thinning

Abdominal CT findings included renal trauma and nephroblastoma (Figure 4) in equal proportions (2.1%). Table 2 shows the CT findings in the study population.

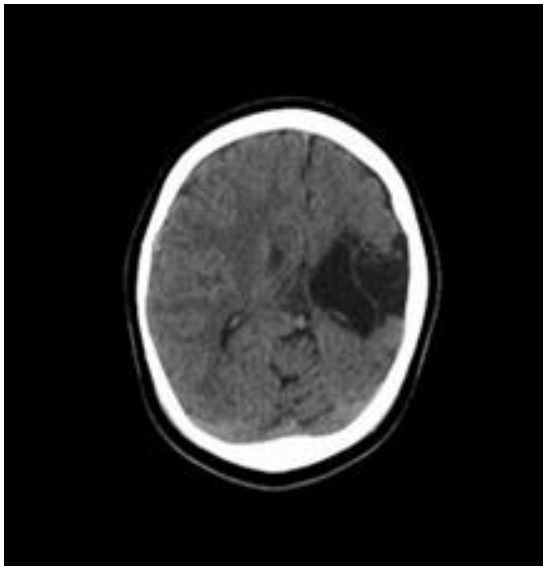

Figure 3: Chronic left temporal lobe infarction

Discussion

After sonography which is the preferred imaging method for screening the central nervous system (CNS) during infancy, CT scan is the next preferred method for all other ages [4]. However, the risk of this method should be considered alongside its advantages and radiation dose reduction should be employed where necessary [3] [4]. However, the small proportion of children with normal scan indicates good selection by referring clinicians.

Table 2: Findings for children referred for CT scan (n = 47)

	Frequency	Percent	Valid percent	Cumulative percent
Normal	8	17.0	17.0	17.0
Cerebral atrophy	26	55.3	55.3	72.3
Cerebral infarction	1	2.1	2.1	74.5
Meningial enhancement	1	2.1	2.1	76.6
Arnold chiari	1	2.1	2.1	78.7
Communicating hydrocephalus	1	2.1	2.1	80.9
Choroid plexus papilloma	2	4.3	4.3	85.1
Cerebral atrophy and infarction	2	4.3	4.3	89.4
Sturge-weber syn	1	2.1	2.1	91.5
Abscess(s)	1	2.1	2.1	93.6
Encephalocele	1	2.1	2.1	95.7
Renal trauma	1	2.1	2.1	97.9
Nephroblastoma	1	2.1	2.1	100.0
Total	47	100.0	100.0	

The study revealed that the most frequent age of those referred for Paediatric CT scan was one year.

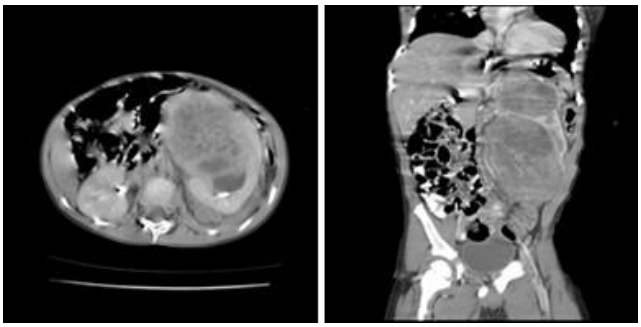

Figure 4: Left nephroblastoma

The most frequent referrals were for a brain scan. This agrees with a study done by Anas et al., [5] in Kano and may be related to the use of cheaper options like plain radiography, contrast studies and ultrasonography in examination involving other parts of the body. A study was done by Nzeh et al., [6] revealed that most prevalent suspected abnormalities of the paediatric brain could be evaluated by ultrasound in much younger children before the fusion of the fontanelles. The use of ultrasonography in the evaluation of pathologies in younger children is very important as children are known to be more radiosensitive than adults as seen in studies by Brenner et al., [3] and Donnelly et al., [4].

Delayed milestones 29 (61.7%), seizures 8 (17.0%), hydrocephalus 2 (4.3%) and trauma 2 (4.3%) were the predominant indications for CT examination in this study. This is similar to the study done by Anas et al., [5] where the most common indications were convulsions (21.43%) followed by trauma (15.71%) and progressive head enlargement (11.43%). The high incidence of birth asphyxia, meningitis and neonatal jaundice as reported by Eyong et al., [7] in our environment may be responsible for this pattern. About 17% of the CT scans in this study showed normal findings. This is less than that reported by Anas et al., [5] (30%), Islam et al., [8] (45%) and Fenton et al., [9] (54%). This work reflects cerebral atrophy as the major CT finding probably due to the high incidence of birth asphyxia from obstructed and prolonged labour followed by choroid plexus papilloma and cerebral infarction. Anas et al., [5] however reported obstructive hydrocephalus as the most common finding which may be due to the high prevalence of meningitis in the northern part of the country [5].

In conclusion, the high yield of diversity of CT scan findings in the children in our study justifies the appropriate use of CT scan in the diagnosis and management of suspected brain pathologies in areas where magnetic resonance imaging scan is unavailable or where ultrasonography cannot be done following the fusion of the fontanelles in younger children. Although cost should be considered, the patient should be given the chance for an appropriate work up to avoid delay in diagnosis. The cost problem can be solved by universal insurance scheme that is inclusive of both the public and private work force. Paediatric CT scan is an important diagnostics tool with lots of potentials and flexibility [6]. It should, however, be used with great caution as stipulated by the “as low as reasonably achievable” (ALARA) principle to reduce radiation dose to the patient [4]. It is obvious from this study that the environment is underserved and therefore Public Private Partnership (PPP) is recommended to expand CT facilities in this environment.

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Case Report

Technical Case Report of Deep Brain Stimulation: Is it Possible Single Electrode Reach to Both of Subthalamic Nucleus and Ventral Intermediate Nucleus in One Stage?

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Keywords: STN; DBS; VIM; Technical report; Tremor

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Abstract

BACKGROUND: The primary target of this operation is Ventral Intermediate Nucleus (VIM); however VIM - Subthalamic Nucleus (STN) were tried to be reached with one electrode, adjusting the angle well, the coronal section; medial of VIM can partially reach the STN. Using the properties of the electrode; we believe we could act on a wide area.

METHODS: An analysis was performed on one patient who underwent VIM Deep Brain Stimulation (DBS) in 3 periods (pre – peri - post-operation).

RESULTS: A 53 – year - old woman diagnosed with Parkinson's disease 8 years earlier including symptoms of severe tremor on the right than left underwent bilateral DBS VIM. Obtaining a satisfactory improvement of tremor, the patient did well, and postoperative complications were not observed. The patient was discharged from hospital on postoperative thirty day

CONCLUSIONS: It is certain that more research and experience are needed. However, we believe that the two targets can reach the same point and the second operations for another target can be avoided.We believe that this initiative is advantageous and promising regarding patient and cost.

Introduction

Surgery treatment is lesioning (Thalamotomy) or DBS (Deep Brain Stimulation) for PD and ET (Parkinson's disease and essential tremor). In this particular case, DBS has been preferred over lesioning because it is safer: for two reasons; no permanent brain tissue damage and controllable side effects from stimulation [1] [2].

In recent years; DBS has had an important place in the treatment of Parkinson's disease (PD), tremor, dystonia and some psychiatric diseases. Subthalamic nucleus (STN) is one of the main targets for DBS in PD. Clinical signs are improved by STN stimulation; thus reducing dopaminergic drug requirements by about 50% [1] [3] [4] [5]. DBS of the ventral intermediate nucleus (VIM) of the thalamus is highly effective for the treatment of tremor. Patients with tremor associated with Parkinson's disease and essential tremor appear to respond best but without benefit on other the cardinal PD symptoms and motor complications [1] [5].

We aim to determine the single electrode reach to both of STN and VIM in one stage. All aspects of the subject have been demonstrated in this article. The closeness of STN and VIM has led us to evaluate the possibility of paving a path to the development of new technologies.

Case Report

A 53 – year - old woman followed with PD for 8 years had tremor mainly over the right side & slightly dyskinesia. Trials of more than four medications and other therapies failed to relieve the tremor.

The medical history of the patient was unremarkable. Preoperative laboratory values were within normal limits. Standard oral medication failed to prove significant tremor control.

The patient was evaluated by a multidisciplinary team of neurosurgeons, neurologists and psychiatrists; she underwent surgery for implantation of DBS - VIM.

Antiparkinsonian drugs were withdrawn 24 hours before the surgery. The patient has fitted a stereotactic frame (Radionics™ CRW Precision™ Arc Stereotactic System, Integra Radionics Burlington - USA**)** with local anaesthesia. A one – mm - thick section of CT image and a two – mm - thick section of MR Image (1, 5 Tesla) was used to identify the coordinates of the target. The target was determined from fused images. MR imaging (T1 - weighted Gd-enhanced) data was used to avoid the puncture of vascular structures and the lateral ventriculus. Brain mapping, direct method and indirect method (anterior-posterior commissure) were used to determined target.

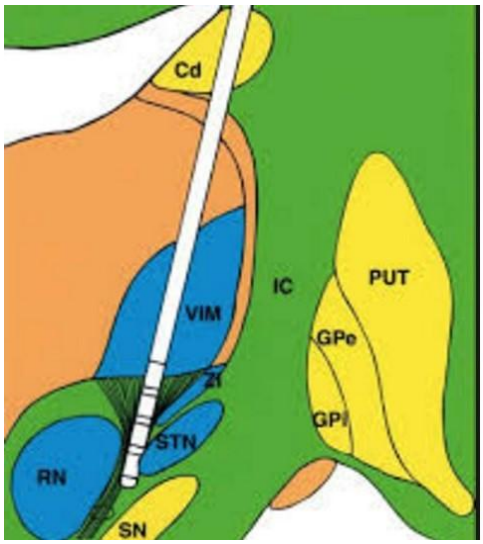

Figure 1: Schematic view of DBS targets (Especially STN and VIM)

A burr hole was made on the frontal skull the singe-track microelectro-recording, macrostimulation were carried out to confirm the effect of stimulation. Boston Scientific DBS 8 contact Lead implantation was decided to adjust each contact from all total 8 contacts, different amplitude and frequency. X-ray system was also used to identify the actual electrode malposition. The electrode has multiple Independent Current Control and Interleaving feature.

During macro stimulation tremor decreased was observed and VIM - STN were detected during MER.

Following implantation of the DBS; generator (The Vercise, Boston Scientific) was placed in a subcutaneous pocket in the infraclavicular region under general anaesthesia.

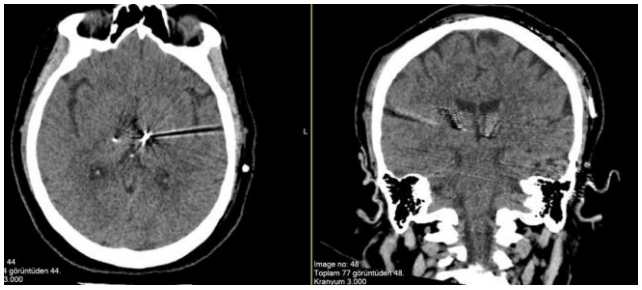

Figure 2: The sagittal an axial sectional view of the target in postoperative CT

Thanks to Postoperative CT images showing any intracranial haemorrhage and we believed that the DBS lead was implanted at the appropriate position. There was no complication in our case. Thus, the patient was discharged from hospital on the postoperative third day. The battery was activated 15 days after the surgery; we received a response at all the contacts.

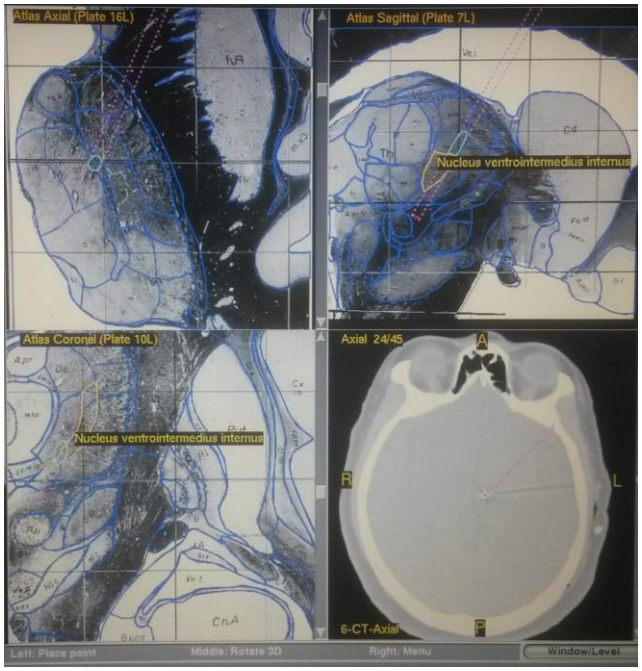

Figure 3: Anatomical relationship of STN and VIM according to Postoperative Brain Mapping

VIM and STN have been reached with one electrode. The primary target was VIM. Adjusting the angle well in Neurosighr Arc with Atlas Plan Module (Integra); the STN from the medial of the VIM could be partially reached. The properties of the electrode made it possible to affect a wide area. We aimed to

place the Lead's Caudal contact points into the STN. We worked in the coronal and axial slices on MRI. The side was found on the brain map; we believed the targets were reachable, STN&VIM were observed with MER during the operation. Clinical response in all our target point was achieved with Macrostimulation. We checked the situation with postop CT that it could affect a wide area. Although the main target VIM, We tried to place. We've worked in. We found our site on the brain map. We thought that the targets were reachable. We observed STN and VIM waves during operation with MER We have a clinical response in all our target point with macro stimulation. We checked the situation with postop CT.

The condition was repeatedly checked with MER, macro stimulation postoperative brain mapping and CT (Figure 1, 2, 3 and 4).

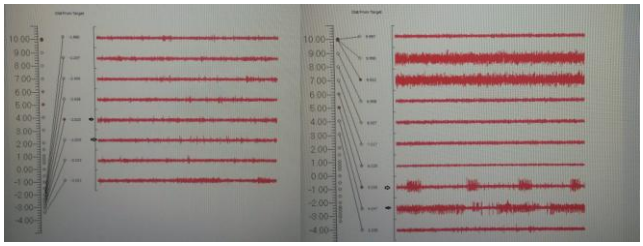

Figure 4: Perioperative MER mapping of the STN and VIM

Stimulation Parameters

During the post-operative process, the stimulation was optimised by utilising the various combinations of frequency, voltage and pulse width. Intraoperatively, system parameters consisted of a frequency of 60 to 130 Hz, the pulse width of 60 İs, and amplitude of 1.0 - 4.0 mA. Optimal tremor suppression was seen with a voltage of 4 – 5.2 mA, a pulse width of 80 - 150 is and a frequency of 100 - 130 Hz.

Discussion

During target determination process electrophysiological and clinical evaluation have been observed as the most reliable methods. VIM - DBS of the thalamus is highly effective for the treatment of tremor. In addition to VIM - DBS patient suffering from tremor associated with PD appear to respond best but without benefit on other the cardinal PD symptoms and motor complications [6].

VIM can be recognised either by MRI (3 Tesla) or the indirect method.In our case, the letter one was chosen [7] [8].

Two electrodes with different angles have been placed for STN and VIM. Arranging this, not only anatomy but also preoperative clinical and electrophysiological assessment has been precedence in our case. We believe that the two main targets have been affected by one single electrode. In consequence, not only the primary tremor but also the possible other PD symptoms may benefit from STN.

Clinical trials studies Show that STN is a good target for ET and Parkinsonian tremor. STN can be accessed directly or indirectly via AC - PC. The relationship between RN (Red Nucleus) and STN has also been a guide for us [1] [9] [10].

RN is easier to recognise in MR than STN. The STN is recognised as a hypointense structure located lateral to the anterior portion of the RN on a coronal section MRI [9].

In Parkinson tremors, STN can be selected because it will contribute to the treatment of other motor symptoms [1] [11]. In our case, the tremor was dominant. VIM - DBS was chosen for the tremor. However, we believed that other cardinal findings of PD would develop over time. At this point, we believed that STN - DBS would increase the clinical utility.

In Literature we have seen that two targets have been reached by two electrodes [1][11][12].

Clinical studies in literature were taken into consideration. The features of new lead were reviewed with 8 contacts. The target has not been evaluated radiological electrophysiological even clinical assessment are of at most importance. The angle was arranged with one electrode in VIM and STN. STN was placed in medial; not only were side effects recognised; which is believed to be the outcome of the effect of the lead. It is possible to activate various contacts through different parameters. For instance, contact point 1, 2, 3, 4 can be activated through different frequencies and voltage. It was observed that this was an advantage. The possibility of stimulation to STN and VIM (together or separately) with this lead, which may be important for the future progress.

It is certain that more studies and clinical trials are needed. However, we belive that reaching the same point with two targets can prevent succeeding operations for another target. We believe that this initiative is advantageous and promising in term of cost and patient benefit.

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Case Report

Pretibial Located Stewart-Treves Syndrome: Uncommon Presentation in a Bulgarian Patient!

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Keywords: (HHV-8); Angiosarcoma; Epithelioid variant; Stewart Treves syndrome; Surgery; Amputation

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Abstract

BACKGROUND: The Stewart-Treves syndrome with localisation in the region of the lower extremities is not something unusual as clinical pathology, but the clinical diagnostics is rather difficult, and it can be further complicated maximally because of: the similar locoregional findings in patients with other cutaneous malignancies.

CASE REPORT: Presented is a rare form of an epithelioid variant of the Stewart Treves syndrome in a woman, aged 81, localised in the region of the lower leg and significantly advanced only for 2 months. The diagnosis was confirmed histologically and immunohistochemically. Amputation of the affected extremity was planned. Discussed are important etiopathogenetic aspects regarding the approach in patients with lymphedema and possibility for development of the Stewart Treves syndrome.

CONCLUSION: Analyzing the evidence from the literature worldwide, we concluded that perhaps the only reliable (to some extent) therapeutic option in patients with Stewart Treves Syndrome is 1) the early diagnostics and 2) the following inevitable radical excision or amputation with the maximal field of surgical security in the proximal direction.

Introduction

Sarcomas are connective tissue tumours, comprising approximately 1% of all malignant tumours [1]. Stewart Treves syndrome is a skin variant of angiosarcoma which has developed based on chronic lymphedema [1].

The disease affects women predominantly, with a higher incidence in the fifth and seventh decades as in the patient described by us [1].

Interestingly, the syndrome could also develop by congenital lymphedema, which implies the need for a serious reflection on the "active" or more aggressive approach in affected groups of patients (at an early age) [2].

Case Report

An 81-year old female was admitted in the Dermatology and Dermatological Surgery department because of pain, edema and burning sensation of 2 months duration in the left lower limb (Fig. 1a). She is under treatment for hypertension and cardiac ischemia and there is a past history of hysterectomy due to multiple myomatous nodules (Fig. 1a).

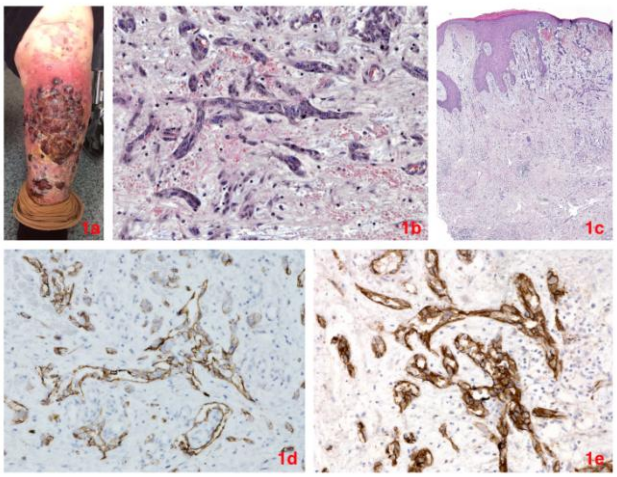

Figure 1: 1a) multiple solitary nodules, 2-3.8 cm in diameter and tendency for confluence and endophyte growth resulting in the formation of a bigger endophyte tumour growth and forming a single tumour lesion with dimensions of about 22 to 16 cm. Perilesional area of erythema around the tumours. Presence of multiple satellite lesions in all directions around the tumour plaque; 1b) vessels with irregular branching consisting of large atypical endothelial cells. Mitotic figures and extravascular red blood cells, H&E x200; 1c) irregular acanthosis. In the upper dermis increased number of vessels. Extravasated red blood cells and oedema. H&E x25; 1d) atypical vessels expressing D2-40. Promontory sign, IHC x200; 1e) 3b CD31 in the atypical cells, IHC x200

General examination revealed marked pallor of the mucous membranes and skin, no other findings could detect. On the other hand, local examination of the lower limbs revealed. Bilateral progressive lymphedema on the lower legs, more pronounced on the left. Edematous left lower leg and foot. Pretibially located tumour conglomerates with dimensions 22/11 cm and with black to dark blue colour, partially confluent with one another. Presence of multiple satellite tumour lesions on an erythemic base near the main tumour formation (Fig. 1a). HIV-1 P24 antigen and HIV-1, 2 antibodies: negative. TPHA test negative, Hb S-Ag, Anti HCV autoantibodies: negative. From the paraclinical and microbiological tests were established: the microbiological smear from lesional smear showed significant bacterial growth of Acinetobacter Spp., E faecalis. LDH: 269 IU/l, CKMB: 30 IU/l, Granulocytes 72.5%, blood sugar 6.4 mmol/l, sediment urine-massively leukocytes and bacteria. Urine culture: significant concentration of *Klebsiella pneumoniae*. X-ray of the lung is with evidence of thickening of the pleura with unclear genesis, on the left, axillary. X-ray of left lower leg and ankle joint: osteoporosis of the visible bones, inhomogeneous structures with areas of thickening and increase in the transparency of the soft tissues of the examined extremity. Doppler of arterial and venous vessels was without evidence of thrombosis or chronic arterial insufficiency as a result of compression by the tumour masses. Histopathological assessment of a biopsy of the lesional tissue: large lobes, dermally based, a malignant vessel-forming tumour with prevailing epitheloid, pleomorphic cells with prominent nucleoli (Figures 1b and 1c). Immunohistochemistry: CD34 positive expression in the malignant population, CD 31 positive (Fig. 1e), D2-40 positive (Fig. 1d), HHV8 negative, S-100 negative, Anti CKA-1, -3 negative. Based on the anamnestic evidence of hysterectomy in the past, the histological and immunohistochemical assessments, the patient was diagnosed with an epithelioid variant of angiosarcoma with lymphedema or Stewart Treves syndrome. Amputation of the lower leg was also planned at a later stage. Due to the patient's refusal to undergo surgical treatment, she was referred to the Oncology for planned chemotherapy and/or eventually radiotherapy.

Discussion

The pathogenesis concerning the origin of cells in angio or lymphangiosarcoma is controversial [3] [4]. Lymphangiosarcoma is a controversial term because a tumour itself originates from the vessels and hence, according to some authors, the definition hemangiosarcoma should be more accurate [1] [2]. Other studies have suggested that a tumour expresses markers characteristic of both lymphatic and vascular capillaries [3] [4].

Stewart-Treves Syndrome is characterised by the presence of lymphangiosarcoma on limb extremities [1] [2]. Rare, it occurs in 0.5% of patients who have undergone radical mastectomy with axillary node dissection [1].

The main cause of the disease seems to be the chronic lymphedema with the subsequent endothelial and lymphatic differentiation, with no direct relationship to other types of cancer [4]. The main etiopathogenesis is chronic lymphedema, and it is believed to occur a neoplastic proliferation with blood and lymphatic endothelial differentiation, but it's not possible to determine definitely which predominates [1] [3].

The extent to which a lymphostasis is capable of leading to a reduced local immunity, followed by degeneration of the cell matrix, and hence to the uncontrollable production of growth/angiogenic factors, is unclear. But at least the hypothesis seems reasonable, though it is speculative. The chronic

lymphostasis is likely to lead to cellular changes and possibly to an early onset of malignant transformation [5] [6]. It is because of this fact that many authors advise that the radiotherapy in the early stages of the illness should be rather avoided hence it can 1) potentiate the DNA changes in the cells and 2) even worsen the lymph flow. These two factors could further trigger the onset of the Stewart Treves syndrome [5] [6]. No racial preponderance has been observed in this syndrome [7]. It has not been shown to be more prevalent in patients with AIDS or any other immunodeficiency disorders, as compared with Kaposi's sarcoma (KS) [7].

Interestingly, some authors postulate that mutations in the DNA repair genes BRCA1 and BRCA2 predispose to angiosarcomas after treatment for breast cancer [8]. Three phases in the development of STS have been reported in the literature: (1) prolonged lymphedema, (2) angiomatosis, and subsequently (3) angiosarcoma [8]. Prolonged lymphedema (lymphostasis) is observed in KS as well as angiosarcoma, resulting in localised immunosuppression and vascular oncogenesis in the presence of human herpesvirus 8 (HHV-8) infections and immunosuppression [9].

Interestingly, lymphedema has also been reported in other cutaneous malignancies including cutaneous melanoma, basal cell carcinoma, squamous cell carcinoma, Merkel cell carcinoma, and cutaneous lymphomas [9] [10].

The most important differential diagnosis of Stewart Treves Syndrome is Kaposi sarcoma (KS). (KS) Differs from (STS), because it does not require the presence of lymphedema for its development [7]. Immunohistochemical testing for the presence of HHV-8 is the primary way to distinguish STS from KS (7). KS is associated with this virus, while STS is not [8].

In conclusion, unclear as a whole is the contribution of the different histological, immunohistological and molecular-biological characteristics (as markers) in this tumour regarding its pathogenesis and its course. This is what makes the rapid diagnosis as well as the choice of treatment regimen rather problematic than standard and easy to apply.

Perhaps the only method to guarantee better survival or a chance for survival of the affected patients is radical surgery or amputation of the affected limb with the maximal field of surgical security in the proximal direction. The aim is to avoid creating new lymphedema, which will prove to be a beneficial basis for reactivation or new Stewart Treves Syndrome (STS).

Chemotherapy is reserved for patients with unresectable disease or refusal of amputation (as in our patient). Radiotherapy could be used as adjuvant therapy in selected advanced cases with Stewart Treves syndrome [7]. The general prognosis of patients with Stewart-Treves syndrome is poor. Local recurrence rate is relatively high, with frequent need for amputation of the limbs [7].

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Case Report

Carnitine Palmitoyltransferase II Deficiency (CPT II) Followed By Rhabdomyolysis and Acute Kidney Injury

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Keywords: Carnitine palmitoyltransferase II deficiency; Rhabdomyolysis; Metabolic disorder; Creatine kinase; Myoglobin; Acute kidney injury; Hemodialysis

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Abstract

BACKGROUND: Carnitine palmitoyltransferase II deficiency (CPT II) is an autosomal recessive disorder and the most common inherited disorder of mitochondrial long-chain fatty acid oxidation, characterised by attacks of myalgia and myoglobinuria. The most common “classic” myopathic form occurs in young adults and is characterised by recurrent episodes of rhabdomyolysis triggered by prolonged exercise, fasting or febrile illness.

CASE PRESENTATION: We present a case of a 22-year-old Caucasian male admitted to our hospital with fever, dyspnea, fatigue, myalgia and dark urine (brown-coloured). The symptoms appeared after viral infection followed by fever. Acute kidney injury (AKI) developed as a complication, and there was a need for treatment with hemodialysis. At the clinical presentation, the patient had plasma creatine kinase (pCK) level of 130.383 U/L and plasma myoglobin level over 5000 μg/L. Genetic testing (molecular analysis) confirmed the diagnosis of inherited rhabdomyolysis, a metabolic disorder of carnitine palmitoyltransferase II deficiency. A previous episode with the same symptoms, the patient had four years ago but did not ask for medical treatment. The patient was discontinued from hemodialysis because of the resolution of acute kidney injury. The patient was discharged from the hospital in good condition, with a recommendation about his future lifestyle in order to prevent similar episodes.

CONCLUSION: Every patient presenting with myalgia, dark urine (brown-coloured), high level of pCK and development of AKI requiring hemodialysis, should be explored for inherited rhabdomyolysis induced by CPT II deficiency.

Introduction

Carnitine palmitoyltransferase II (CPT II) deficiency is a genetic disorder of mitochondrial fatty acid oxidation. Long-chain fatty acids are required for fueling the skeletal muscles, and they are only able to cross mitochondrial membrane after esterification with carnitine in a reaction with the enzyme CPT II [1] [2]. The damages are the result of an increased intracellular free ionised cytoplasm and mitochondrial calcium. This might be caused by depletion of adenosine triphosphate (ATP), and/or by direct injury and rupture of the plasma membrane. ATP depletion leads to myocyte injury and the release of intracellular muscle constituents, including creatine kinase (CK) and other muscle enzymes, myoglobin, and various electrolytes [3]. Three types of CPT II deficiency are recognised: a lethal neonatal form, a severe infantile hepatocardiomuscular form and myopathic form in which the onset ranges from infancy to adulthood [1] [2] [4]. The third one is the most common disorder of lipid metabolism affecting skeletal muscle and is the most frequent cause of hereditary myoglobinuria.

In vivo investigation of fatty acid oxidation in CPT II deficiency individuals, by indirect calorimetry and stable isotope methodology, shows impaired oxidation of long chain fatty acids during low-intensity exercise, with normal oxidation at rest. In addition, this method can only highlight low-fat oxidation but not diagnose CPT II deficiency. Almost all individuals with the myopathic form suffer from myalgia. Approximately 60% of them have muscle weakness during the attacks. The muscle cramps also occur

during the attacks, although they are not typical of this disease [5]. Myoglobinuria with brown-coloured urine occurs during the attacks in approximately 75% of the patients [6]. The first description of this disease in adults with exercise-induced rhabdomyolysis was made by Di Mauro in 1973 [7]. The symptoms start in childhood, whereas the attacks with myoglobinuria mostly emerge in adolescence or early adulthood. The most severe complications are massive rhabdomyolysis followed by acute kidney injury (AKI) requiring hemodialysis, acute hepatic lesion, respiratory insufficiency and paroxysmal heart arrhythmias.

Case presentation

We present a case of a 22-year-old Caucasian male, who until this hospital admission, had never asked for medical treatment. From the family medical history, the father of the patient died 10 years ago from cancer. The patient presented with fever, dyspnea, myalgia, fatigue, and dark urine (brown-coloured), followed by the development of acute kidney injury with oliguria. The laboratory analyses showed: plasma creatine kinase (pCK) level of 130.383 U/L, plasma myoglobin level over 5000 µg/L, plasma lactate dehydrogenase (pLDH) level of 2607 U/L, plasma alanine aminotransferase (pALT) level of 1104 U/L, plasma aspartate aminotransferase (pAST) level of 2920 U/L, plasma creatinine level of 574 µmol/L, and plasma blood urea nitrogen level of 20.6 mmol/L. The patient was initiated with hemodialysis treatment via a temporary vascular access-venous femoral catheter. The hemodialysis sessions were performed without complications.

Genetic testing (molecular analysis) for an inherited metabolic disorder was done, and there was a confirmation of the diagnosis CPT II deficiency. Deoxyribonucleic acid (DNA) sequencing analysis of exons 3 of the CPT II gene revealed that the patient had homozygote active mutation TCG>TTG for Ser 113 Leu and it was associated with family myoglobinuria.

During the hospitalisation, six hemodialysis treatments were performed. At day 12 of the hospitalisation, the patient was discontinued from the hemodialysis because of recurrence of diuresis with declining plasma levels of creatinine and blood urea nitrogen (Table 1). Ultrasonography examination of the kidneys showed normal kidney size and echogenic structure. Urine culture was sterile. The immunological testing (ANA, anti-dsDNA, cANCA) were negative. Continuous abdominal pain in the epigastric region with vomitus was also present. With upper digestive endoscopy, chronic gastritis with superficial ulcers was diagnosed. During the whole period, the plasma levels of calcium and potassium were in the normal range (Table 1). At the hospital admission the level of myoglobin was over 5000 µg/L, but at the end of the hospitalisation, it was 258.6 µg/L. Also, the level of pCK was 130.383 U/L, but at the end, it was 329 U/L (Table 1).

Table 1: Presentation of the laboratory findings during the patient's hospitalisation

	14 march	15 march	16 march	17 march	18 march	19 march	24 march	26 march	27 march	30 march
	Day (1)	Day (2)	Day (3)	Day (4)	Day (5)	Day (6)	Day (11)	Day (13)	Day (14)	Day (17)
P CK U/L		130.383	38.251	9.865		1.306	155			329
P CK-MB U/L		1626	473			30				
P Myoglobin µg/L		>500	>5000	>3000		1297	258.6			
P LDH U/L		2607	1554			471	460			
P AST U/L		2920	1590	869		139	46			
P ALT U/L		1104	955	697		357	134			
P Creatinine µmol/L	574	555	601					641	671	480
P Urea mmol/L		20.6	21.7					11.5		
P Potassium mmol/L	4.7	5		4.2		4.1		3.8	3.9	4
P Calcium mmol/L		2.08		2.32		2.3	2.33	2.34		2.35
P Phosohat mmol/L		2.2								
White blood cells count 10*9/L		25.2	16.1	13.4	15.9		18.1			10.1
Platelet count 10*9/L		214	198	194	246		341			412
P Total proteins g/L		58	62	61		59	67			
P Albumins g/L		38	37	35		35	38			
P Globulins g/L		20	25	26		24	29			
C3 g/L						1.27				
C4 g/L						0.204				
Diuresis ml		100		100		200	500	1900	2800	4800

The patient was discharged from the hospital in good condition, with recommendations about his future lifestyle in order to prevent similar episodes. The advice was given to his family members about the genetic metabolic disorder, CPT II deficiency.

Discussion

We presented a patient with fever, dyspnea, myalgia, fatigue, and dark urine (brown-coloured), with the acute hepatic lesion, without respiratory or heart failure. The symptoms occurred after a viral infection followed by fever. The patient also developed acute kidney injury requiring hemodialysis. Laboratory data suggested that it was due to massive rhabdomyolysis. The diagnosis was established by genetic testing (molecular analysis) for an inherited metabolic disorder which confirmed the diagnosis CPT II deficiency.

A few years ago, at the Hospital of Cardiology in Skopje-Macedonia, another patient (20 years old) with the same genetic inherited metabolic disorder (CPT II deficiency) was treated. The patient developed acute kidney injury, acute hepatic lesion, respiratory insufficiency and cardiomyopathy with volume overload. He was treated with plasmapheresis, hemodialysis and supportive therapy. The patient was discharged from the hospital in good condition with advice for a lifestyle modification [8]. One clinical study summarizes the clinical features of this disease, analysing data of 28 patients with

biochemically and genetically confirmed CPT II deficiency. It is noticeable that exercise was the most important trigger factor for attacks. The authors also noted that infections were more frequent trigger factors than fasting. CPT II deficiency was characterised with a male predominance of 86%, due to the X-chromosomal modifier genes or hormonal factors such as estrogen that might be a regulator of CPT [9]. Another clinical study presented that this enzymatic defect was detected in 47% of 77 patients, who underwent biopsy for idiopathic myoglobinuria. The carnitine palmitoyltransferase II deficiency was the most common disorder in the group of the biopsied patients [10].

It is necessary to detect the aetiology of rhabdomyolysis and start with the medical treatment**.** Although some genetic mutations have already been defined, the prevalence of these mutations is still unknown. It might be that the mutant enzyme is thermolabile in the adult form, causing the episodes of rhabdomyolysis during the acute febrile illness [11].

Diagnosing CPT II deficiency can be done by acylcarnitine analysis using tandem mass spectrometry (peak at C16 is indicative of the condition). Measurement of the CPT II activity can be performed as well as many laboratory findings, such as low carnitine levels, increased serum plasma creatine kinase and transaminase, which can be associated with the disease. For a definitive diagnosis, sequencing of the CPT II gene for mutation analysis is recommended [8]. Prenatal diagnosis may be offered for pregnancies at a 1/4 risk of infantile/severe-type CPT II deficiency [2].

Prevention includes protection from infections, avoidance of some medications (ibuprofen, diazepam, valproic acid) and general anaesthesia, toxins, heat and stress. Treatment is based on avoidance of fasting (more frequent meals) and exercise. A low-fat diet enriched with medium chain triglycerides and carnitine is recommended [12]. During acute infections, an infusion of glucose can be administered. Oral carnitine supplementation can be considered as an adequate therapy. The medium-chain fatty acid triheptanoin may be effective in the adult-onset CPT II deficiency [13].

In conclusion, inherited genetic metabolic disorder (CPT II deficiency) followed by massive rhabdomyolysis with acute kidney injury requiring hemodialysis might be a life-threatening condition. It could cause severe organ damage with a need for intensive medical treatment. The definitive diagnosis of this condition is achieved by genetic testing. Whenever a patient suffers from recurrent episodes of myalgia, followed by myoglobinuria due to rhabdomyolysis, the possibility of the presence of this rare condition should be considered.

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Case Report

Treatment of a Patient with Merkel Cell Skin Carcinoma Using Radiation Therapy - A Case Report

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Abstract

BACKGROUND: Merkel cell carcinoma (MCC) is a rare, very aggressive tumour. The pathogenesis remains unclear, but UV radiation, immunosuppression, and the presence of Merkel cell polyomavirus in the tumour genome appear to have a key role. Merkel cell carcinoma is a highly aggressive tumour that often has a lethal end.

CAS REPORT: A patient at 93 years of age comes for an examination by a dermatologist due to a rapidly growing nodular tumour growth in the forehead area. A tumour was about 3 cm in size. It had no signs of basal-cell carcinoma, no arborising vascularisation, no pigmentations on dermoscopy. Clinically, an eventual Merkel cell carcinoma was considered for the patient, but other primary skin tumours had to be excluded, as well as the possibility that regarding the patient's age, it may be a metastatic deposit. A skin biopsy was performed, as well as H-E examination and immunohistochemical analyses (positive CD56, positivity of neuroendocrine markers synaptophysin, chromogranin) which were in favour of Merkel cell carcinoma of the skin. After setting the diagnosis, our patient was treated with therapy which led to a complete withdrawal of a tumour. However, after 3 months the patient had repeated relapse of a tumour at the same site on the forehead and metastases in the retroauricular lymph nodes bilaterally. It shows that the radiotherapy as monotherapy has a great effect on the removal of the tumour formation, but unfortunately, it has no impact on lesion recurrence. It is also compatible with the literature data.

CONCLUSION: In many adult patients, as our case suggests, radiotherapy could be a good palliative treatment opportunity that should be considered, as well as a combination of radiation therapy with other oncologic therapeutic options.

Introduction

Merkel cell carcinoma (MCC) is a rare, very aggressive tumour, with quite common local or regional recurrences and with high metastatic potential. MCC usually develops in areas of the skin exposed to sunlight, in patients of advanced age. Its incidence has grown four times over the past decades due to the ageing of the population and immunohistochemical techniques leading to the diagnosis. The pathogenesis remains unclear, but UV radiation, immunosuppression, and the presence of Merkel cell polyomavirus in the tumour genome appear to have a key role. Toker was the author who first described a tumour in 1972. He used the term trabecular carcinoma of the skin, suggesting a possibility of glandular origin. Ultrastructural studies made 6 years later by Tang and Toker indicated a presence of electron-dense granules in the cytoplasm of the tumour cells. They suggested a neuroendocrine origin, similar to Merkel cells in the epidermis. The term cutaneous neuroendocrine carcinoma may be the one that best describes the immunohistochemical and ultrastructural phenomena of these tumours, but the most widely used and ultrastructurally accepted name in the literature is MCC [1].

Merkel cell carcinoma (MCC) is typically presented as a painless, rapidly growing, cubist red or purple nodule of sun-exposed areas of the skin, such as the head and the neck, or the upper limbs. Aetiology is multifactorial, with immunosuppression, UV-induced skin damage and viral factors [2].

According to the NCCN, resection of a tumour in healthy tissue is the basic therapy. Patients at high risk may also undergo adjuvant radiotherapy. The role of chemotherapy is unclear. The incidence of Merkel cell carcinoma in the United States is estimated to be 0.32/100.000 [3].

Merkel cell polyomavirus (MCPyV) was discovered in 2008 and still is the only human

polyomavirus that can be causally associated with human malignancy, i.e. Merkel cell carcinoma [4].

Merkel cell carcinoma is a highly aggressive tumour that often has a lethal end. Clonal colonisation with Merkel cell polyomavirus in the host genome may have a role in the carcinogenesis or the UV-induced carcinogenesis. Viral-encoded oncoproteins and UV-induced mutations affect the related signalling pathway such as RB restriction of cell cycle progression or p53 inactivation. Although its relatively low incidence Merkel cell carcinoma has drawn much attention recently due to immunogenetics and immunomodulatory treatments [5].

MCC is characterised by the acronym (from English words) AEIOU.

Most cases of MCC occur in a population at obvious high risk of developing this tumour. AEIOU features can be useful in identifying a suspected lesion [6] [7].

"A" is for asymptomatic. MCC is typically asymptomatic compared to an inflamed cyst, which it may sometimes resemble.

"E" is for expanding rapidly; a node that increases in 1-2 months.

"I" is for immunocompromised. 92% of patients with MCC are not immunocompromised, but those with long-standing T cell dysfunction (HIV, leukaemia, chronic immunosuppression) are at a much greater risk to develop MCC although they represent less than 10% of the cases.

"O" is for patients older than 50 years. The risk of MCC increases with age due to the immunosenescence (the immune system is less capable of detecting immunogenic cancer).

"U" is for the ultraviolet radiation-exposed fair skin.

Ninety of the patients have 3 or more of the AEIOU features. But this is not specific since some lipomas or cysts can meet 2 or 3 criteria.

Treatment is based on multidisciplinary management, although the optimal therapy is still controversial due to lack of data. Aggressive surgery, which is often associated with adjuvant radiotherapy, improves the locoregional recurrence and overall survival [8]. According to some authors, surgery and radiotherapy achieve excellent locoregional control. However, a certain percentage of patients develop a disseminated disease that is incurable. Chemotherapy has a great response in metastatic disease, but the response is short-lived, and the survival impact has not been established [9].

There are rare cases of metastatic disease. The disease usually metastasises in the local lymph nodes, but there are also cases of pleural metastases. [10] Immunohistochemical analysis of a section stained with hematoxylin and eosin indicates tumour cells with infiltrative growth and hyperchromatic nuclei that have been positive for CK20, CD56, chromogranin and synaptophysin [11]. Survival at 5 years is 51% for local disease and is low as 14% for distant disease, which underscores the aggressive nature of this tumour and challenges in management [12].

Case Report

The clinical examination is the first step in MCC diagnosis. The nonspecific and varied clinical features of this tumour pose certain diagnostic challenges even for an experienced dermatologist. Unlike basal cell carcinoma or melanoma, which may be clinically or dermatoscopically apparent, the “classic” MCC lesion does not exist [12].

A patient at 93 years of age comes for an examination by a dermatologist due to a rapidly growing erythematous nodule growth in the forehead area. A tumour was about 3 cm in size. It had no signs of basal-cell carcinoma, no arborising vascularisation, no pigmentations on dermatoscopy. The polymorphous vascular pattern has been observed.

Clinically, an eventual Merkel cell carcinoma was considered for the patient, but other primary skin tumours had to be excluded, as well as the possibility that regarding the patient’s age, it may be a metastatic deposit [13].

A skin biopsy was performed, as well as H-E examination and immunohistochemical analyses (positive CD56, positivity of neuroendocrine markers synaptophysin, chromogranin) which were in favour of Merkel cell carcinoma of the skin.

The biopsy specimen was formalin fixed and paraffin moulded. Besides the routine hematoxylin and eosin stained tissue sections, additional immunohistochemical analyses were performed on DAKO Autostainer link 48, an automatic immunohistochemical stainer, using monoclonal ready to use antibodies from Agilent Technologies.

Histopathological analysis of the biopsy specimen detected a presence of neoplastic cells in the dermis, arranged in solid sheets with areas of "crush effect", composed of relatively uniform cells. The neoplastic cells had small basophilic nuclei and very scant cytoplasm consistent with the histopathological finding of small cell variant of MCC (Figure 1A). Numerous mitotic figures were evident. To confirm the neuroendocrine nature of the tumour cell, additional immunohistochemical analyses were performed. The immunohistochemical analysis indicated that tumour cells were positive for neuroendocrine markers chromogranin (Figure 1B) and synaptophysin (Figure 1C), whereas they were

negative for cytokeratins 5/6 (Figure 1D), cytokeratin 7, CD45, TTF1, as well as for cytokeratin 20 (Figure 1E). Ki-67 proliferative index was more than 90% (Figure 1F).

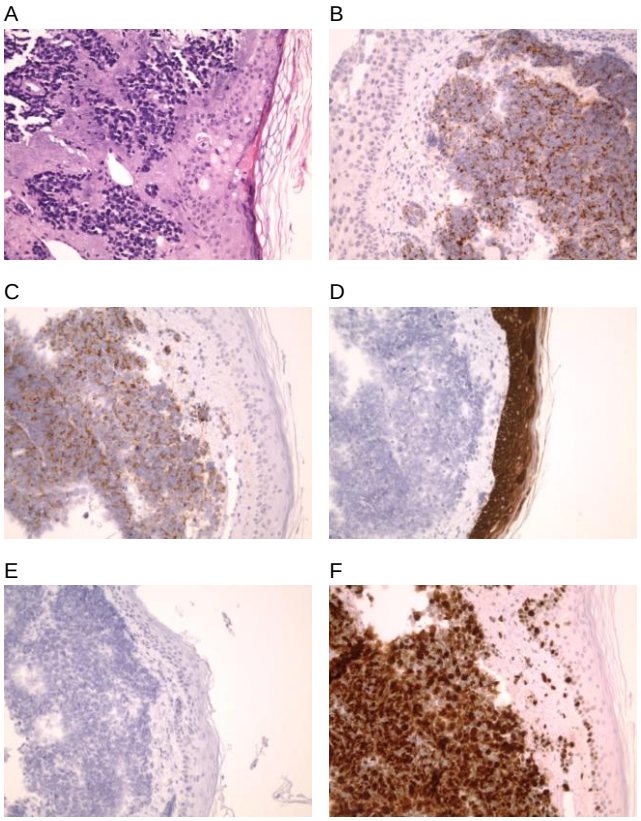

Figure 1: A) Histological appearance of the tumor (hematoxilin and eosin, x200); B) Neoplastic cells show positivity for Chromogranin (x200); C) Neoplastic cells show positivity for Synaptophysin (x200); D) Neoplastic cells are negative for cytokeratins 5/6 (x200); E) Neoplastic cells are negative for cytokeratin 20 (x200); F) Ki-67 proliferative index is >90% (x200)

The performed CT examination indicated no presence of other tumour formations in the deep tissues of the head and the neck. The finding was normal.

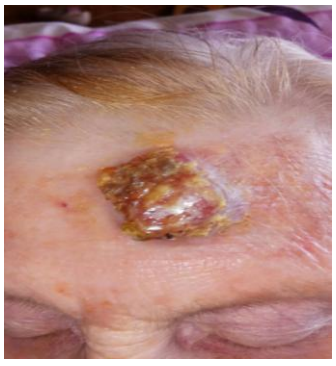

Figure 2: Patient before treatment

After CT simulation in the patient, a plan for radiation of the skin efflorescence in the frontal area was made. The treatment used X-rays with an energy of 6MV and a total dose of 40 Gy during 10 fractions. The patient achieved a full-scale remission of the lesions. There was no tumour recurrence in the monitoring period of 3 months.

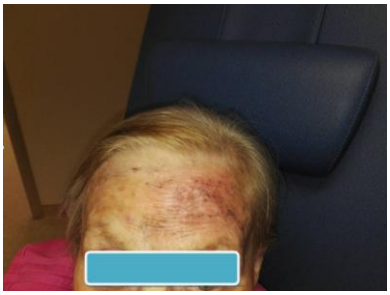

Figure 3: Patient after radiation treatment

Discussion

Merkel cell carcinoma (MCC) usually has a clinical presentation in the form of a solitary, solid, well-defined nodule which is erythematous or purple, and mobile about the subcutaneous tissue.

Staging classification of the disease is done according to the American Joint Committee on Cancer.

Table 1: TNM Classification of MCC according to the American Joint Committee on Cancer

Tumour	Node	Metastasis
Tx, the tumour cannot be assessed	Nx, lymph node involvement cannot be assessed	Mx, metastasis cannot be assessed
T0, no evidence of a primary tumour	N0, no lymph node involvement	M0, no metastasis
This, primary tumour in situ	-cN0, no clinical signs of lymph node involvement (on inspection, palpation, and radiograph)	M1, distant metastasis
T1, primary tumour ≤ 2 cm	-pN0, no lymph node involvement detected by a pathologist	-M1a, metastasis to the skin, subcutaneous cellular tissue, or distant lymph nodes
T2, primary tumour>2 cm and ≤ 5 cm	-pNx, no histology of lymph nodes	-M1b, metastasis to the lung
T3, primary tumour>5 cm	N1a, micrometastasis[b]	-M1c, metastasis to other visceral organs
T4, a primary tumour affecting bone, muscle, fascia, or cartilage	N1b, macrometastasis[c]	
	N2, in-transit metastasis[d]	

Abbreviations: MCC, Merkel cell carcinoma; a - Adapted by [1]; b - Micrometastases refer to clinically undetectable lymph nodes that are affected by the disease found in a sentinel lymph node or by elective dissection; c - Macrometastases refer to clinically evident and pathologically proven regional lymph nodes that are affected by the disease, using dissection or punch biopsy; d - In-transit metastases refer to metastases that are found between the primary tumor and the regional lymph nodes or distally of the primary tumor.

After setting the diagnosis, our patient was treated with therapy which led to a complete withdrawal of a tumour.

However, after 3 months the patient had repeated relapse of a tumour at the same site on the forehead and metastases in the retroauricular lymph nodes bilaterally. It shows that the radiotherapy as monotherapy has a great effect on the removal of the

tumour formation, but unfortunately, it has no impact on lesion recurrence.

Table 2: Staging of MCC according to the American Joint Committee on Cancer

Stage	Tumour	Lymph Nodes	Metastasis	5-Year Survival, %[a]
0	This	N0	M0	100
IA	T1	pN0	M0	79
IB	T1	cN0	M0	60
IIA	T2/T3	pN0	M0	58
IIB	T2/T3	cN0	M0	49
IIC	T4	N0	M0	47
IIIA	Any T	N1a	M0	42
IIIB	Any T	N1b/N2	M0	26
IV	Any T	Any N	M1	18

Abbreviations: MCC, Merkel cell carcinoma.

It is also compatible with the literature data. A study of 1.227 patients has proven that survival of 5 years is greater in patients with performed resection of the tumour formation in healthy tissue, compared to the definite radiotherapy of the disease [11].

In conclusion, an increase of MCC incidence in recent years has drawn attention to this malignant disease that attacks older people. [14] This is especially relevant to the increase in patient's lifespan. Treatment of Merkel cell carcinoma on the head and the neck requires an early and adequate diagnosis so that proper treatment can start. AEIOU (acronym) rule can be of great help in raising suspicion to this tumour. It includes surgery, radiotherapy and/or combined chemotherapy. An adequate stage assessment of the cervical lymph node is of supreme importance prior starting the definitive plan for treatment of the disease [15].

Partial or complete regression of the tumour is also observed but is a rare phenomenon. Regression is accompanied by dense lymphocytic infiltrate predominantly from CD8 phenotype and apoptosis [16].

Definitive radiation monotherapy is an alternative to surgery, for patients who are poor surgical candidates, or those in whom surgery would result in significant functional compromise. The outcomes of radiation monotherapy may be inferior compared to complete surgical resection. Overall survival is decreased (37-39%, 5 years survival with radiation monotherapy) compared to complete surgical resection [17]. In many adult patients, as our case suggests (93 years old patient, 3 cm tumour size), radiotherapy could be considered as treatment opportunity, but is not preferable, since tumour relapse is seen soon afterwards. However, only if we can - not perform a wide surgical resection, because of functional disability and/or other co-morbidities, we could consider it as the first option, because Markel cell carcinoma is a radiosensitive tumour.

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Case Report

Innovative One Step Melanoma Surgical Approach (OSMS): Not a Myth-It's a Reality! Case Related Analysis of a Patient with a Perfect Clinical Outcome Reported from the Bulgarian Society for Dermatologic Surgery (BULSDS)!

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Keywords: melanoma surgery; innovations; treatment options; survival; surgical approach

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Abstract

BACKGROUND: With the newly described one step melanoma surgical approach, some patient groups could be successfully treated within one surgical session. Depending on the tumour thickness (measured preoperatively) at a later stage (also depending on the ultrasound findings of the locoregional lymph nodes) the respective surgical intervention is planned with the respective field of surgical safety (one-stage melanoma surgery with or without removal of lymph nodes). The innovations could make to some extent some of the already existing algorithms more difficult (due to the introduction of a high-frequency ultrasound to determine the tumor thickness preoperatively as an absolute prerequisite for dermosurgical centres), but it would also lead with absolute certainty to better or least optimal results regarding the prognosis, the side effects and the financial factor also.

CASE REPORT: We present a patient from the Department of Dermatology, Venereology and Dermatologic Surgery at the Medical Institute-Ministry of Interior (MVR-Sofia), treated with the one-step melanoma surgery method with perfect final results. The preoperative tumour thickness determined via ultrasound and the postoperatively measured histological tumour thickness was identical: between 0.98 and 1 mm, which allowed removal of the melanoma lesion with a field of surgical security of 1 cm in all directions and did not require additional removal of a draining lymph node or excisions.

CONCLUSION: Thanks to this new approach, some patients could avoid one surgical intervention, which could be interpreted as a significant advantage or probably also survival benefit. This methodology and its successful application were first officialised by the representatives of the Bulgarian Society for Dermatologic Surgery-(BULSDS), and the purpose of this action, in general, is to fully improve clinical management of patients suffering from cutaneous melanoma in terms of compactness by 1) reducing the number of unnecessary surgeries or the number of surgical interventions in general; 2) reducing side effects occurring in surgeries and 3) introducing a serious optimization in terms of financial resources needed or used in the second hospitalization of patients. The question remains open whether the accepted or the current recommendations for surgical treatment of melanoma will be transformed or adapted for the matching patient groups.

Introduction

We are informing the dermatosurgical society about newly introduced therapeutic approaches that are different from the standard guidelines for treatment of melanoma in the international society. With the new described approach, the patients are successfully treated within one surgical session and are not subjected to the recommendations of the European and American guidelines for treatment of cutaneous melanoma. This is made possible by the careful measurement of the tumour thickness (preoperatively), as a mandatory initial condition is that the clinical and dermatoscopic characteristics of the lesion speak explicitly in favour of cutaneous melanoma.

The case is indicative of the new approach that is being presented and could be one of the

possible major innovations in the field of melanoma surgery today [1] [2] [3].

Case Report

We are reporting for a 72-years-old patient who was admitted to the Department of Dermatology, Venereology and Dermatologic Surgery at the Medical Institute of Ministry of Interior, (MVR-Sofia) because of a new lesion, which is localised in the head region – right parietal region. The complaints are from around 2 years. Reason for the hospitalisation - increase in the lesion size and the pigmentation intensity over the last 2 months. During the clinical and dermatoscopic examination were established the typical features of a malignant melanocytic lesion, namely: heterogeneous lesion regarding the colour gamut: light brown to black in places, appearing dark grey; comparatively clearly demarcated from the healthy tissue; with areas of elevation (with central localization); with diameter of 3.3 cm to 2.7 cm; mostly plaque-like or rather endophytically growing; with irregular shape, uneven distribution of the pigment; with areas of regression.

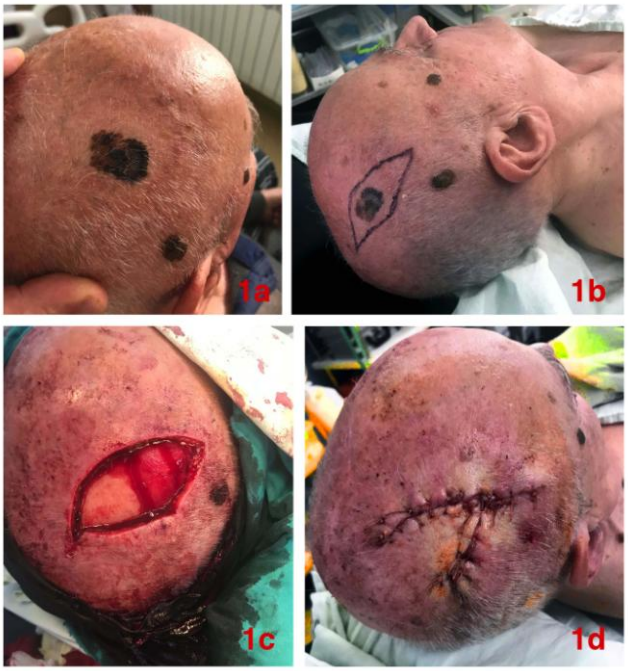

Figure 1: 1a) Patient with cutaneous melanoma; 1b) Surgical margins with 1 cm surgical safety filed, preoperative conditions; 1c) Intraoperative status; 1d) Postoperative clinical status

The measured with a high frequency ultrasound (15 MHz) (in an external institution) tumor thickness was established that this is most probably a malignant melanocytic lesion with tumor thickness less than 1 mm (0.98 to 1 mm). The histologically established postoperative tumor thickness was 1 mm, with no ulcerations, no signs of angiolymphatic invasion, no increased number of mitoses.

Arterial hypertension as co-morbidity was noticed. Systemic medication of Amlodipine 5 mg (0/0/1), Moxonidine 0\.2 mg (0/0/1), Irbesartan 0.3 g (300 mg) (1/0/0) was prescribed. There is no anamnestic evidence of allergies to food and medications. Mildly enlarged several lymph nodes above the right common carotid artery established via ultrasound, without definitive evidence of metastatic involvement. Performed was a surgical treatment with field of surgical security of 1 cm in all directions, the defect was initially closed with expandable flap, as intraoperatively (because of the inability to fully adapt the wound edges) a decision was made to perform additional progressive (advancement) flap, the skin flap was mobilized from the proximal part of the skin of the skull and translocated in distal direction (Fig. 1a-1d). The defect was closed in the form of the letter Y (Y-plastic). The postoperative course was without complications. In order to monitor the enlarged lymph nodes, ambulatory antibiotic therapy was planned with clarithromycin 500 mg for a period of 10 days and repeated ultrasound control.

Discussion

Until now it was recommended to remove any melanocytic lesion with a field of surgical security of 0.4 to 0.5 cm, and subsequently to perform re-excisions with or without parallel drainage lymph node (depending on the established postoperative tumor thickness) [1]. Thanks to this new approach, some patients could avoid one (or the secondary re-excision) surgical intervention, which could be interpreted as a significant advantage or probably also survival benefit [1] [2] [3].

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Dental Science

Free Gingival Graft versus Mucograft: Histological Evaluation

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Keywords: Gingival recession; Free gingival graft; Mucograft; Elastic fibres; Collagen fibres; Connective tissue

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Abstract

INTRODUCTION: The correction of the gingival recession is of esthetical and functional significance, but the tissue regeneration can only be confirmed by a histological examination.

AIM: This study aims to make a comparison between the free gingival graft and the autograft.

MATERIAL AND METHODS: This study included 24 patients with single and multiple gingival recessions. Twelve patients were treated with a free gingival graft and the other twelve with a micrograft. Six months after the surgical procedure, a micro-punch biopsy of the transplantation area was performed. The tissue was histologically evaluated, graded in 4 categories: immature, mature, fragmented and edematous collagen tissue. The elastic fibres were also examined and graded in three categories: with a normal structure, fragmented rare and fragmented multiplied.

RESULTS: Regarding the type of collagen tissue that was present, there was a significant difference between the two groups of patients, with a larger number of patients treated with a micrograft showing a presence of mature tissue, compared to the patients treated with a free gingival graft. A larger number of patients in both of the groups displayed elastic fibres with a rare fragmented structure; 33.3% of the patients showed a normal structure; 50% demonstrated a normal structure.

CONCLUSION: The patients treated with a free gingival graft showed a larger presence of fragmented collagen tissue and fragmented elastic fibres, whereas a mature tissue was predominantly present in the surgical area where a Geistlich Mucograft was placed.

Introduction

Despite the form, size and colour of the teeth [1], the anatomical characteristics of the gums have a great impact on the aesthetics and visual appearance of every individual. Regarding the previously stated, it is only logical that the recession of the gums is responsible for problems in the physiognomy, root hypersensitivity and the patient's fear of tooth loss.

However, the treatment of gingival recession is a quite complicated procedure, where the successful outcome depends on many factors, such as the initial condition of the gums, the biological capacity of the tissue, the type of surgical technique, the blood supply of the tissue, the regenerative potential of the periodontal tissue, etc. [2].

The biotype of the gums is also a critical factor that determines the outcome of the dental treatment, i.e. it has an impact on the therapeutic prognosis, which is based on the restorative, regenerative and implant treatment. The biotype of the gums is in a direct correlation with the appearance of gingival recession and the surgical techniques that can be used in the corrective procedures.

In the studies, guided tissue regeneration (GTR) is mostly recommended as a method of choice, because its main benefit is the formation of a new periodontal tissue attachment, which is also histologically confirmed. The newly formed attachment is colonised by ligament cells that eventually produce a new connective tissue [3].

According to Chambrone et al., [4] from a histological point of view, the use of CTG increases

the amount of keratinised tissue, providing much better protection from marginal inflammation and trauma.

From a histological aspect, the use of periodontal surgical methods should provide full or partial coverage of the exposed root surfaces with an actual periodontal regeneration [5]. Despite the quite predictable clinical results that derive from the use of various periodontal flaps in combination with a free gingival graft or a Mucograft, the histological results can be compared only by performing a biopsy from the transplantation area.

Many foreign doctors face difficulties when it comes to convincing the patients to agree on a tissue biopsy, despite the fact that this procedure is minimally invasive. Most of the comparisons derive from animal studies and biopsies [6] performed after extracting the teeth that have previously been treated with a free gingival graft or a micrograft. These biopsies display an obvious periodontal regeneration, which is confirmed by a histological examination.

The adhesion between the root surface and the graft indicates that the tissue recovery occurs primarily with the formation of a new periodontal tissue attachment between the root and the graft [7].

The goal of this study was to make a histological evaluation and comparison of the surgical area, six months after the procedure, between the uses of two types of graft: a free gingival graft and a Mucograft.

Material and Methods

The surgical and histological protocols were approved by the Ethics Committee of the Faculty of Dentistry in the University “St. Cyril and Methodius” in Skopje - Republic of Macedonia. Also, every patient received a thorough explanation, concerning the surgical procedure, prognosis and possible complications. If all of this were in order with the patient's expectations, we would receive their consent to start the procedure.

This study includes a total of 24 patients with single and multiple recessions of the gums, divided into two groups: group A (12 patients treated with a free gingival graft) and group B (12 patients treated with a Mucograft).

Every oral - surgical procedure was performed with the application of a 3% anaesthetic - Scandonest in the form of local infiltration anaesthesia with the use of a carpal syringe for the maxillary and mandibular nerves. The single and multiple gingival recessions were treated with Carl Martin GmbH instruments for periodontal surgery (Solingen - Germany). With number 15 surgical scalpels, an incision was made 2-3 mm from the gingival margin on the medial side of the upper first molar, going in depth right down to the periosteum, while maintaining a parallel course with the tooth position. In length, the incision spread all the way to the distal side of the canine, without including the palatal rugae, as not to compromise the esthetic results.

Because the path of the incision was parallel to the longitudinal axis of the tooth, damage to the palatal artery was avoided, and a separation of the connective tissue from the periosteum and epidermis was made in the desired length, according to the size of the recession, where the graft will be later placed. By size, the obtained graft was compatible to the recipient location and fixated to it with Vicryl absorbable sutures with a 5-0 thickness. The remaining part of the gingiva was sutured with non-absorbable sutures.

On the market, the Geistlich Mucograft can be found packed in two sizes: 15 mm x 20 mm and 20 mm x 30 mm, and which size will be used depending on the dimensions of the surgical region. The advantage of this collagen matrix is that it provides a simple application without any need of previous preparation and hydration.

After the dimensions of the surgical area are measured, the dry autograft is easily cut in the appropriate size and placed in the area of interest. Its hydrophilic capability enables it to be easily hydrated by the patient’s blood. The sutures are absorbable and fixate the graft with the adjacent tissue without any tension.

The micro punch gum technique was used for performing a biopsy of the area of transplantation (2 mm length), after which the tissue sample was sent for histological examination. The biopsy was performed six months after the surgical procedure with a previous application of a 3% local anaesthetic - Scandonest. The tissue sample was processed with a fixative agent in a formalin solution that was neutrally buffered and placed inside Eppendorf tubes for 6 to 18 hours and processed with embedding it in paraffin. 4 - 6 microsections were coloured with hemalaon eosin of LEICA automatic stainer. Afterwards, the tissue sample was submitted to a histological analysis to determine the structural characteristics, or more precisely the collagen and elastic fibres.

Histological verification of the tissue sample of every examinee was made, thus grading it in four categories: a) immature collagen tissue; b) mature (normal) collagen tissue; c) fragmented collagen tissue; d) edematous tissue. Regarding the structure of the elastic fibres, the tissue samples were divided into three groups: a) with a normal structure; b) rare fragmented structure; c) fragmented multiplied structure.

SPSS 17.0 was used for processing the acquired data. Factors of relations, proportions and rates were used for analysing the attribute series, thus presenting them as absolute and relative numbers. The numerical (quantitative) series were processed with the use of measures for central tendency (average, median, minimal value, maximal value) and a measure of dispersion (standard deviation). Pearson, Chi-square test, Fisher – Freeman - Halton exact test and Fischer exact test were used to help determine the difference between certain attribute marks. The Mann Whitney U test was used for analysing the significance of the difference between certain numeric variables. The significance of the p-value was set to 0.05.

Results

From a total of 12 patients, who were treated with a free gingival graft, a fragmented collagen tissue (Figure 1a) was present in 8 (66.7%) of them, whereas a normal (mature) collagen was seen in the remaining four patients (33.3%). None of the patients from this group displayed immature collagen tissue in the histological examination in the following checkup period.

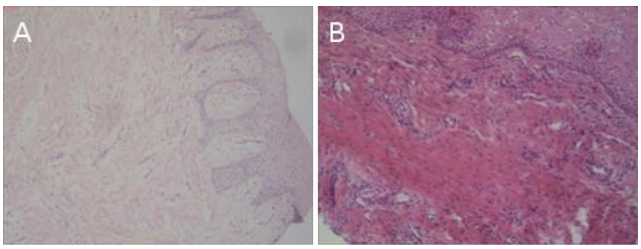

Figure 1: Fragmented collagen fibres in tissue samples, staining is H & E, shot with a 200x magnification Nikon Eclipse 80, six months after the surgical procedure: A) in a free gingival graft; B) in a Mucograft

In the other group of patients, where an autograft was used, in 9 (75%) of them was present a mature (normal) collagen tissue, whereas only 3 (25%) had an immature collagen tissue. There was no patient from this group that was registered with a fragmented collagen tissue.

Evidently, in the two groups of patients, there was no case with an appearance of edematous changes (not regarding its presence in the early stages of tissue formation). For a value of $p < 0.05$, a significant difference was confirmed between the two groups, regarding the present type of collagen tissue (Fisher – Freeman -Halton exact test: $p = 0.0009$), displaying a significant increase in the number of patients with a mature (normal) tissue, where a Mucograft was a method of choice (Table 1).

Table 1: Histological verification according to the type of collagen tissue in both of the groups, six months after the surgical procedure

Type of collagen tissue		Group Examined (FGG)	Controlled (Mucograft)	Total
Immature	Number	0	3	3
	%	0%	25%	
Mature (normal)	Number	4	9	13
	%	33,33%	75%	
Mature fragmented	Number	8	0	8
	%	66,67%	0%	
Total	Number	12	12	24
	%	50%	50%	100 %

Fisher - Freeman - Halton exact test: $p = 0.0009$*; *significance for a value of $p < 0.05$.

The analysis of the elastic fibres structure proved that no fragmented increased elastic fibres were present in any of the tissue samples. In the first group of patients, where an autograft was placed, 8 of them showed elastic fibres that were fragmented rare, whereas in 4 of them the elastic fibres were with a normal structure. In the second group of patients, where a Mucograft was used 6 of them had elastic fibres with a normal structure, whereas the other 6 had fragmented rare elastic fibres (Figure 2a).

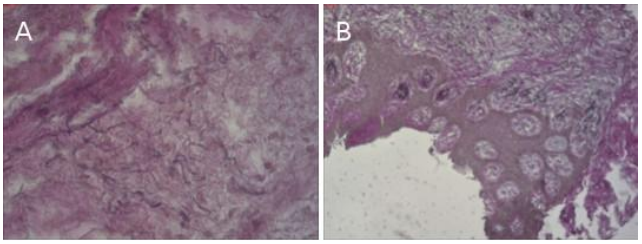

Figure 2: Elastic fibres with a rare fragmented structure in patients tissue samples, staining is H & E, shot with a 400x magnification Nikon Eclipse 80 six months after the surgical procedure: A) in an FGG; B) in a Mucograft

For a value of $p > 0.05$, no significant difference was present between the two groups, regarding the present type of elastic fibres (Fisher exact two-tailed test: $p = 0.6802$).

Table 2: Prevalence of the elastic fibres in the tissue samples according to their type, six months after the surgical procedure

Type of collagen tissue		Group Examined (FGG)	Controlled (Mucograft)	Total
Normal structure	Number	4	6	10
	%	33,33%	50%	
Fragmented rare	Number	8	6	14
	%	66,67%	50%	
Total	Number	12	12	24
	%	50%	50%	100 %

Fisher exact two tailedtest: $p = 0.6802$; *significance for a value of $p < 0.05$.

Discussion

The free gingival graft is the oldest surgical technique that is used in the periodontal surgery, where the graft is obtained from the palate or the maxillary tuber. Obtaining the graft from the maxillary

tuber region is justified with the fact that the operative site is small; the healing of the donor place is much more simple and faster than when the graft is taken from the palate. The only problem in obtaining a palatal graft is the risk of damaging the palatal artery, so a very precise preoperative evaluation of the maximal dimension of the palatal tissue is required [8].

Ensuring the stability of the postoperative area is a key factor that has a direct impact on the successful outcome of the surgical procedure. The initial adhesion of the blood clot on the root surface is of a great significance because it provides the much-needed pressure and support for the healing process. This is why the surgical method of choice and the first week after the procedure are crucial for accomplishing therapeutic success [9].

In this study, the final results were noted six months after the surgical procedure. Unfortunately, the assessment of the healing process and the histological characteristics of the tissue samples are not well documented in the available dental literature. Because of this, we had no possibility of comparing the results we detected in this study with results from other authors.

For determining the thickness of the palatal tissue, the CT [10] and ultrasound are considered to be the most advanced methods of choice in the everyday practice.

Other types of graft material that are used in the periodontal surgery are the collagen grafts, of which the most preferable is the Geistlich Mucograft, because its composition of collagen matrix is specifically designed to initiate soft tissue regeneration and ensure stability for the sutures with immediate support for the blood clot and early colonization of soft tissue cells (blood and nerve cells) [11]. The compact outer layer, which is comprised of collagen fibres with protective cell capabilities, doesn't only protect against bacterial invasion but also has a certain elasticity that simplifies the suturing process. The second layer is comprised of a dense, porous, spongy collagen structure, which enables an easy coagulum formation and helps promote angiogenesis and tissue integration [12].

The Geistlich Mucograft ® 3D collagen matrix is the ideal biomaterial for soft tissue regeneration. The collagen from the graft membrane provokes the host's fibroblasts to start producing new collagen fibres [13] [14]. The porosity of the Mucograft enables an enhanced infiltration of mesenchymal cells inside the area of transplantation. Unlike the typical reaction to foreign bodies (production of gigantic multinuclear cells, lymphocytes and granulation tissue), the host doesn't reject the Mucograft tissue and accepts it with no severe consequences.

The data that was gathered in this study has shown similarities with the results in Schmitt, et al., a study [15], in which he also examined the differences between a Mucograft and a free gingival graft, from a histological point of view. Schmitt suggests that the membrane of the Mucograft [16] is a promising alternative for regeneration of the keratinised mucosa, i.e. the Mucograft is a sufficient substitute for the free gingival graft when it comes to increasing the keratinised mucosa. Our study has shown concordance with Schmitt, regarding the fact that the Mucograft provokes similar clinical reactions in the early stages of regeneration with the natural tissue and that it has a more natural histological and clinical appearance that the free gingival graft. Schmitt also declared that a precise prediction of the duration and stability of the transplanted graft tissue cannot be proven yet, due to the lack of scientific studies in this area. Similar results are also found in McGuire and Scheyer study [17]. Their study confirms that the Mucograft collagen matrix is characterised by a shorter epithelium and an ability to successfully incorporate itself into the adjacent connective tissue of the host.

In conclusion, six months after the surgical procedure, a fragmented collagen tissue and fragmented elastic fibres were dominantly present in the tissue samples of the patients that were treated with a free gingival graft. However, a mature (normal) collagen tissue was found in the tissue samples of the patients that were treated with a Mucograft.

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Dental Science

An Anthropometric Study of Cranio-Facial Measurements and Their Correlation with Vertical Dimension of Occlusion among Saudi Arabian Subpopulations

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Keywords: Anthropometric measurements; the Vertical dimension of occlusion; Cranio-facial measurements; Full mouth rehabilitation

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Abstract

BACKGROUND: Determining and restoring physiological vertical dimension of occlusion (VDO) is the critical step during complete mouth rehabilitation. The improper VDO compromises the aesthetics, phonetics and functional efficiency of the prosthesis. Various methods are suggested to determine the accurate VDO, including the facial measurements in the clinical situations with no pre-extraction records. The generalisation of correlation between the facial measurements to VDO is criticised due to gender dimorphism and racial differences. Hence, it is prudent to verify the hypothesis of facial proportion and correlation of lower third of the face to remaining craniofacial measurements in different ethnic groups. The objective of the study was to evaluate the correlation of craniofacial measurements and OVD in the Saudi-Arabian ethnic group.

METHODOLOGY: Total of 228 participants from Saudi-Arabian Ethnic group were randomly recruited in this cross-sectional study. Fifteen craniofacial measurements were recorded with modified digital Vernier callipers, and OVD was recorded at centric occlusion. The obtained data were analysed by using the Spearman's correlation and linear regression analysis.

RESULTS: The Mean OVD in male participants was higher (69.25 ± 5.54) in comparison to female participants (57.41 ± 5.32). The craniofacial measurement of Exocanthion-right labial commissure and the Mesial wall of the right external auditory canal-orbitale lateral had a strong positive correlation with VDO. The strong correlation was recorded with a trichion-upper border of right eyebrow line and trichion-Nasion only in males. Meanwhile, the length of an auricle recorded the positive correlation in female participants.

CONCLUSIONS: Being simple and non-invasive technique, craniofacial measurements and linear equations could be routinely utilised to determine VDO.

Introduction

The prosthodontist has an important role in geriatric health care for an ever-increasing elder population with accompanying dental disabilities. The number of patients requiring prosthetic rehabilitation for complete or partial edentulism is continuously growing due to improved life expectancy across the world. The efficient prosthesis to replace the missing teeth helps to palliate the functional, aesthetic and psychological disabilities of the patient.

Complete denture prosthesis includes the replacement of the lost natural dentition and the associated structures of the maxilla and the mandible. Establishing the optimum maxilla-mandibular relations, including correct occlusal vertical dimension is crucial for the successful clinical performance of complete denture [1], implant-supported prosthesis [2] and full mouth rehabilitation for excessive dental attrition. According to the Glossary of Prosthodontic Terms, the occlusal vertical dimension (OVD) is defined as the distance between two selected anatomic or marked points (usually one on the tip of

the nose and the other on the chin) when in maximal intercuspal position [3]. Inaccurate OVD leads to multiple adverse effects on aesthetics, functional efficiency, temporomandibular joints, and masticatory muscles [4]. Furthermore, the improper dimension of the lower third of the face adversely affects the facial expression of the individual. Lack of reliable parameters makes the determination of OVD subjective and challenging task for the clinicians. Majority of researchers are of the opinion; the vertical dimension during denture rehabilitation ideally is similar to the OVD prior to the edentulous situation [5]. The methods to determine the OVD are broadly classified as Pre and Post extraction methods. The proposed pre-extraction methods are pre-extraction diagnostic casts [6], pre-extraction phonetics [7], cephalometric radiograph [8], profile tracing of the lower third of the face [9]. Reliability of these techniques are entirely dependent on the patient presentation in a dentate state. The pre-extraction methods are effective provided acceptable OVD, stable occlusion and satisfactory aesthetics prior to edentulism. Researchers have recommended numerous post extraction methods like physiologic rest position [10], facial aesthetic appearance [11], deglutition [12], cephalometric radiographs [13], post-extraction phonetics [14], measurement of the former dentures [15], fingers length [16] and anthropometric measurement. Anthropometry is the science of measuring the weight, size, and proportions of the human body, providing valuable and objective insights into how to characterize phenotypic variation and dysmorphology [17]. The anthropometric measurements are routinely obtained directly from subjects by utilising the callipers and measuring tapes. The philosophy of anthropometric measurement to determine OVD was derived from the Leonardo Da Vinci drawings, later explored further by researchers like Ivy, Good Friend and Willis. The additional advantages of anthropometry technique are simple, non-invasive, low-risk, and inexpensive

The generalisation of the correlation between craniofacial distances and the OVD is criticised owing to the likelihood of variation due to gender dimorphism and racial differences [18]. The knowledge of the correlation between Anthropometric measurement and OVD in local population will add the objectively during the full mouth rehabilitation with a complete denture, implant-supported prosthesis or fixed prosthesis. The studies on the correlation between Cranio-facial landmark's measurements and OVD in the Saudi-Arabian population are sparse in dental literature.

Hence the present study was designed with an objective to examine the correlation between the Occlusal vertical dimension and Craniofacial measurements in the Saudi-Arabian ethnic group. The objective was also to evaluate the variation of these correlations between male and female gender.

Material and Methods

The study proposal was approved by the institutional research ethics committee, at College of Dentistry, King Khalid University, Saudi Arabia (SRC/ETH/2016-17/037). The study design was a descriptive cross-sectional, and it was conducted between June 2017 to December 2017 in King Khalid University, Abha, Kingdom of Saudi Arabia. Total of 228 participants from Saudi Arabian ethnic groups, consisting of 72 males, 156 females were included in the study. The participants were randomly selected during their visits to King Khalid University dental clinic. The participants were in the age group of 20-40 years and residents across the Southern part of Saudi Arabia. The participants were explained about the study objectives and methodology; the informed consent was obtained. The participants with a full complement of permanent dentition without partial denture, and angle class I jaw relations were included in the study. The individuals with previous orthodontic or prosthodontic treatment, major stomatognathic surgery, temporomandibular disorders, facial deformities, asymmetry and scars in the lower part of the face were excluded from the study.

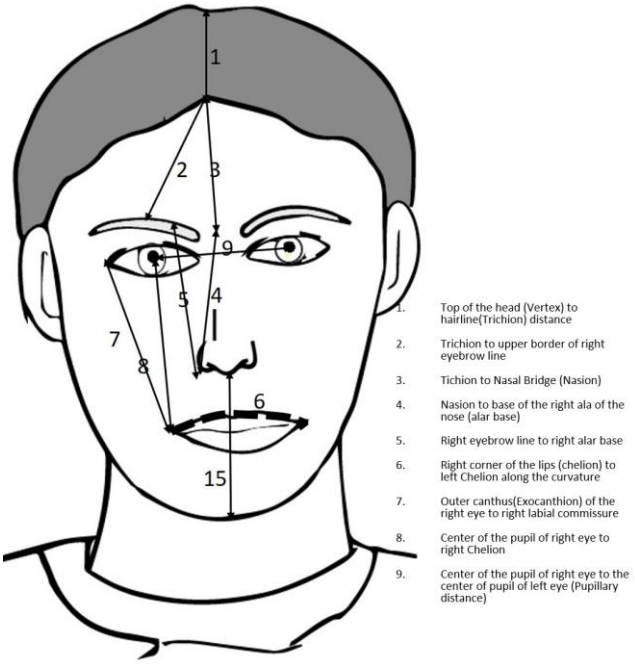

Figure 1: Figure displaying the craniofacial landmarks from 1-9

Before the craniofacial measurements, measurement training methods regarding the position of the participants, instrument handling, landmarks identification and recording procedures were confirmed and unified among the researchers. The digital callipers were calibrated with calibration rods. The facial measurements were recorded by two investigators. The inter-observer reliability was calibrated with a pilot study on 15 participants before the study. Intra-class correlation coefficient test was

performed (ICC value = 0.98, P < 0.01) to confirm the inter-observer reliability. The readings were independently recorded by two investigators and average was considered as final measurement.

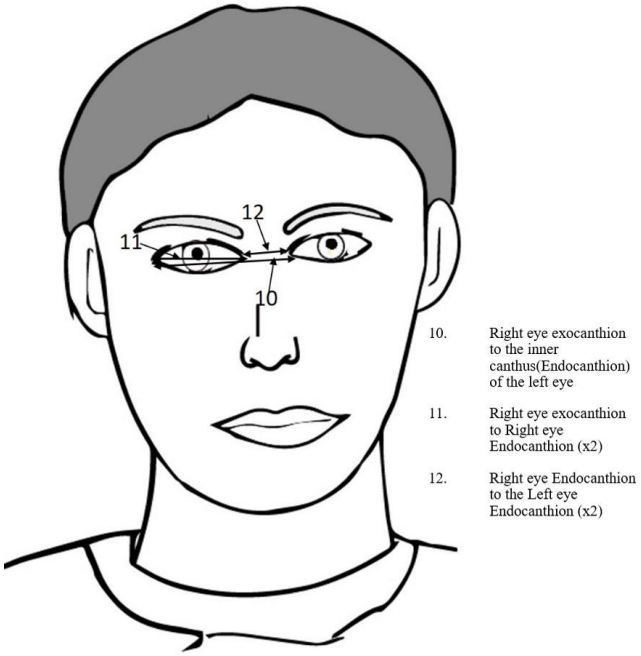

Figure 2: Figure displaying the craniofacial landmarks from 10-12

Participants were requested to sit in an upright position, head unsupported and occlusal plane parallel to the floor. It was verified with air bubble within the spirit level at the middle. The participants were guided to close at centric occlusion, and the soft-tissue points were marked by an indelible pencil. The facial measurements were registered with modified digital Vernier calliper (Mitutoyo 500-196, Illinois, USA). The Cranio-facial measurements [19] recorded during the study are as follows.

1) Top of the head (Vertex) to hairline (Trichion) distance

2) Trichion to the upper border of right eyebrow line

3) Tichion to Nasal Bridge (Nasion)

4) Nasion to the base of the right ala of the nose (alar base)

5) Right eyebrow line to the right alar base

6) Right corner of the lips (chelion) to left Chelion along the curvature

7) Outer canthus (Exocanthion) of the right eye to right labial commissure

8) Center of the pupil of the right eye to right chelion

9) Center of the pupil of the right eye to the centre of the pupil of the left eye (Pupillary distance)

10) Right eye exocanthion to the inner canthus(Endocanthion) of the left eye

11) Right eye exocanthion to Right eye Endocanthion (x2)

12) Right eye Endocanthion to the Left eye Endocanthion (x2)

13) Mesial wall of the right external auditory canal to lateral corner of the bony orbit (orbitale lateral)

14) Superior surface of right ear to inferior surface of the right ear (length of the auricle)

15) The lower border of the septum of the nose (Subnasale) to most under the surface of the mandible (Gnathion). This measurement was considered as an occlusal vertical dimension for comparison with other cranio-facial dimensions.

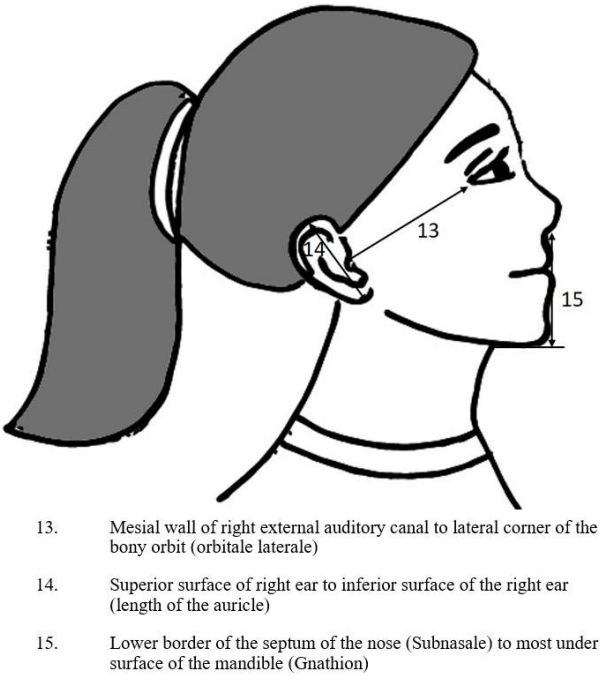

Figure 3: Figure displaying the craniofacial landmarks from 13-15

The statistical analysis was performed using the SPSS 19 software (IBM Corporation, Armonk, New York, USA). All the Cranio-Facial measurements were recorded in millimetres, and the mean, standard deviation were calculated. The correlation between the craniofacial measurements and the OVD were assessed by Spearman's rank correlation coefficient. The linear regression analysis was performed to determine the model fit equation to predict the OVD. The level of statistical significance was determined at P < 0.05.

Results

The descriptive values of all the craniofacial measurements recorded during the study for both gender are presented in Table 1. The Mean OVD (Subnasale-gnathion distance) for Saudi-Arabian ethnic male was recorded at 69.25 (5.54) mm, while the mean OVD for females was 57.41 (5.32) mm. There was a significant difference (Table 2) in the Mean OVD scores for males and female; t (226) = 15.400, p = 0.000.

Table 1: Descriptive statistics of vertical dimension of occlusion, and cranio-facial measurements for male participants (mm)

Cranio-facial landmarks	Mean (SD)	
	Male	Female
Subnasale-gnathion(ovd)	69.25 (5.54)	57.41 (5.32)
Vertex-trichion	113.15 (12.63)	85.58 (13.76)
Trichion-upper border of right eyebrow line	61.34 (8.65)	61.82 (7.94)
Trichion-nasion	65.30 (9.38)	65.00 (8.50)
Nasion-right alar base	61.83 (4.25)	63.32 (4.91)
Right eyebrow line-right alar base	63.22 (4.30)	63.10 (4.85)
Right Chelion-left Chelion along the curvature	65.01 (5.59)	62.45 (5.99)
Exocanthion of the right eye-right labial commissure	72.52 (4.45)	70.50 (4.02)
Center of the pupil of the right eye to right Chelion	64.42 (2.92)	64.86 (3.78)
Pupillary distance	65.87 (4.40)	57.79 (4.14)
Exocanthion of right eye-Endocanthion of the left eye	67.51 (4.06)	57.00 (5.04)
Exocanthion of right eye-Endocanthion of right eye (x2)	69.22 (5.43)	49.51 (11.16)
Endocanthion right eye-Endocanthion of left eye (x2)	66.37 (6.77)	54.37 (12.13)
Mesial wall of right external auditory canal-orbitale laterale	67.59 (4.34)	68.05 (4.54)
Length of the auricle	62.44 (3.50)	61.82 (4.44)

The Spearman's correlation test was performed (Table 3) to identify the presence of a relation between OVD and other craniofacial measurements. The results indicated the presence of statistically significant correlation ($p < 0.05$) between the OVD and Mesial wall of the right external auditory canal to Orbitale laterale, exocanthion of the right eye to Endocanthion of the left eye in both genders.

Table 2: Independent T-test to compare the OVD in male and female participants

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
OVD	Equal variances assumed	0.258	0.612	15.400	226	0.000	11.83333	0.76839	10.31921	13.34745
	Equal variances not assumed			15.164	133.083	0.000	11.83333	0.78038	10.28978	13.37689

The recorded measurements between Right eyebrow line -alar base, Nasion-alar base on right side and exocanthion of the right eye-right labial commissure showed the positive correlation with OVD in both genders. The positive correlation between OVD and distance between Trichion to the upper border of right eyebrow line, trichion to nasion was observed only in males. The OVD was significantly correlated with measurements between Exocanthion of right eye-Endocanthion right eye and Endocanthion of right eye-Endocanthion of the left eye in Females.

Table 3: Spearman's correlation matrix between OVD and other craniofacial measurements in both gender

Measurement	Male		Female	
	r	P	r	P
Vertex-Trichion	-0.107	0.372	0.057	0.479
Trichion-upper border of right eyebrow line	0.467	0.000*	0.140	0.080
Trichion-Nasion	0.568	0.000*	0.131	0.103
Nasion-right alar base	0.304	0.009*	0.198	0.013*
Right eyebrow line-right alar base	0.517	0.000*	0.243	0.002*
Right Chelion-left Chelion along the curvature	0.499	0.000*	0.080	0.323
Exocanthion of right eye-right labial commissure	0.578	0.000*	0.365	0.000*
Center of the pupil of right eye to right Chelion	0.373	0.001*	0.251	0.002*
Pupillary distance	0.386	0.001*	0.274	0.001*
Exocanthion of right eye-Endocanthion of left eye	0.315	0.007*	0.236	0.003*
Exocanthion of right eye-Endocanthion of right eye (x2)	0.148	0.214	0.173	0.031*
Endocanthion right eye-Endocanthion of left eye (x2)	0.217	0.067	0.230	0.004*
Mesial wall of right external auditory canal-orbitale laterale	0.362	0.002*	0.221	0.006*
length of the auricle	0.013	0.910	0.209	0.009*

*p < 0.05.

The Regression analysis was performed for prediction of OVD using craniofacial measurements (Table 4) and to obtain the linear regression equations. The significant regression equations were found with all craniofacial measurements, except vertex-trichion distance, Exocanthion of the right eye to Endocanthion of the right eye in both genders.

Table 4: Summary of Simple Regression Analyses for Variables Predicting OVD and Cranio-facial measurements (Male N = 72, Female N = 156)

Variable	Male				Female			
	R^2	B	*SE B*	*β*	R^2	*B*	*SE B*	*β*
Vertex-trichion	0.026	-0.071	0.052	-0.16	0.001	0.013	0.031	0.032
Trichion-upper border of right eyebrow line	0.134	0.235	0.071	0.367*	0.003	0.034	0.054	0.052
Trichion-nasion	0.229	0.283	0.062	0.479*	0.003	0.035	0.050	0.057
Nasion-right alar base	0.132	0.473	0.145	0.363*	0.021	0.158	0.086	0.146
Right eyebrow line-right alar base	0.205	0.583	0.137	0.453*	0.032	0.197	0.087	0.180*
Right Chelion-left Chelion along the curvature	0.196	0.438	0.106	0.442*	0.007	0.073	0.071	0.082
Exocanthion of right eye-right labial commissure	0.294	0.675	0.125	0.542*	0.127	0.471	0.100	0.356*
Center of the pupil of right eye to right Chelion	0.119	0.655	0.213	0.345*	0.061	0.347	0.110	0.247*
Pupillary distance	0.126	0.464	0.140	0.369*	0.074	0.348	0.099	0.271*
Exocanthion of right eye-Endocanthion of left eye	0.087	0.402	0.156	0.295*	0.038	0.207	0.083	0.196*
Exocanthion of right eye-Endocanthion of right eye (x2)	0.030	0.176	0.120	0.173	0.003	0.027	0.038	0.057
Endocanthion right eye-Endocanthion of left eye (x2)	0.139	0.306	0.091	0.373*	0.011	0.047	0.035	0.106
Mesial wall of right external auditory canal-orbitale laterale	0.077	0.354	0.147	0.277*	0.050	0.253	0.092	0.224*
Length of the auricle	0.000	0.009	0.189	0.006	0.039	0.238	0.095	0.199*

*p < 0.05 Abbreviations: the unstandardized beta-*B;* the standard error for the unstandardized beta *SE B*, the standardised beta β.

The prediction for OVD was insignificant with the length of the right auricle in males. Meanwhile, the poor prediction was observed with Endocanthion of right eye-Endocanthion of the left eye, and all facial measurements from trichion in females. The linear regression equations to predict the OVD from different cranio -facial measurements are presented in Table 5.

For the male subjects, OVD could be determined from other cranio-facial measurements utilizing following equations: Trichion-upper border of right eyebrow line: Y = 0.235 (IV) + 54.83, Trichion – Nasion = 0.283 (IV) + 50.78, Nasion-right alar base: Y = 0.473 (IV) + 40.01. The liner regression formula to predict OVD in both gender were: Right eyebrow line-right alar base: Y = 0.583 (IV) + 32.37 (males), Y = 0.197 (IV) + 44.97 (females), Exocanthion of right eye-right labial commissure: Y = 0.675 (IV) + 20.323 (males), Y = 0.471 (IV) + 24.22 (females), Center of

the pupil of right eye to right Chelion: Y = 0.655 (IV) + 27.07 (males), Y = 0.347 (IV) + 34.91 (females) and Pupillary distance: Y = 0.464 (IV) + 38.68 (males), Y = 0.348 (IV) + 37.30 (females).

Table 5: The regression equation to predict the OVD from different Cranio-facial measurements

	Male	Female
Variable	Regression equation	Regression equation
Vertex-trichion		
Trichion-upper border of right eyebrow line	Y=0.235 (IV)+ 54.83	
Trichion-nasion	Y=0.283 (IV)+ 50.78	
Nasion-right alar base	Y=0.473 (IV)+ 40.01	
Right eyebrow line-right alar base	Y=0.583 (IV)+ 32.37	Y=0.197 (IV)+ 44.97
Right Chelion-left Chelion along the curvature	Y=0.438 (IV)+40.74	
Exocanthion of right eye-right labial commissure	Y=0.675 (IV)+20.323	Y=0.471 (IV)+24.22
Center of the pupil of the right eye to right Chelion	Y=0.655 (IV)+27.07	Y=0.347 (IV)+34.91
Pupillary distance	Y=0.464 (IV)+38.68	Y=0.348 (IV)+37.30
Exocanthion of right eye-Endocanthion of left eye	Y=0.402 (IV)+42.12	Y=0.207 (IV)+45.63
Exocanthion of right eye-Endocanthion of the right eye (x2)		
Endocanthion right eye-Endocanthion of left eye (x2)	Y=0.306 (IV)+48.95	
Mesial wall of right external auditory canal-orbitale laterale	Y=0.354 (IV)+45.31	Y=0.263 (IV)+39.54
Length of the auricle		Y=0.238 (IV)+42.72

Abbreviations: OVD: occlusal vertical dimension; IV: Independent variable.

Discussion

Determination of accurate vertical dimension of occlusion is a critical clinical step during full mouth rehabilitation. It is challenging task for the clinicians to predict the appropriate OVD due to lack of teeth or the excessive loss of coronal tooth structure. The multiple clinical techniques are suggested by the researchers to determine accurate OVD. Cranio-facial measurements to predict the height of the lower third of the face is suggested by numerous authors. The hypothesis is on the premise of facial proportions and relationship of the lower third of the face with other craniofacial dimensions [20]. Multiple researchers have attempted to find the correlation between OVD and other craniofacial measurements in various ethnic groups. Since there is noticeable genotypic and phenotypic variation between ethnic groups [21], it is prudent to scrutinise the hypothesis in the different ethnic groups. Hence, this study was conducted to understand the association between OVD with craniofacial dimensions in Saudi-Arabian population.

In 1950 Fenn et al. [22], suggested the utilisation of dimension between outer canthus of an eye-angle of mouth to determine the accurate OVD. Several studies are conducted to evaluate the correlation between various craniofacial distances to predict the OVD and results are inconsistent. Boyanov [23] suggested the length of the upper lip to the distance measured from the tubercle of the mouth to the lower border of the chin is reliable for determining the OVD. The Chou et al. [24] proposed the eye – ear distance, but Al-Dhaher et al. [25], reported a non - significant correlation between the clinical OVD and eye-ear distances in males.

The study results indicated the mean OVD for a male was higher than the female counterparts. The Mean OVD recorded during the study was similar to the results described by Al-Hamdany et al. [26], in Iraqi male (68.25 ± 6.13). The OVD amongst the female participants was analogous to the investigation conducted among Indian population by Ladda et al. [16], (56.7 ± 3.0 mm) and Kulkarni et al. [27], (54-59 mm). However, the Mean OVD in male gender was higher in the current study. Majority of craniofacial measurements in the males were significantly higher than the female subjects; the altered growth and development due to the sex-related characteristics are attributed to the difference [28].

The distance between the Nasion to Alar base and eyebrow line to the Alar base showed the significant correlation with OVD in both genders. The research results are in confirmation with the recommendation of Earl Pound et al. [29], regarding the complete denture construction and anterior teeth placements. The distance between the chelion along the curvature was found to be positively correlated ($p = 0.000$, $r = 0.499$) with OVD only in the male. As suggested by Abhishek Nagpal et al. [30], it is highly inconsistent to be used to predict OVD. The results were in agreement with the findings of the anthropological study conducted within the Pakistani population [31]. The present study showed the strong correlation between Right eyebrow line to right alar base measurement and OVD; the similar observation was recorded by Majeed et al. [31].

The statistically significant correlation was observed between OVD and the dimension between pupils to the chelion in both genders. The studies conducted by the Nur Emalina et al. [32], among Sundanese population observed the similar strong correlations between the OVD and the pupil-rima oris distance. The inter pupillary distance between left-right eye also had the strong correlation with OVD with a p-value of 0.001. The Exocanthion of the right eye-Endocanthion of left eye distance also indicated the strong predictive capability for OVD. The Mesial wall of the right external auditory canal to orbitale lateral (eye to ear width) and centre of the pupil of the right eye to Chelion showed the statistically significant correlation with OVD, a similar observation was reported by Basent el al. [7]. The Chou et al. [24], Abdul-Rassol [34] opined eye-ear distance is a reliable predictor for OVD estimation. Subsequently, Delic et al. [18], recommended the eye-ear distance as a dependable method to determine the OVD. Al - Dhaher et al. [25], reported the significant variation in the correlation between ear-eye distance and stated the positive correlation only in female groups.

There was a significant positive correlation between Exocanthion of right eye-right labial commissure and OVD in both genders. Nagpal et al.

[30], evaluated multiple craniofacial measurements to predict the OVD accurately, and they proposed the distance from the Exocanthion of the eye to the labial commissure and the distance from the distal canthus of the eye to the tragus of the ear as valuable adjuvants to determine the OVD.

Based on paleoanthropology, modern humans from Africa colonise Eurasia through the northern Sinai Peninsula and Bab al Mandab strait routes. The researchers indicate the Saudi-Arabian ethnic groups genetic flow from its African and Asian surrounding areas, including Africa, India, Indonesia. This could be the reason for the craniofacial measurements were similar to other nationals [35].

The cephalometric analysis of Saudi Arabian national indicated the convex profile with increased ANB angle, retrognathic mandible and bi maxillary protrusion in comparison to Caucasians [36]. The difference in correlation between male and female craniofacial measurements are due to the more prognathic mandibles and steeper mandibular angle in male in comparison to females, although the anterior lower face height is similar for both genders [37].

The results of the study are in agreement with the observation of Chou et al. [24], that the left facial measurements were more reliable in predicting OVD than right side measurements. The variation could be due to right hemisphere dominance for emotional expressions. The mobility of facial expression also exhibits facial asymmetry, and studies indicated the left side of the face is most commonly dominant in both males and females [38].

The study findings revealed the unreliability craniofacial measurements involving the trichion in estimating the OVD. The positive correlation was recorded with a trichion-upper border of right eyebrow line and Trichion-Nasion only in males. Meanwhile, the length of the auricle of the right ear recorded the positive correlation with OVD in female groups.

The direct in-vivo measurement is still considered as a gold standard due to multiple advantages like simple, low-cost and do not require complex instruments. The facial soft-tissue dimensions, spatial positions and proportions are helpful for the clinician to develop the treatment plan, analyse the final treatment outcome. The thickness of soft tissue covering the teeth and bone varies in a different location; hence dento-skeletal measurement alone is insufficient for clinical evaluation. An additional disadvantage of radiological evaluation is exposing individuals to radiation. The anatomic anthropometric description will help in quantifying craniofacial deformities to treat the congenital or traumatic facial disfigurements. Hence ethnically specific facial measurement is of great assistance for orthodontists, maxillofacial surgeons, pedodontist, plastic surgeon to identify, assess and develop appropriate the treatment plan to treat facial disharmony. The anthropometric database is also utilised to identify the genetic disorders and recognize the individuals during forensic examinations. All facial measurements included in the present study excluding the measurement between Subnasale-Gnathion (OVD) is not affected due to teeth extraction. Hence, the unaffected craniofacial measurement will be helpful in predicting the OVD in edentulous patients and clinical situations with loss of occlusal dimension.

The limitation of the study was that only the Saudi-Arabian nationals were included in the study. Hence the results and regression formula cannot be extrapolated to other ethnic groups. The participants with Angles class I occlusion were included in the study; hence, the correlation in other malocclusion needs further investigation.

Within the limitations of the study, following conclusions were drawn.

The craniofacial measurement of Exocanthion of the right eye to right labial commissure and the Mesial wall of the right external auditory canal to orbitale lateral can be used a method for OVD approximation. The dimension between the centre of the pupil of the right eye to Chelion and Right eyebrow line to right alar base showed the strong positive correlation with OVD.Being simple and non-invasive technique, cranio-facial measurements could be routinely utilised to supplement the accurate determination of OVD. Researchers suggest the construction of ratios among all these craniofacial measurements to facilitate the subjective measurements of OVD, as using a calliper routinely is not highly applicable. It is recommended to utilise the linear regression equation and craniofacial measurements as an adjunct to determine the OVD in Saudi Arabian population.

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Haemostasis in Oral Surgery with Blue-Violet Light

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Keywords: Tooth extraction; Bleeding; Bleeding control; Haemostasis; LED irradiation

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Abstract

BACKGROUND: The invasive dental procedures usually result in wounds accompanied by physiological bleeding. Even though the bleeding is easily manageable, it is still one of the major concerns of the patients and a reason for their subjective discomfort. Recently, a novel approach with light-emitting diode (LED) was introduced to control the bleeding. This study aims to examine the effectiveness of the irradiation with blue-violet light LEDs on the haemostasis.

MATERIAL AND METHODS: The study included 40 patients with an indication for tooth extraction, divided into two groups: examination group (n = 30) and a control group (n = 10). The site of the extraction socket in the examination group was irradiated with LED (410 nm) until the bleeding stopped. The patients from the control group were treated by conventional gauze pressure to stop the bleeding (control group). The duration of irradiation and gauze pressure was measured and compared. The statistical analysis was performed with Student T-test.

RESULTS: The examination group showed the shorter duration of bleeding compared to the control group for 13.67 seconds and 156 seconds, respectively. The most of the cases in the examination group were irradiated in 10 seconds (70%), followed by irradiation of 20 seconds (23.3%) and 30 seconds (6.6%). In the control group, the average time to stop the bleeding by the conventional method was 156 second.

CONCLUSION: The blue-violet LED light shortens the bleeding time from the extraction socket after tooth extraction and may be a promising method for achieving haemostasis.

Introduction

Light presents a spectrum of electromagnetic particles that travel in waves. The shorter the wavelength is, the higher the energy. On one end of the visible light spectrum, there are the blue light rays with the shortest wavelengths (and highest energy) and this area of light is called blue-violet or violet light. This is why the invisible electromagnetic rays just beyond the visible light spectrum are called ultraviolet (UV) radiation (Figure 1). The blue light has a wavelength of approximately 380nm to 500nm; making it one of the shortest and highest-energy wavelengths.

The blue light heightens the reaction times, elevates moods, boosts awareness and increases the feeling of well-being. Artificial sources of blue light include electronic devices (cell phones, laptop computers) as well as energy-efficient fluorescent bulbs and light-emitting diode (LED) lights [1]**.** A LED is a two-lead semiconductor light source (p-n junction diode) which emits light when activated [2]. The "p" (positive) side contains an excess of holes, while the "n" (negative) side contains an excess of electrons.

When a suitable voltage is applied to the leads, electrons can recombine with electron holes within the device and energy is released in the form of photons. This effect is called electroluminescence, and the colour of the light corresponds to the energy of the photon [3].

Blue LEDs were first developed by Herbert Paul Maruska in 1972 using gallium nitride (GaN) on a sapphire substrate [4] [5]. Nakamura, Hiroshi Amano and Isamu Akasaki were awarded the Nobel Prize in Physics in 2014 for the invention of the blue LED [6]

[7].

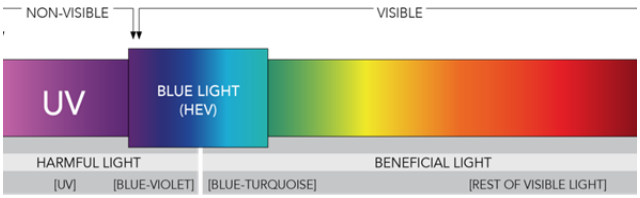

Figure 1: Location of blue light wavelengths in the spectrum

The invasive dental procedures usually result in bone and soft tissue wounds accompanied with physiological bleeding during the first few minutes. The most common technique to stop the postoperative bleeding is the superficial tamponade, i.e. placing gauze and applying pressure on it in the duration of 10-15 minutes. In patients without systemic diseases, disorders of the bleeding, coagulation or blood vessels and those who do not take any medications for altering the bleeding, this approach is appropriate enough to control the bleeding. However, even though the bleeding is easily manageable, it is still one of the major concerns of the patients and a reason for their subjective discomfort. Post-extraction dental sockets are filled with blood immediately after tooth extraction. The haemoglobin from the red blood cells has the strong light-absorbing capacity [10]. The absorbing haemoglobin can transform the light from the LED irradiation into heat energy on the bleeding surface (photocoagulation).

Some devices that are used in everyday dental practice for photopolymerisation of the composite fillings (LEDs) emit blue-violet light with a wavelength of 380-515 nm with two peaks (410 nm and 470 nm). These wavelengths can cover most of the absorption ranges of haemoglobin (430 nm). Control of bleeding is a major problem during oral-surgical interventions.

The study aims to examine the effectiveness of the new revolutionary method of irradiation with blue-violet light (LEDs) in achieving rapid and effective haemostasis.

Material and Methods

The study included overall 40 patients at the Clinic for Oral Surgery and Implantology at University Dental Clinical Centre ”St. Pantelejmon” in Skopje, scheduled in appropriate time periods for oral surgical interventions. The written informed consent (Declaration of Helsinki**)** was obtained from all patients with their signature before starting with the treatment protocol.

In the questionnaire, appropriate data were noted, such as age and sex of the patient, type of intervention, localisation of intervention, duration of intervention and postoperative condition. The age of the patients varied from 20 to 80 years. The reasons for extraction were gangrenous dental radices, periodontitis, chronic apical periodontitis (HAP) and prosthodontic reasons for extraction (teeth that were not included in the prosthodontic treatment). Exclusion criteria were as follow: patients with the compromised general medical condition, patients under 18 years, pregnant women, patients who received anticoagulation medication and patients with systemic bleeding disorders.

Dental extractions were performed under local anaesthesia Scandonest 2% (Septodont, United Kingdom), consisting of mepivacaine hydrochloride 2% with epinephrine 1:100.000. After the tooth extraction, the surgical debridement (granulation tissue curettage) was done. The LED irradiation was performed on 30 patients over the extraction socket for 10 seconds and an additional 10 seconds in those clinical cases where bleeding continued. The same operator irradiated the bleeding socket with LED light from a distance of 1 cm from the surgical site (Figure 2a, b). Another 10 patients were treated by conventional gauze pressure to stop the bleeding (control group).

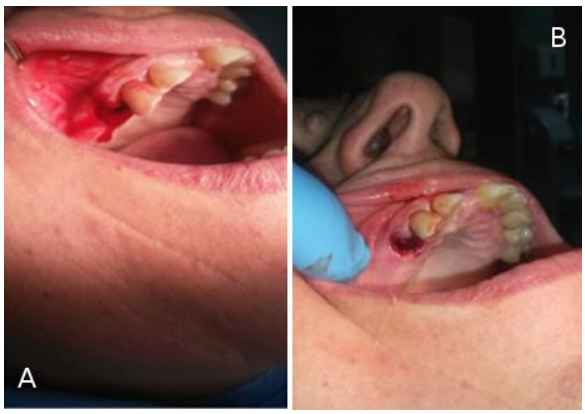

Figure 2: A) Extraction socket just after extraction of the maxillary right second premolar with obvious bleeding B) Extraction socket after LED irradiation

The study used LED device (Ivoclar Vivadent-Bluephase C5) with wavelengths of 380-515 nm and two peaks (410 nm and 470 nm) according to the protocol of Prof. Isao Ishikawa (Tokyo Women’s Medical University, Tokyo, Japan) [11].

Protocol for blue-violet LED (380-515 nm) irradiation (Prof. Isao Ishikawa) includes:

- Light guide aperture: 1 cm in diameter (0.785 cm^2).

- 750 mW/cm^2 at a distance of 1 cm from light emitter aperture.

- Irradiation area: approximately 1.25 cm^2.

- The irradiation time of 10 sec equals energy 50 joules and an energy density of 7.5 J/cm2.

- The irradiation time of 20 sec equals energy 100 joules and an energy density of 15 J/cm^2.

Bleeding time for both procedures was measured. Bleeding time was defined as the length of needed time to stop the bleeding from the extraction socket after a gentle wipe with a piece of gauze. After seven days the extraction sockets were reexamined to confirm the healing process.

The obtained data and values of the duration of bleeding were collected and statistically analysed using the statistical software SPSS Statistics, v. 20. Student t-test was used to compare the means of the values. The significance for p-value was set to 0.05.

Results

The mean age of the patients in the experimental group was 51, while in the control group it was 53 years. In the examination group, most of the teeth were indicated for extraction due to periodontal disease and prosthodontic needs. In the control group, the most frequent reason was the periodontal disease (Table 1).

Table 1: Distribution of the indications for tooth extractions in both groups

Indication for extraction	*Examination group (n=30)*		*Control group (n=10)*	
	Count	Percentage	Count	percentage
Periodontal disease	9	30%	4	40%
Proshtodontic needs	9	30%	2	20%
Remained necrotic root	5	16.6%	3	30%
Chronic periapical disease	7	23.3%	1	10%

The examination groups showed the shorter duration of bleeding compared to the control group for 13.67 seconds vs 156 seconds, respectively. The statistical difference was very strong (p = 0.000), (Tables 2 and 3).

Table 2: Means of the duration of bleeding in both groups

	Group	*N*	*Mean*	*Std. Deviation*	*Std. Error Mean*
Duration of bleeding	Examination group	30	13.67	6.149	1.123
	Control group	10	156.00	57.966	18.330

The most of the cases in the examination group were irradiated in 10 seconds (70%), followed by irradiation of 20 seconds (23.3%) and 30 seconds (6.6%). In the control group, the average time to stop the bleeding by the conventional method was 156 second.

Table 3: Significance of the values of bleeding between the groups (Student T-test)

		t	*df*	*Sig. (2-tailed)*
Duration of bleeding	Equal variances assumed	(13.574)	38	0.000
	Equal variances not assumed	(7.750)	9.068	0.000

The means of the duration of surgical procedure and significance of the difference of the values of duration of surgical procedures are presented in the Tables 4 and 5.

Table 4: Means of the duration of surgical procedure between the 10 seconds and > 10 seconds LED irradiated patients from the examination group

	Duration of irradiation	*N*	*Mean*	*Std. Deviation*	*Std. Error Mean*
Duration of surgical procedure	10 seconds	21	15.3571	1.63663	0.35714
	>10 seconds	7	19.2857	4.00892	1.51523

Discussion

The haemostasis is a complex process consisting of different steps, involving some cells and factors. The main purpose of this process is to stop bleeding by mechanical mechanisms of reparation of the injured blood vessels and clotting of the leaked blood. The first step is the vascular spasm as a first response to the injury.

Table 5: Significance of the difference of the values of duration of surgical procedures between the cases irradiated for 10 seconds and those irradiated for longer than 10 seconds, in the examination group

		t-test for Equality of Means			
		T	*df*	*Sig. (2-tailed)*	*Mean Difference*
Duration of surgical procedure	Equal variances assumed	(3.748)	26	0.001	(3.92857)
	Equal variances not assumed	(2.524)	6.679	0.041	(3.92857)

The vessels get constricted, and the blood flow decreases. Then, the platelets aggregate and release factors which further improve the vasoconstriction. The second step consists of the formation of the platelet plug, which depends on some factors from the plasma. The third step is the process of coagulation, which can be enhanced by the intrinsic or extrinsic pathway. Some coagulation factors are involved, and the final result is the formation of fibrin around the platelet plug, representing the blood clot.

The process of haemostasis after tooth extraction is enhanced by applying dressing pressure with gauze, for the duration of 10 minutes. The normal ranges of bleeding time by Duke are from 2-5 minutes [12]. In this time frame, it is expected the bleeding to stop. However, because the wound that is caused by such invasive procedure is sometimes bigger than just a simple blood vessel injury, the gauze is left in place for extended period of time. The other methods to control the bleeding are used usually when extensive bleeding is present.

The electrocoagulation is an effective way to stop the bleeding [13]. This is a physical method

which includes heat and causes almost immediate bleeding stop. However, the risk of damaging the surrounding soft and bone tissues make this approach only acceptable where very extensive and uncontrolled bleeding is present.

Various materials, such as hemostatic agents, gelatin sponges, hemostatic gauze and collagen have been used to help stop bleeding [14]. Also, various lasers are used in oral surgery because of their excellent cutting and hemostatic effects. The laser's effects are due to three phases included in the mechanism of photocoagulation. The first phase consists of heating effects, followed by formation of spherocytes and met-haemoglobin. Finally, extended coagulum formation happens due to spherocyte rupture [11]. Thermal damage and carbonisation of the underlying soft and hard tissues could be possible complications after laser irradiation [15]. The high financial cost of the laser apparatus could be a barrier for its wide application in dentistry.

LEDs are used for the photopolymerization of composite resin fillings. The device Ivoclar Vivadent-Bluephase C5 was used in our study because it emits a wide range of blue-violet specific wavelengths (380-515 nm) and two peaks (410 nm and 470 nm). These wavelengths can cover most of the absorption ranges of haemoglobin (430 nm).

Blue-violet LED irradiation immediately controlled postextraction bleeding in our study. The photothermal interaction caused superheating of the blood, absorption and scattering of the energy, surface condensation of proteins, and vaporisation of blood fluid followed by coagulation.

In the statistical analysis of the results, we obtained a significant association of the analysed parameters. Irradiation with blue-violet LEDs causes immediate haemostasis of extraction socket after 10 sec; some cases still needed additional 10 sec. A double length of irradiation was needed in 23.3% of the cases, while the triple length in only 6.6% of the cases. The examination group showed almost immediate bleeding stop after applying LED light, compared to the control group. These findings are very similar to the findings from author Isao Ishikawa et al., which tested the effects of LED on postoperative bleeding in oral surgery [11]. The duration of the irradiation needed to stop the bleeding in our study was also similar to his results.

A conventional method stops the bleeding for 2-5 min (mean interval 180 sec). LED irradiation of the extraction alveoli caused the immediate formation of a blood clot and achieved stable haemostasis. The LED application provided rapid haemostasis and better coagulum filling than conventional pressure dressing.

- Postoperative side effects were not noted

- The healing of the extraction site was uneventful

The transmission electron microscopy of the blue-violet LED irradiated blood clot found out a unique structure of the clot (photothermal interaction with the blue-violet LED). On the surface of the LED irradiated clot, there was a thin amorphous layer of denatured plasma proteins (about 50-1500 nm) [11]. This amorphous layer is clinically presented as a shiny surface of the LED irradiated blood clot. Beneath the layer, many agglutinated platelets and other cellular elements (red blood cells, leukocytes) supported the layer and formed a blood clot. Red blood cells seemed to keep their normal morphologies. In laser irradiation, the spherocytes and their rupturing are present extensively, and it crosses the phase of absorption of the wavelength from the haemoglobin. In our study only wavelength that is enough to cause photocoagulation was used. Therefore, the LED light has considerably slighter effects on the wound healing, compared to the laser light [11]. The LED application is less harmful to the eyes than lasers and is less expensive. However, it is known that the light is often unable to penetrate more than 2-3mm into the composite [16]. LED irradiation never causes adverse effects on the surrounding tissues.

LED photocoagulation may produce not only rapid haemostasis but also the acceleration of periodontal tissue healing. The LED application has the potential to benefit periodontal photo-engineering and bone repair processes. This better coagulum formation may result in early tissue repair with minimal alveolar bone resorption. Pinheiro and Gerbi [17] reviewed the recent studies of photo-engineering of accelerated bone repair processes by low-level laser irradiation and mentioned that fibrin on a coagulum would act as a framework for cell migration during bone healing. Based on these findings, it seems possible that the role of photomedicine, including photo engineering, will be recognised and applied in oral treatment shortly.

The results of a study where LED irradiation was implemented in combination with gelatin sponge in patients taking warfarin showed effects of enhanced haemostasis in 30 seconds [18]. This finding is of particular importance because no sutures and further invasive measures were undertaken in this group of patients, who usually need additional measures of bleeding control due to the changed coagulation process. The study supports the photocoagulation effects on the platelet aggregation and further improved blood clotting, which is the late phase of the haemostasis process. However, these statements should be further examined in more details.

Another study showed that LED light enhances the cell and immune response to some of the bacteria specimens involved in the developing of the periodontal disease [19]. It is well known that the stable and appropriate coagulation is a first and a very important step in the process of wound healing. Favourable healing means no postoperative

complications and less bone defect and resorption. When all these factors are combined, it becomes clear that the LED irradiation may positively affect both the immediate and later wound healing.

In conclusion, the blue-violet LED light with a wavelength of 410 nm significantly shortens the bleeding time from the extraction socket after a tooth extraction. It may be a promising method for controlling the bleeding in healthy patients. There is a need for future research in this field, and the possible effects in patients with bleeding disorders should be evaluated, as well.

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Dental Science

Anxiety, Stress and Coping Patterns in Children in Dental Settings

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Abstract

BACKGROUND: Fear of the dentist and dental treatment is a common problem. It can cause treatment difficulties for the practitioner, as well as severe consequences for the patient. As is known, the level of stress can be evaluated thought electrodermal activity, cortisol measure in saliva, or indirectly by psychometric tests.

AIM: The present study examined the psychological influence of dental interventions on the child as well as coping patterns used for stress diminution.

METHODS: We examined two matched groups of patients: a) children with orthodontic problems (anomalies in shape, position and function of dentomaxillofacial structures) (N = 31, mean age 10.3 ± 2.02) years; and b) children with ordinary dental problems (N = 31, mean age 10.3 ± 2.4 years). As psychometric instruments, we used: 45 items Sarason's scale for anxiety, 20 items simple Stress - test adapted for children, as well as A - cope test for evaluation coping patterns.

RESULTS: Obtained scores confirmed the presence of moderate anxiety in both groups as well as moderate stress level. For Sarason's test obtained scores for the group with dental problems are 20.63 ± 8.37 (from max 45); and for Stress test 7.63 ± 3.45 (from max 20); for the orthodontic group obtained scores are 18.66 ± 6.85 for Sarason's test, while for the Stress test were 7.76 ± 3.78. One way ANOVA confirmed a significant difference in values of obtained scores related to the age and gender. Calculated Student t - test shows non-significant differences in obtained test results for both groups of examinees. Coping mechanisms evaluated by A - cope test shows that in both groups the most important patterns used for stress relief are: developing self-reliance and optimism; avoiding problems and engaging in demanding activity.

CONCLUSION: This study confirmed that moderate stress level and anxiety are present in both groups of patients (orthodontic and dental). Obtained scores are depending on gender and age. As more used coping patterns in both groups are developing self-reliance and optimism; avoiding problems and engaging in demanding activity. Some strategies for managing this problem are discussed.

Introduction

Dental fear usually indicates an unpleasant emotional reaction to specific threatening stimuli occurring in situations associated with dental treatment, while dental anxiety is an excessive and unreasonable negative emotional state experienced by dental patients.

Anxiety, fear and perceived stress in the dental setting are common worldwide. These problems are particularly related to the pediatric dentistry. It was assumed that fear and anxiety have a mean prevalence between 10% and 20%, being very high in the earliest ages [1].

Statistics show that people from low socioeconomic status groups reported a higher level of dental fear than those individuals from high socioeconomic groups. The reported incidence of high dental fear and anxiety was 10% in an Icelandic study, but slightly higher in Singaporean population (17.1%). A cross-cultural study of Chinese and Danish patients reported moderate to high dental fear in 30% of Chinese and 15% of Danish participants. In 2009, a study of dental fear prevalence in the Netherlands reported 24.3% of the participants had moderate to high dental fear. Dental fear studies on German

populations have reported a mean Dental Anxiety Score of 8.6 and a dental phobia incidence of 11%. The highest prevalence of dental fear appears to be in Japan, where a study of 3041 students and adults reported that 42.1% had high dental fear [2].

However, perceived stress in dental setting can arise in both, patients as well as in dental practitioners. Many studies demonstrated that stress in dental practitioners arises from pediatric examination and treatment and can be broadly divided into those produced by the child and those produced by the child's guardian (usually the mother). On the other side, the patients (children) usually manifest some discomfort in a dental setting, which could be manifested as anxiety, worry or stress. In some patients, this anxiety can be so high and be presented as odontophobia. Fearful patients might neglect their teeth and oral hygiene and avoid any treatment procedures.

Processes known to contribute to the aetiology of dental fear and phobia include a variety of genetic, behavioural, and cognitive factors. Genetic vulnerability factors may interact with other etiological elements that cause a phobia.

The theory of classical conditioning explains acquired fear as a result of previous negative or traumatic experiences. Consequently, negative experiences during dental treatment are possible factors that promote dental anxiety, and several studies have findings that support this [3].

In our previous study [4] in a sample of 50 schoolers, we showed the presence of high anxiety level among all children undergoing dental intervention. There were differences in anxiety scores between girls and boys, girls having higher scores. Personality characteristics (evaluated with Eysenck personality questionnaire) showed low psychopathological traits, moderate extroversion and neuroticism, but accentuated insincerity (evaluated with L scale). We did not find a correlation between personality traits (obtained scores for EPQ) and anxiety, except for the neuroticism which was positively correlated with the level of anxiety.

This study aimed to evaluate anxiety and perceived stress in two groups of patients: orthodontic and dental, and to elaborate the patterns of coping mechanisms patients use to mediate the stress level.

Methodology and Sample

The evaluated sample comprised two groups of schoolers: a) children with orthodontic problems (anomalies in shape, position and function of dentomaxillofacial structures) (N=31, mean age 10.3 ± 2.02 years); and b) children with ordinary dental problems (N= 31, mean age 10.3 ± 2.4 years). Both genders were presented equally. Examinees were randomly selected.

The following psychometric tests were used: Sarason's General Anxiety Scale, Stress test for children and A-Cope questionnaire for assessing coping style.

The Sarason's General Anxiety Scale for Children (GASC) is a 45-item yes/no scale for use with children (grades 1-9). It measures chronic, generalised anxiety. The obtained score of 12 or below ranks in the low anxiety range. A score of 12-20 ranks in the medium range. Any score above 20 signifies high anxiety. Scoring 15 or higher is a good indication that a child experiences considerable discomfort about the situation in which he is [5].

Stress-test is a simple yes/no 20-item questionnaire where the higher scores are related to higher stress level [6].

The A-COPE is a coping inventory designed to explore children's coping behaviors that result from the normal stress associated with trying to create a balance between being connected to and at the same time independent from one's family [7]. The coping inventory identifies the behaviors children find helpful in managing problems or difficult situations. The A-COPE can be used as one single scale or broken into 12 sub-scales that reflect 12 different coping patterns: 1) ventilating feelings (like yelling and blaming), 2) seeking diversions (like sleeping or watching TV), 3) developing self-reliance and optimism (like organizing his/her life), 4) developing social support (like helping others solve their problems), 5) solving family problems (like working through family rules), 6) avoiding problems (like substance use or ignoring the problems), 7) seeking spiritual support (like talking to clergy), 8) investing in close friends (like boyfriends or girlfriends), 9) seeking professional support (like getting help from a counselor), 10) engaging in demanding activity (like strenuous physical activity or academically challenging activity), 11) being humorous (like making a joke of the situation), and 12) relaxing (like listening to music). Psychological tests in this study were applied prior to dental intervention. Children were usually accompanied by their mothers and they gave prior consent for the study.

For statistical calculations, the online package Statistics 8 was used.

Results

Two groups of examinees were included: a) 31 children with orthodontic problems, mean age 10.3 ± 2.02 years; and b) 31 children with simple dental problems, mean age 10.3 ± 2.4 years. Examinees

were matched by age and gender.

Evaluated by Sarason's anxiety test the obtained scores for the group with dental problems were: 20.63 ± 8.37 (from max 45); these results correspond to moderate anxiety level. Evaluated by Stress test 7.63 ± 3.45 (from max 20), which correspond to small stress level. The obtained scores in the orthodontic group were: 18.66 ± 6.85 using Sarason's anxiety tests and 7.76 ± 3.78 using Stress test (Fig. 1).

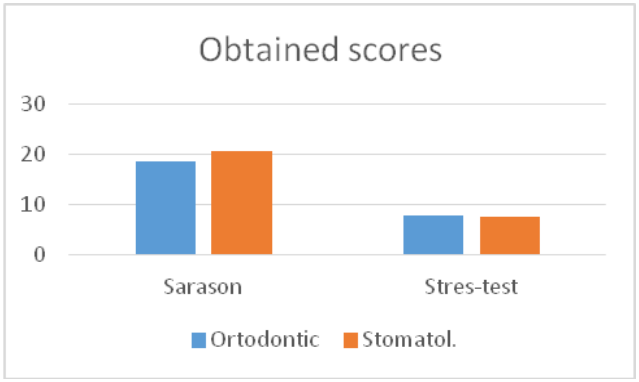

Figure 1: Obtained scores for both psychometric tests in orthodontic and dental patients

Calculated one-way ANOVA showed a significant variance in scores obtained using Sarason's anxiety scale related to age in both groups of patients.

Calculated one-way ANOVA for the significance of age in stress test is presented in Table 1. In this calculation results also confirmed the influence of the age on the variance of obtained scores.

Table 1: ANOVA-related-age and scores using stress-test in both groups

a)

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	2229.803	1	2229.803	400.6634	0.000000
Stres test	110.125	11	10.011	1.7989	0.129696
Effor	100.175	18	5.565		

b)

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	2514.056	1	2514.056	539.8604	0.000000
Stres test	43.800	12	3.650	0.7838	0.660721
Effor	79.167	17	4.657		

Correlation between age and scores evaluated by Sarason's anxiety test is shown in Fig. 2. There was a small positive correlation between the two mentioned variables.

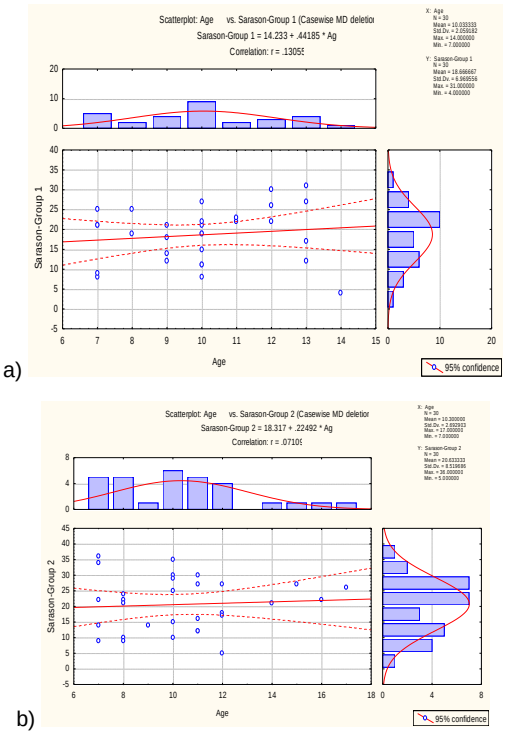

Figure 2: Correlation between age and scores obtained with Sarason's anxiety test

Correlation between scores obtained with Stress-test for both groups of patients is presented on Fig. 3.

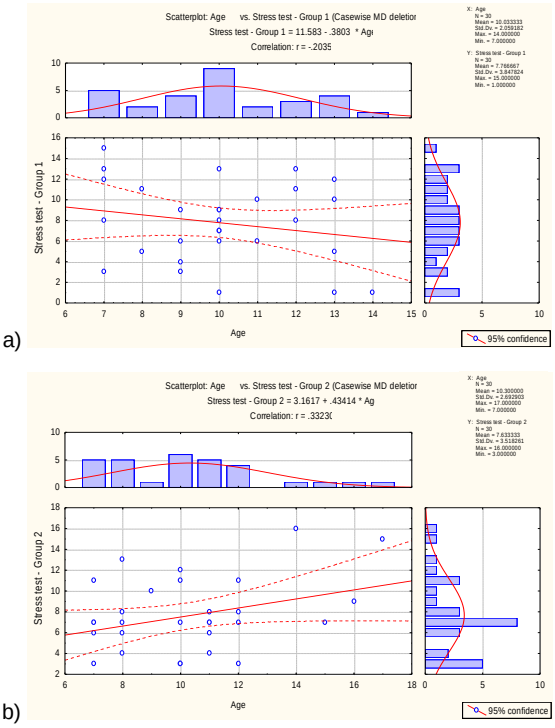

Figure 3: Correlations between age and obtained scores with Stress-test in both groups of examinees

As it can be seen, the correlation between age and obtained scores using Stress-test is negative

for orthodontic patients but positive for dental patients.

Finally, we used Student's t-test for obtained scores in both groups for both psychometric tests (Fig 4 and 5).

T-test for Independent Samples (Copy of sarakinova-excel 97)
Note: Variables were treated as independent samples

Group 1 vs. Group 2	Mean Group 1	Mean Group 2	t-value	df	p	Valid N Group 1	Valid N Group 2	Std.Dev. Group 1	Std.Dev. Group 2	F-ratio Variances	p Variances
Sarason-Group 1 vs. Sarason-Group 2	18.6666	20.6333	-0.97861	58	0.331834	30	30	6.969556	8.51968	1.494297	0.285216

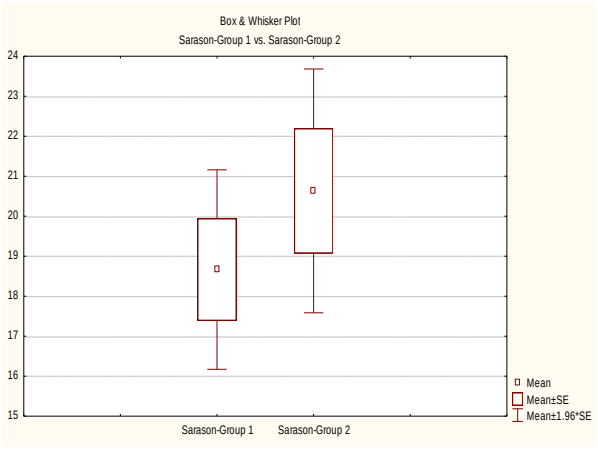

Figure 4: T-test for scores obtained with Sarason's anxiety test in both groups

The Student's t-test showed no significant differences in obtained scores for both tests in both groups of examinees.

T-test for Independent Samples (Copy of sarakinova-excel 97)
Note: Variables were treated as independent samples

Group 1 vs. Group 2	Mean Group 1	Mean Group 2	t-value	df	p	Valid N Group 1	Valid N Group 2	Std.Dev. Group 1	Std.Dev. Group 2	F-ratio Variances	p Variances
Stress test - Group 1 vs. Stress test - Group 2	7.76666	7.63333	0.140061	58	0.889096	30	30	3.847824	3.518261	1.196111	0.632837

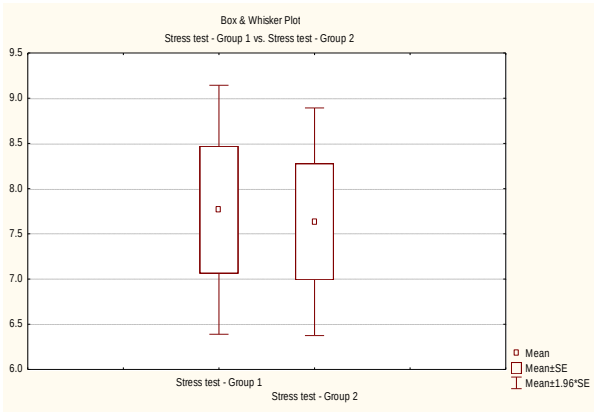

Figure 5: T-test for scores obtained with Stress-test in both groups

Coping can be defined as a set of cognitive and effective actions that arise in response to a particular concern. They represent an attempt to restore the balance or remove the turbulence for the individual. This may be done by solving the problem (removing the concern) or accommodating the concern without bringing about a solution.

The A-COPE applied in this research can be used as one single scale or broken into 12 sub-scales that reflect 12 different coping patterns: 1) ventilating feelings (like yelling and blaming), 2) seeking diversions (like sleeping or watching TV), 3) developing self-reliance and optimism (like organizing his/her life), 4) developing social support (like helping others solve their problems), 5) solving family problems (like working through family rules), 6) avoiding problems (like substance use or ignoring the problems), 7) seeking spiritual support (like talking to clergy), 8) investing in close friends (like boyfriends or girlfriends), 9) seeking professional support (like getting help from a counselor), 10) engaging in demanding activity (like strenuous physical activity or academically challenging activity), 11) being humorous (like making a joke of the situation), and 12) relaxing (like listening to music).

The obtained all 12 coping patterns for patients in both groups are presented in Fig. 6.

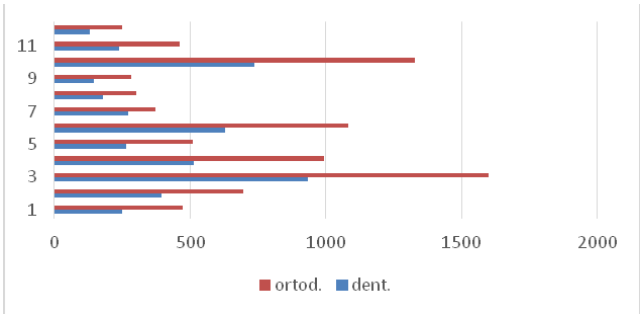

Figure 6: Obtained 12 coping patterns in both groups of patients

Coping mechanisms evaluated by A-cope test showed that in both groups the most important patterns used for stress relief were: developing self-reliance and optimism (3); avoiding problems (6) and engaging in demanding activity (10).

Discussion

The results of our study revealed the presence of moderate anxiety and relatively low-stress level in evaluated school children in both groups (orthodontic and dental settings). These results differ from our previous study [8] where they obtained anxiety scores were more accentuated and higher for girls compared to boys. We suppose that pretreatment preparation organised in the last period, as well as teaching children for the necessity of oral hygiene in elementary school, are very important for diminishing anxiety/stress manifestations. Our results are quite similar to the other studies in this context.

In a recent study published by Nelson et al., 2015 [8], the aim was to identify factors related to young children's distress during preventive oral health visits. The study showed that the majority of parents report that young children experience moderate to severe distress during preventive dental treatment. Pre-examination distress and difficulty with prior medical examinations and immunisations are

significantly associated with distress during dental exam. Additionally, it was suggested that dental providers could help parents of young children to develop a habit of routine pediatric preventive care by anticipating child behaviours, informing parents about possible child reactions, providing parent coaching, and altering their style to facilitate a positive experience.

A similar study related to anxiety in dental practice was performed by Storjord et al., 2014 [3]. The authors compared dental anxiety in students of dentistry, biology, and psychology and showed that dental students demonstrated a lower degree of dental anxiety compared to psychology students and biology students. Senior dental students with clinical experience also showed a lower dental anxiety level than junior dental students. The practice-oriented dentistry education at the university might contribute to the differences in anxiety levels between new and experienced dentistry students.

Having in mind that dental fear and anxiety are strong negative emotions associated with dental treatment especially among children and adolescents, Cainetti et al., 2017 [9] conducted a meta-analysis about interventions used to diminish these problems. Two main techniques were analysed: pharmacological and non-pharmacological. Non–pharmacological interventions can be theoretically grouped into improved communication skills, rapport and trust building; behaviour modification techniques; cognitive behavioural therapy and physical restraints. Authors supported the second approach as more available and useful. In an earlier study, Hunter et al., 1990 [10] proposed rectally administered methohexital as a safe, effective sedative to ameliorate the stress of the dental surgical experience for the uncooperative child.

Unlike dental care for adults, care for young children necessitates a triadic relationship among the patient, parent, and clinician. Research demonstrates that the dental fear of a child who is 8 years or younger is significantly related to the dental fear of the parent. Emotional aspects of the dental experience, for the child and the parent, influence a parent's decision to return for subsequent dental visits [11].

Recommendations derived from the literature suggest that medical providers' use of distraction, nonprocedural talk, breathing exercises, specific directions to the child, and physical contact such as bouncing, patting, and rocking may improve a child's reaction to care. It has also been suggested to use the live or Filmed modelling technique as an effective intervention to prepare the child for a dental visit [12].

Interventions which can be useful for fear diminution are cognitive behavioural therapy, relaxation therapy, computer-assisted relaxation learning, hypnotherapy, group therapy, individual systematic desensitisation, pharmacological, flooding (implosion), and swallowing relaxation. These forms of treatment are essentially a form of counter conditioning to reverse the fear/anxiety into a state of acceptance and calm. Our own experience with peripheral biofeedback procedure showed a very positive effect to stress diminishing in different groups of children-patients [13] [14]. We strongly recommend the use of this technique.

In conclusion, the study confirmed moderate anxiety and relatively normal stress level in school children undergoing orthodontic and dental interventions. The obtained scores for psychometric tests are significantly different according to age (one-way ANOVA). No significant differences were obtained between mean values of scores in both groups of examinees and for both psychometric tests (t-test was > 0.05). Using the Sarason's anxiety test a very small positive, but not significant correlation was obtained for age and scores ($r = 0.13$; $r = 0.7$, respectively). Using the Stress-test, calculated correlations between age and scores differ: it was positive for dental ($r = 0.33$) and negative for an orthodontic group of patients ($r = -0.20$), but without statistical significance. Three main coping patterns were used for stress mediation in the evaluated children: developing self-reliance and optimism (3); avoiding problems (6) and engaging in demanding activity (10).

We recommend the use of peripheral biofeedback for diminishing anxiety and stress as an easy to apply and highly cost-benefit procedure in children.

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Dental Science - Review

Exploring the Gingival Recession Surgical Treatment Modalities: A Literature Review

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Abstract

Gingival recessions present complex soft tissue pathology, with a multiple aetiology and a high prevalence which increases with age. They are defined as an exposure of the root surface of the teeth as a result of the apical migration of the gingival margin beyond the cementum-enamel junction, causing functional and aesthetic disturbances to the affected individuals. Aiming to ensure complete root coverage and satisfying aesthetic outcomes, a wide range of surgical techniques have been proposed through the decades for the treatment of the gingival recessions. The following literature review attempts to provide a comprehensive, structured and up-to-date summary of the relevant literature regarding these surgical techniques, aiming to emphasise for each technique its indications, its long-term success and predictability, its advantages and disadvantages about each other.

Introduction

Gingival recessions present one of the most common aesthetic and functional problems of the periodontium, but also one of the most complexes regarding the aetiology and the treatment modalities. They are defined as an exposure of the root surface of the teeth as a result of the apical migration of the gingival margin beyond the cementum-enamel junction [1] [2] [3]. It is very common: 50% of subjects in the populations studied have at least one or more sites of 1 mm of root exposure or more[1] [4] [5] [6]; it affectspatients with both good and poor oral hygiene [7] but with a higher prevalence in males [8] and in older ages [7]. It may be localized or generalized and it can affect one or more tooth surfaces, with the buccal ones being most frequently affected [7].

Besides aesthetic shortcomings [7] [8], gingival recessions have a high predisposition to be associated with functional problems related to root exposure, such as dentinal hypersensitivity [9] [10] [11], plaque retention, gingival inflammation, root caries [12] [13] [14] [15] [16], alveolar bone loss and eventually tooth loss [16] [17].

Like in many other periodontal conditions, the aetiology of gingival recessions is multifactorial and complex, with its exact mechanism not fully understood yet. It intertwines predisposing anatomic risk factors-such as bone dehiscence [18], gingival width and thickness insufficiency, tooth malposition

[19] [20], aberrant attachment of the labial frenulum [1] [21] [22] with precipitating factors such as inflammation related to plaque, improper tooth brushinghabits [6] [21] [22] [23] [24], smoking [10], chronic trauma because of traumatic incisor relationship and iatrogenic factors related to improper restorative, prosthetic, orthodontic and periodontal procedures [25] [26].

Considering the high prevalence of this condition, the aesthetic and functional problems related to it and the challenges its treatment presents, a thorough understanding of the disease and its treatment modalities is of crucial importance, to manage it successfully and with predictable long-term outcomes.

Many attempts have been made by different authors [27] [28] [29] to provide a comprehensive classification system regarding gingival recessions. Miller [28] proposed useful recession defect classification based on the height of the interproximal papillae and interdental bone adjacent to the defect area, and the relation of the gingival margin to the mucogingival junction. This classification is useful when deciding on treatment options [30]. Nowadays, it is the most widely used.

Class I: Marginal tissue recession not extending to the mucogingival junction (MGJ). No loss of interdental bone or soft tissue

Class II: Marginal recession extending to or beyond the MGJ. No loss of interdental bone or soft tissue

Class III: Marginal tissue recession extends to or beyond the MGJ. Loss of interdental bone or soft tissue is apical to the CEJ but coronal to the apical extent of the marginal tissue recession.

Class IV: Marginal tissue recession extends to or beyond the MGJ. Loss of interdental bone extends to a level apical to the extent of the marginal tissue recession.

The key factors which determine the successful management of gingival recessions are the identification of its etiologic agents and their elimination, the assessment of the degree of tissue involvement and last but not least, the selection and the careful implementation of the appropriate surgical procedure in order to achieve optimal root coverage, improved soft tissue aesthetics and reduced sensitivity.

The selection of the surgical technique is influenced by some important factors related to the anatomy of the defect such as the size of the defect, the width of the keratinized gingiva apical to the recession, the thickness of the flap, the level of the interdental papilla and the alveolar bone, the vestibular depth and the position of the labial frenulum.

Evidence shows that the size of the initial recession defect will determine the amount of root coverage achieved [31]. Miller class I defects can achieve complete root coverage in 100% of cases, whereas in class II defects complete root coverage is seen in 88% of cases [28]. Larger recession defects rarely achieve full coverage. One study showed recession defects of 3-5 mm only managed to attain 80.6% coverage and recessions[32] greater than 5 mm only attained 76.6% root coverage with free gingival grafts. Nelson[33]reported 100% root coverage in recession defects less than 3 mm, 92% root coverage in recession defects of 4-6 mm and 88% in recession defects of 7-10 mm. Overall better results regarding the percentage of complete and mean root coverage can be achieved if defects are less than 4 mm [31].

Since 1960, a wide range of surgical techniques have been proposed for the treatment of the gingival recessions such as: the free gingival epithelialized graft [27]or free partially epithelialized graft [34], pedicle flaps such double papilla rotational flap [35] laterally repositioned "stimulated" osteoperiosteal pedicle [36], laterally advanced flap [37] [38], coronally advanced flap [39] [40] [41], subepithelial connective tissuegraft [42] (the so-called envelope technique)and their modifications [43] [44]. Other authors have also combined some of the above-mentioned techniques, especially the coronally advanced flap technique, with enamel matrix derivative [45] [46], non-resorbable membranes [47] [48], resorbable membranes [49] [50], acellular dermal matrix allografts [51], xenogeneic collagen matrix [52] [53], platelet-rich plasma [54] [55] and living tissue-engineered human fibroblast-derived dermal substitute [56].

In general, the surgical procedures can be broadly classified in pedicle flap procedures, free graft procedures and guided tissue regeneration procedures either with resorbable or non-resorbable membranes [25]. Several modifications to the conventional techniques have been developed in an attempt to obtain optimal root coverage and better aesthetics.

The pedicle flap was the first periodontal plastic surgery procedure proposed in 1956 for root coverage [37]. This procedure consists in the repositioning of the donor tissue from an area adjacent to the recession defect to cover the exposed root surface. Since the flap remains attached at the base, it retains its blood supply, facilitating the revascularisation with the recipient site. Pedicle flap procedures involve:

a) Rotational flap procedures, which include a laterally positioned flap and the double papillae flap.

b) Flap advancement procedures such as the coronally advanced flap.

Pedicle flap surgical techniques offer long-term predictability and satisfying aesthetic results in cases of relatively shallow single or multiple recession defects (< 5 mm) and if there is adequate keratinised tissue close to the recession defect. They are contraindicated in cases with an inadequate width of the keratinised gingiva, in subjects with a shallow vestibulum, or with a high insertion of the frenulum.

The laterally positioned flap procedure was the first pedicle graft procedure that was used for the treatment of gingival recessions [37]. It was first introduced by Grupe and Warren in 1956 [37] and later modified by Grupe [57]. It was a full-thickness flap prepared from the adjacent site on the side of the recession and repositioned to cover the defect. This was later modified by Hattler [58] who used a split-thickness flap repositioned in a similar way to cover multiple exposed root *surfaces*. Pfeifer and Heller [59] advocated the use of this split-thickness flap to minimise the potential risk for development of dehiscence at the donor's tooth.

The success rate reported for this technique was 69%- 72% [60]. Literature findings suggest that several factors contribute to the success of the procedure. Factors such as the existence of a shallow recession defect [60] the adequate height and width of the keratinized tissue lateral to the recession [25], the wide dimensions of the pedicle and the adequate tissue thickness of the flap [55] are critical in order to achieve predictable root coverage and good aesthetic results. The main advantages of the laterally positioned pedicle graft that it is relatively easy and not time-consuming, it produces excellent aesthetic results and avoids the need for a second surgical site [61]. The disadvantages, however, include the fact that it is applicable only for single-site recession, that there is a possible risk of gingival recession, dehiscence, or fenestration at the adjacent donor site, and that an adequate amount of keratinised tissue at the neighbouring donor site and a deep vestibule are needed.

Double papillae flap (DPF) procedures

This procedure was introduced by Cohen and Ross [35] to overcome the limitations presented by the laterally positioned flap regarding adequate width and height of keratinised gingiva. Since the procedure consists in the coverage of the exposed root by the interproximal papillae of both sides, it can be used in cases where there is insufficient keratinised gingiva on any one side of the recession defect. The excellent aesthetic result thanks to the perfect colour matching of the donor tissue with the recipient is the main advantage of this technique. Anyway, the procedure presents some major drawbacks such as its limitation to single recession defects and its poor predictability [62].

Coronally advanced flap (CAF) procedures

This procedure was first presented by Bernimoulin et al., [41] and it involves the coronal repositioning of the gingival tissue that lies apical to the recession defect. In cases representing shallow recession defects, a thick gingival biotype and a sufficient amount of keratinised gingiva, it can be performed as a one-stage procedure [40]. In other cases, when the thickness and/or amount of the keratinised gingival tissue are an issue, there is the need first to increase the thickness and the amount of the gingiva using a free gingival graft, a connective tissue graft or a resorbable/non-resorbable membrane (guided tissue regeneration). At a second stage, after three months of healing, the tissue can be coronally advanced to cover the recession defects. Since the soft tissue used to cover the root exposure is similar in colour, texture and thickness and blends perfectly with the in-situ gingiva, the coronally advanced flap procedures provides great aesthetic results [63], as long as some critical criteria-such as the presence of adequate keratinized tissue apical to the root exposure, the presence of adequate sulcular depth and no interproximal bone loss- are met [55].

The coronally advanced flap can be used with great reliability and predictability for the treatment of Miller Class I and II recession defects [25] [40] [64]. Zuchelli and de Sanctis [65] have also proposed a modified approach for the treatment of multiple recession defects in cases with high aesthetic demands.

The mean root coverage achieved with a single stage coronally repositioned flap varies between 55-99% and complete root coverage ranges from 24-95% of sites [25] [66]. According to Huang et al., [55], several factors such as the height of the interdental papilla, the amount of keratinised gingiva, the presence of gingival cleft extending in the alveolar mucosa, the deep cervical wear, the frenulum attachment, and the vestibular depth-might have an impact in the outcome.

Pini-Prato et al., [67] concluded that to achieve 100% root coverage with a coronally repositioned flap, the flap should be overcompensated by 2-2.5 mm and sutured tension-free. However, this may be difficult in cases where there are a large recession defect and a shallow sulcus depth. The coronally advanced flap is often used together with a subepithelial connective tissue graft and has proven to be the standard golden treatment in the treatment of

recession defects [68]. In Miller's Class I defects, this combination has been shown to provide complete root coverage of the recession defect [69].

Newer approaches that involve the combination of CAF with acellular dermal matrix graft, enamel matrix derivative, platelet rich fibrin, or collagenous membrane are described further in this article.

Free graft procedures consist in the harvesting of soft tissue from the palatal mucosa, the maxillary tuberosity area or an edentulous ridge and placing it over a recession defect. This technique presents several differences compared to pedicle grafts such as the need of two surgical sites, the lack of the graft's blood supply, making it reliant on the vascularisation offered by the recipient site. For this to occur, there needs to be an adequate overlap of the graft tissue with the soft tissue around the recession defect at the recipient site. Immobilization of the graft at the recipient site is also essential. The success of this technique depends on the thickness of the graft tissue obtained. Therefore, the thickness and volume of the tissue to be grafted from the donor site are important factors in determining the appropriate treatment method and for predicting the prognosis [70] [71].

The commonly used free graft techniques include an epithelialized free gingival graft and a subepithelial connective tissue graft placed either with a pedicle flap, an envelope technique or a tunnelling technique.

The free gingival graft was first described by Bjorn in 1963 [72], and Sullivan and Atkins in 1968 [27] and it was initially used to increase the amount of the attached gingiva and extend the vestibular depth. Later it was used to cover exposed root surfaces.

Free gingival grafts can be used in either one stage procedure, where the graft is placed directly over the root surface, or in a two-stage procedure when the gingival biotype is thin at the recipient site. In this case, the graft is placed apical to the recession defect, and following healing, a pedicle flap is raised and moved coronally to cover the exposed root surface.

Wennstrom [25] reports that the success of free gingival grafts in root coverage is lower compared to other surgical procedures. The mean root coverage achieved with an epithelialized free gingival graft has been shown to vary between 9-87%, and complete root coverage varies between 9-72% of sites [66].

Several factors such as the adequate blood supply from tissues adjacent to the graft bed, the dimensions, border characteristics, thickness and the immobilization of the graft [73] and also the smoking habits of the patient (more than 10 cigarettes/day) [74] have been reported to influence the success of the procedure.

Besides of offering several advantages such as the simplicity of the technique, the possibility to be used in situations that need an increase of the amount of the attached gingiva, this procedure comprises several disadvantages as well, such as the colour mismatch between the donor and recipient tissues, the increased discomfort and the potential for post-operative bleeding from the donor area because of the large wound that heals by secondary intention [75].

The subepithelial connective tissue graft is a bilaminar procedure designed to maximise the supraperiosteal and gingival blood supply of the grafted tissue. The subepithelial connective tissue graft that is usually harvested from the palate is placed over the recession area, while nutrients and revascularisation are derived from the recipient bed, the interdental papillae, and the overlying flap. This method is suitable for covering recessions of both single and multiple adjacent teeth and is especially indicated when aesthetics is the primary consideration. As well as providing root coverage, the subepithelial connective tissue graft can also be used to increase the thickness of the gingival tissues in areas of the gingival recession to reduce the risk of further recession in the future.

Subepithelial connective tissue grafts were first introduced by Langer and Calagna in 1980 [76] and further described in detail by Langer and Langer in 1985 [43]. It was presented as an alternative that overcame the limitations of the free gingival graft since it provided great aesthetic results, lower morbidity of the donor site because of its healing by primary intention and most importantly it offered excellent predictability of the results. For any given site, Nelson reported mean root coverage of 88% [33] while both Levine [77] and Harris [78] reported ~97% root coverage, whereas Tozum et al., [79] reported 96.4%. According to a study focused on the long-term results (27.5 months) of the subepithelial connective tissue grafts, these graft have been shown effective in obtaining a mean of 98.4%root coverage in 100 patients with 146 Miller class I or II recession defects [80]. Other studies on the subepithelial connective tissue graft have considered it to be a predictable method to obtain root coverage in recession defects on molars [81] and on other sites [51]. Chambrone et al., [82], conducted a systematic review and the results showed that the subepithelial connective tissue grafts provided significant root coverage and significant clinical attachment and keratinised tissue gain. Overall comparisons allow us to consider the subepithelial connective graft in combination with the overlying flap as the golden standard procedure in the treatment of recession-type defects [82].

Various modifications of the original technique have been proposed, including connective tissue graft with or without an epithelial collar, partially or covered by a pedicle flap, with an envelope or tunnel design preparation [83] [84].

The main advantages of this procedure are that: it maintains a blood supply to the graft and therefore has good predictability; it provides good aesthetics with preservation of the original flap tissue; the donor site wound is less haemorrhagic and painful and can be healed by primary intention; it is simultaneously applied to both single and multiple recessions. However, the main disadvantage is the fact that this technique is technically demanding and more time-consuming.

Table 1: Comparison of different root coverage techniques in terms of advantages, disadvantages and success rate

Technique	Advantages	Disadvantages	Success rate
LPF [19] [25] [37] [38] [57] [58] [60] [61]	Easy and not time-consuming. No need for a second surgical site. Good aesthetic results.	Applicable only for single-site recessions. Possible risk of gingival recession, dehiscence, or fenestration at the adjacent donor site. An adequate amount of keratinised tissue at the neighbouring donor site and a deep vestibule are needed.	69-72%
DPF [35] [62]	Perfect colour matching of the donor tissue with the recipient. Excellent aesthetic result.	Applicable only for single-site recessions. Poor predictability.	34-82%
CAF [25] [40] [41] [55] [63] [64] [66] [68] [69]	Effective. Excellent aesthetic results. Applicable for single- site and multiple- site recessions.	Presence of adequate keratinised tissue apical to the root exposure, adequate sulcular depth and no interproximal bone loss are needed.	55-99%
FGG [25] [27] [66] [72] [73] [74] [75]	Simplicity of the technique. Can be used in situations that need an increase in the amount of the attached gingiva.	Need of two surgical sites. Lack of the graft's blood supply, relying on the vascularisation offered by the recipient site. Increased discomfort for the patient. The potential for post-operative bleeding. Lower success rate. Colour mismatch.	9-72%
SCTG [43] [51] [76] [77] [78] [79] [80] [81] [82] [83] [84]	Applicable for single- site and multiple- site recessions. Great predictability. Excellent aesthetic results. Lower morbidity of the donor site compared to FGG thanks to the healing by primary closure.	Need of two surgical sites. A limited quantity of graft that can be harvested. Technically demanding. Time-consuming.	88-97%
GTR [85] [86] [87] [88] [89] [90] [91]	Good aesthetics. No need for a second donor site.	No added clinical benefit for the patient treatment in comparison to other traditional root coverage techniques. Need of a second surgical stage when non-resorbable membrane are used. High postoperative membrane exposure rate with potential infection and difficulties with wound closure. Applicable only to single-site recession defects.	45-81%
CAF +ADMA [51] [93] [94] [95] [96] [97] [98]	Unlimited availability. No need for a second donor site. Similar results as SCTG regarding the mean root coverage and the aesthetic outcome Lower postoperative discomfort for the patient. Applicable for single- site and multiple- site recessions.	Not as effective as the SCTG technique in increasing the width of the attached gingiva.	88.7-97 %
CAF+ EMD [45] [69] [99] [100] [101] [102] [103] [104] [105]	Unlimited availability. No need for a second donor site. Similar results as SCTG regarding the mean root coverage and the aesthetic outcome Lower postoperative discomfort for the patient. Applicable for single- site and multiple- site recessions.	Significant variation in the clinical outcomes.	62-89%
CAF+ PRF [106] [107] [108] [109] [110] [111] [112] [113] [114]	Unlimited availability. No need for a second donor site. Lower postoperative discomfort for the patient. Healing biomaterial with great potential for bone and soft tissue regeneration. Enhanced wound healing. Similar clinical results in solving gingival recession problems	More extensive studies are needed to prove its predictability.	72.1-92.7%
CAF + CM [52] [53] [115] [116] [117] [118] [119]	Unlimited availability. No need for a second donor site. Lower postoperative discomfort for the patient. Increases gingival thickness and the width of the keratinised gingiva.	More extensive studies are needed to prove its predictability.	88.5-94.32

The guided tissue regeneration technique consists in the placement of a non-resorbable or resorbable membrane between the recession defect and the exposed bone on one side and the coronally advanced flap on the other, with the aim to allow the selective repopulation of the root surface by periodontal ligament cells that can form new connective tissue attachment between the root surface and the alveolar bone. The first authors that have studied the use of guided regeneration techniques in the treatment of gingival recessions were Tinti and collaborators [85] [86] [87] [88].

In spite of providing several advantages, such as good aesthetics, the absence of the need for a second donor site, a realistic opportunity for true regeneration of the lost periodontal attachment, this technique also has some major drawbacks. Several literature reviews [89] [90] have concluded that GTR doesn't provide an added clinical benefit for the patient treatment in comparison to other traditional root coverage techniques such as the connective tissue graft or the coronally advanced flap procedure. Moreover, two meta-analyses conducted by Al-Hamdan et al., [90] and Clauser et al., [91], concluded that conventional mucogingival surgery resulted in statistically better root coverage, the width of keratinised gingiva, and complete root coverage compared to GTR. Other disadvantages are also the need of a second surgical stage when non-resorbable membrane are used, and the high postoperative membrane exposure rate is resulting in colonisation by oral microbiota [92], potential infection and difficulties with wound closure [89] [90]. Furthermore, the application of this technique is still restricted to single recession defects due to limitations concerning the membrane design, the properties of the membrane material, and the possibility mentioned above of membrane exposure.

Even though the combination of CAF with connective tissue grafts has been demonstrated to be the golden standard in the achievement of predictable root coverage of the recession defects, there are several limitations related to the harvesting of soft tissue autografts, such as the postoperative discomfort associated with an extra surgical site and the limited quantity of soft tissue that can be harvested. To overcome these limitations, Silverstein and Callan [93] advocated the use of an acellular dermal matrix allograft as a substitute for soft tissue autografts. The acellular dermal matrix allograft is biocompatible, safe and non-immunogenic since it is prepared by the removing of the cell components from the human donor skin and the preservation of the ultrastructural integrity.

Many clinical studies revealed the effectiveness of ADMA in the treatment of gingival recession defects [51] [94] [95] [96] [97] both in the short-term and in the long term. The use of the acellular dermal matrix produced a thicker marginal tissue and yielded a higher percentage of root coverage than a CAF alone [95]. Compared to CAF+ SCTG several authors [51] [96] demonstrated that there was no statistically significant difference in the

mean root coverage obtained between the two procedures and that they were both aesthetically acceptable to the patients. A meta-analysis comparing the efficacy of ADM-allografts to other methods concluded that ADM-based root coverage therapy could be used successfully to repair gingival recession defects and to increase the width of the keratinised gingiva [98].

Thus, recession defects might be successfully covered using either an ADMA or SCTG, with no practical difference in the root coverage results or the aesthetics and with the advantage that ADMA offers unlimited availability of donor tissue, making it possible to treat many sites in one single surgical procedure, which improves patient case acceptance and reduces postoperative discomfort. The only drawback of the procedure is the fact that in spite of similar root coverage rates, AMDA technique is not as effective as the SCTG technique in increasing the width of the attached gingiva[51].

Enamel matrix derivative is an extract of the enamel matrix and contains amelogenins of various molecular weight, which according to several authors, are considered to play a particular role not only in enamel formation, but also in the formation of the cementum, periodontal ligament and alveolar bone [99] [100] [101].

Studies have shown that EMD enhances the proliferation and protein production by human periodontal ligament cells in vitro. Moreover, two histological studies showed the formation of new cementum, organising PDL fibres and newly formed bone after treating recession with SCTG+EMD or CPF+EMD [45] [102]. A randomised controlled trial that compared the treatment of Miller Class I and II defects with either EMD+CAF or SCTG+CAF revealed that at the 10-year follow-up evaluation, both techniques provided stable, clinically effective results and that they were similar to each other on all measured parameters [103].

Also, a review article from Cairo et al., [69] concluded that SCTG or EMD in conjunction with CAF enhances the probability of obtaining complete root coverage in Miller Class I and II single gingival recessions.

According to many recent studies, the combination of EMD with CAF produces similar results as the combination of SCGT with CAF regarding the predictability of the treatment of gingival recessions [104] [105].

A recent innovation in dentistry is the preparation and use of platelet-rich fibrin, an autologous leukocyte-platelet-rich fibrin matrix prepared from centrifuged blood without any addition of anticoagulant and bovine thrombin [106].

PRF was first developed in France by Choukroun et al., [107] for special use in oral and maxillofacial surgery.

These growth factors are involved in wound healing and act as promoters of tissue regeneration.A number of studies have confirmed that the specific dense three-dimensional (3D) structure of the fibrin gel in PRF and the action of cytokines and growth factors trapped in the mesh fibrin matrix upregulate cellular activity, promote neoangiogenesis [108] [109], bone growth and maturation and periodontal regeneration in vivo [110]. Some studies have demonstrated that PRF is a healing biomaterial with great potential for bone and soft tissue regeneration, without inflammatory reactions and may be used alone or in combination with bone grafts [111] [112]. Moreover, PRF used in the treatment of gingival recession problems provides several advantages related to the avoidance of a donor site surgical procedure, advanced tissue healing for the first 2 weeks post-surgery, and a major decrease in patient discomfort during the early wound healing period.

Moreover, studies that evaluated the clinical efficacy of PRF in comparison to SCTG concluded that both procedures provided similar clinical results in solving gingival recession problems. No difference could be found between PRF and SCTG procedures in gingival recession therapy, except for a greater gain in keratinised tissue width obtained in the SCTG group and enhanced wound healing associated with the PRF group [113] [114].

The need to avoid palatal donor sites and to have unlimited material availability has inspired researchers to search for alternative options to treat gingival recessions. One of these new approaches is the use of a xenogeneic collagen matrix (CM) of porcine origin (Mucograft®, Geistlich, Wolhusen, Switzerland) in combination with the coronally advanced flap. Structurally, it is composed by two functional layers: an outer compact collagen layer which contributes to structure integrity, protection against infections and allows for better control during suturing, and an inner spongious layer which provides a suitable environment for early vascularisation and promotion of cellular recruitment.

Several studies have concluded that the collagen matrix of porcine origin has proven to be as effective and predictable as the connective tissue graft for increasing the width of KG and to be associated with significantly lower patient morbidity [52] [115] [116] [117].

In 2009, Sanz et al., [115] conducted a randomised retrospective clinical trial consisting of 20 patients followed for 1, 3 and 6 months about keratinised tissue gained through SCTG vs CM augmentation. They found a statistically significant amount of keratinised tissue achieved with both grafting materials (2.6 mm and 2.5 mm respectively) and lower patient morbidity associated with the collagen matrix. Similarly, in one of the first clinical

studies that have compared CM to SCTG, McGuire et al., [52] found that for both techniques, parameters such as the mean clinical attachment level, the periodontal depth and the keratinised gingiva width, improved significantly compared to baseline. All parameters tested for differences between treatment groups also showed equivalence, and at 6 months, no difference could be made in regards to colour or texture.

Another study by Cardaropoli et al., [53] comparing the CM+CAF technique and the SCTG+ CAF technique, demonstrated that both these methods provided a significant reduction of the recession depth after 12 months and that there was no significant difference between them regarding all the clinical parameters that were investigated. The authors concluded that CM could deservedly be considered a substitute for the subepithelial connective tissue graft regarding the treatment of gingival recessions.

Anyway, a study by Jepsen et al., [118] which has compared the CM+CAF technique with CAF technique alone, has found that CM+CAF combination is not superior to the other technique regarding root coverage, but it improves the gingival thickness and the width of the keratinised gingiva significantly.

A more recent multicentre clinical trial concerning the treatment of isolated recessions proved that the combination of CM+CAF significantly increased the marginal soft tissue thickness and the patient satisfaction compared to coronally advanced flaps alone [119].

However, since the CM+CAF technique is relatively new, more studies are needed to determine its effectivity. If it proves to be as effective as the SCTG in providing adequate root coverage, adequate recession reduction and increased width of the keratinised gingiva, then it will undoubtedly be a priceless asset to the clinician in the treatment of gingival recessions.

In conclusion, gingival recessions present challenging soft tissue pathology, with multiple aetiology and a high prevalence which increases with age. Its successful surgical management is closely related to the identification and the elimination of its etiologic factors, the careful selection of the surgical technique and its correct implementation because the procedure is very technique-sensitive. A wide range of surgical techniques has been proposed for the treatment of the gingival recessions, each with its advantages and disadvantages. To provide predictable and long-term results, it is of paramount importance that the surgical technique is individually selected, taking into account several crucial factors such as the size of the defect, the width of the keratinised gingiva apical to the defect and the thickness of the flap. So far, the combination of the subgingival connective tissue graft with the coronally advanced flap represents the gold standard in the treatment of the gingival recessions. More recent techniques such as the combination of CAF with enamel matrix derivative, or with platelet-rich fibrin or with xenogeneic collagen membrane, need further evaluation through more extensive studies.

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The Predictive Effects of Protection Motivation Theory on Intention and Behaviour of Physical Activity in Patients with Type 2 Diabetes

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Abstract

INTRODUCTION: Theory-based education tailored to target behaviour and group can be effective in promoting physical activity.

AIM: The purpose of this study was to examine the predictive power of Protection Motivation Theory on intent and behaviour of Physical Activity in Patients with Type 2 Diabetes.

METHODS: This descriptive study was conducted on 250 patients in Rafsanjan, Iran. To examine the scores of protection motivation theory structures, a researcher-made questionnaire was used. Its validity and reliability were confirmed. The level of physical activity was also measured by the International Short - form Physical Activity Inventory. Its validity and reliability were also approved. Data were analysed by statistical tests including correlation coefficient, chi-square, logistic regression and linear regression.

RESULTS: The results revealed that there was a significant correlation between all the protection motivation theory constructs and the intention to do physical activity. The results showed that the Theory structures were able to predict 60% of the variance of physical activity intention. The results of logistic regression demonstrated that increase in the score of physical activity intent and self - efficacy increased the chance of higher level of physical activity by 3.4 and 1.5 times, respectively OR = (3.39, 1.54).

CONCLUSION: Considering the ability of protection motivation theory structures to explain the physical activity behaviour, interventional designs are suggested based on the structures of this theory, especially to improve self - efficacy as the most powerful factor in predicting physical activity intention and behaviour.

Introduction

According to the World Health Organization, type 2 diabetes is the third most common cause of death in the world and the biggest challenge for today's modern life [1]. The increase in the incidence of type 2 diabetes represents the disease as a global epidemic [2]. According to the World Health Organization, some people with type 2 diabetes in Iran will exceed 6 million by the year 2030 [3]. Increased diabetes in the world is associated with increased inactivity and obesity [4].

Several studies consider physical activity as the key to lifestyle behaviours, both in prevention and control [5]. Regular physical activity improves blood glucose control and has positive effects on blood lipids, blood pressure, cardiovascular complications, as well as the quality of life. It reduces mortality and disability in these patients [6]. Regarding physical activity, diabetic patients are recommended to perform 30 minutes of moderate exercise five days a week [7].

Although diabetes patients are usually encouraged to exercise, they usually do not succeed in doing so, and health system recommendations about physical activity are barely followed by diabetes [8].The cause of low level of physical activity is the chronic nature of the disease. Studies have shown that, after six months, lack of compliance with the

recommendations is rapidly rising [9].On the other hand, some patients cannot motivate themselves to continue their physical activity. There are numerous personal and environmental barriers that cause instability in physical activity [10]. Therefore, designing appropriate interventions for physical activity seems necessary in this group. Using health education Theory as a framework to help educate and change their behaviour and survival is necessary.

One of the most important theory's for changing the behaviour is the theory of protection motivation (PMT). This theory is designed by Rogers to explain the effective and ineffective adaptive behaviours at the time of feeling threatened with health status [11]. Based on this Theory, two types of threat appraisal and coping appraisal determine the intent of individuals for protective behaviours. Threat appraisal includes perceived severity and perceived sensitivity. The third factor added is the reward which is the result of adopting a health-neutral behaviour. Therefore, a recommended behaviour will be accepted that has a high perceived severity and sensitivity regarding the consequences of not doing so, and the rewards resulting from the implementation of incompatible behaviour are minimal. The coping appraisal is based on self - efficacy, response efficacy, and cost of response [12]. In general, based on this Theory, if individuals feel more threatened regarding the consequences of not performing a behaviour and, at the same time, adapt to this threat, there is a motive for changing behaviour [13].

The motivation for physical activity is considered as an effective concept in interventions related to physical activity. While motivation is considered as the best predictor of following recommendations related to physical activity, studies have shown that the motivation for physical activity is low [14]. Many studies have examined the effectiveness of the protection motivation Theory for promoting physical activity. As found by Mirkarimi et al., education based on this Theory has increased physical activity intent and weight loss in obese women in comparison to the control group [15]. There are controversies in this regard. Milne showed that there was no difference between the motivation group with the control group about intent and practice of physical activity in students [16].

Nevertheless, limited studies have examined the impact of this Theory on promoting physical activity in patients, and in particular, patients with type 2 diabetes. As at the time of the present study, no study of physical activity has been done on patients with diabetes in the Iranian population based on this Theory. Due to the chronic nature of type 2 diabetes, as well as the physiological and psychological characteristics of these patients, the importance of regular physical activity is emphasised in these patients.

For this purpose, the effects of protection motivation theory on physical activity are investigated.

Method

This descriptive - analytical study was performed on 250 patients with type 2 diabetes who had a health record in health centres in Rafsanjan in 2017. The sampling method was random cluster sampling. The samples were randomly selected from four clusters of eight health centres and then, using random numbers, they were selected to reach the sample size.

The criteria for entering a diagnosis of type 2 diabetes were the medical records and consent to participate in the study. Exclusion criteria included having psychological problems such as depression, as well as physical and medical failure to perform the recommended physical activity for diabetic patients. The individuals were informed of the study, and the researchers assured people that the information provided by them would be confidential. To determine the level of physical activity, the International Physical Activity Questionnaire-Short Form (IPAQ-SF) was used, which included all physical activities in the working environment, sports activities and daily activities of life. This standard questionnaire was prepared by the World Health Organization, and its validity and reliability were confirmed in various countries [17]. In Iran, the Persian version of this questionnaire was also used in multiple cases, and its validity was confirmed [18]. This self - report questionnaire includes three categories of physical activity: walking activity, moderate intensity activity and high - intensity activity. Calculation of the total score with the sum of the time and number of days of the week spent on these activities were done, and they were converted to Mets (equivalent to the metabolic rate per minute). This questionnaire is divided into three levels of low, moderate and high activity.

To investigate the constructs of the protection motivation theory, a researcher - made questionnaire was used. The questionnaire was designed based on the opinions of the health education specialists (two people), interview with some diabetics and physicians and staff of the diabetes clinic was involved in the training. After the initial formulation, content validity ratio (CVR) and content validity index (CVI) were used for determining the validity and reliability. The questionnaire was completed by ten health education experts regarding validity and reliability, and questions that did not get the required criteria were removed. Finally, a questionnaire was designed with 36 questions in seven constructs: perceived severity,

perceived Susceptibility, self - efficacy, response efficiency, cost, reward, and protection motivation with Cronbach's alpha of 0.76 - 0.80.Questions were designed in the form of a five-point Likert spectrum. Perceived sensitivity was assessed with six questions, perceived severity with five questions, self - efficacy and response efficacy with six questions, and reward structures and perceived costs by four questions. Protection motivation was considered in the form of three questions of varying intentions. A 5 - point Likert scale was used ranging from completely agree to completely disagree (completely agree = 5, Agree = 4, No idea = 3, Disagree = 2, and completely disagree = 1). Perceived severity Responses ranged from 1 (Very low) to 5 (Very high). Data were analysed by SPSS software version 18 using correlation coefficient, logistic regression, linear regression and chi-square test. The assessment of the normality of data distribution was confirmed by Kolmogorov - Smirnov test ($p > 0.05$). The significance level in the tests was considered to be 0.05.

Results

The majority of participants were women (190 persons; 76%). The age of the participants was between 28 and 65, and the mean was 52. Moreover, about half of the subjects under study were overweight (46.4%). The sample characteristics are listed in Table 1.

The results of physical activity behaviour showed that about half of women (53.2%) and 26.7% of men with diabetes were in the inactive group. Also, 87% of women in the inactive group had a low level of education, and 75.6% of inactive people reported having a low income. Chi-square test showed that there was significant difference between the male and female groups and in people with different economic status and with different levels of education in physical activity level ($p < 0.001$) Table 1.

Table 1: Characteristics and level of physical activity in diabetic patients

Variables		N (%)	Inactive N (%)	Minimally active N (%)	P value
Sex	Male	190 (76)	16 (26.7)	44 (73.3)	P < 0.001*
	Female	60 (24)	101 (53.2)	89 (46.8)	X2 = 12.85
Age	25-40	15 (6%)	6 (40.0)	9 (60.0)	
	40-50	69 (27.6)	27 (39.1)	42 (60.9)	
	50-60	138 (55.2)	68 (49.3)	70 (60.9)	P = 0.327
	60-65	28(11.2%)	16 (57.1)	12 (42.9)	
Education	Elementary	78 (31.2)	56 (71.8)	22 (28.2)	
	Middle school	125 (50)	50 (40.0)	75 (60.0)	P < 0.001*
	Diploma and Postgraduate	45 (18.8)	11 (23.4)	36 (76.6)	X2 = 32.23
Income	Weak	69 (37.6)	52 (75.4)	17 (24.6)	P < 0.001*
	Moderate	173 (66.9)	63 (36.4)	110 (63.9	X2 = 31.62
	Good	8 (3.2)	6 (75%)	2 (25)	
Diabet history	1-3 year	208 (52)	27 (23.1)	25 (18.8)	
	3-5 year	92 (36.8)	39 (33.3)	53 (39.8)	P = 0.512
	Above 5 year	106 (42.4)	51 (43.6)	55 (41.4)	
BMI	Below 18.5	3 (1.3)	1 (0.9)	2 (1.6)	
	18.5 – 24.9	66 (26.4)	28 (23.9)	38 (29.5)	
	25.0 – 29.9	116 (46.4)	54 (46.2)	62 (48.1)	P = 0.41
	30.0 and Above	61 (24.6)	34 (29.1)	27 (20.9)	

* Significant p-value.

The results of protection motivation structures showed that the highest score was related to the response efficacy (21.8 ± 3.05) and the lowest score for perceived costs (9.02 ± 1.82). The results also showed that there was a significant correlation between all the protection motivation structures and the intention to do physical activity. The highest correlation was related to self-efficacy (r = 0.716), and the lowest was related to Perceived severity (r = 0.171). The results showed that patients had a higher degree of coping appraisal compared to the threat appraisal. Also, rewards and perceived cost structures had a reverse and negative relationship with the intention to perform physical activity (Table 2).

Table 2: Descriptive statistics and intercorrelations of PMT construct in diabetic patients

Variable	1	2	3	4	5	6	7	8	9
1.Vulnerability	--								
2. Severity	0.283**	--							
3.Self- Efficacy	0.076	0.062	--						
4.Response Efficacy	0.136*	0.134*	0.549**	--					
5.Perceived Cost	-0.121	-0.053	-0.503**	-0.435**	--	.			
6. Reward	-0.198**	-0.186**	-0.629**	-0.444**	0.440**	--			
7.Coping Appraizal	0.127*	0.101	0.878**	0.836**	-0.709**	-0.630**	--		
8.Threat Appraizal	0.823**	0.639**	0.325**	0.317**	-0.271**	-0.584**	o.373**	--	
9.Protection Motivation	0.171**	0.149*	0.716**	0.520**	-0.576**	-0.567**	0.741**	0.400**	--
Mean	20.2	16.9	20	21.79	9.02	10.18	32.80	26.88	10.98
SD	4.05	2.51	3.41	3.05	1.82	2.42	6.87	6.36	1.96

*p < .05. **p < 0.01.

The results of linear regression showed that the constructs were able to predict the physical activity behaviour. In Model 1 that included the six PMT constructs simultaneously, self - efficacy and perceived cost structures predicted behaviour ($p < 0.001$). Increasing the self-efficacy score and reducing perceived cost scores led to an increased score of physical activity intention. Model 2 involved the two PMT pathways simultaneously.

The result showed that predictive power of coping appraisal was higher in predicting physical activity intention ($p < 0.001$). Finally, after controlling for confounding factors (Model 3), there was no change in the ability to predict the intention to do physical activity, and model structures were able to predict 60% of the variance in physical activity intention (Table 3).

Table 3: PMT Predictors of Physical Activity Intention in Diabetic Patients

PMT Constructs	Physical activity intention (P.M)		
	Model l	Model 2	Model 3
Vulnerability	0.058		0.05
Severity	0.074		0.072
Self- Efficacy	0.467***		0.465***
Response Efficacy	0.090		0.85
Perceived Cost	-0.247***		-0.249***
Reward	-0.099		-0.112
Coping Appraisal		0.68***	
Threat Appraisal		0.14**	
SEX			-0.37
R2	0.60	0.56	0.60

Model 1 includes the 6 PMT construct scores; Model 2 includes the 2 PMT pathway scores; Model 3 Adjusted for sex; $^{*}p < 0.05$. $^{**}p < 0.01$. $^{***}p < 0.001$.

The results of logistic regression test (Table 4) after modifying the confounding factors (Model 2) showed that physical activity behaviour is predictable by three variables: behavioural intention, self - efficacy

and gender (p < 0.001). Based on the results of Table 4, high physical activity intention and self - efficacy scores increased the chances of higher levels of physical activity by 3.4 and 1.5 times, respectively. Also, the gender (female) reduced the chances of having physical activity by 13%.

Accordingly, the most important predictor of behaviour was physical activity intention. The results of Hosmer and Lemeshow showed that the model was able to fit the physical activity after modifying the confounding factors (p = 0.52).

Table 4: PMT predictors of physical activity level in diabetic patients

Variables	Model I				Model 2			
	B	SE	Unadjusted OR	P value	B	SE	Adjusted OR	P value
Vulnerability	0.081	o.068	1.08	0.232	0.081	0.076	1.08	0.287
Severity	-0.082	0.092	.92	0.368	-0.073	0.106	0.930	0.492
Self-efficacy	0.382	0.105	1.46	0.000***	0.434	0.119	1.54	0.000***
Response efficacy	0.052	0.083	1.05	0.532	0.054	0.096	1.05	0.578
Cost	-0.040	0.139	.96	0.774	0.007	0.147	1.00	0.965
Rewards	-0.092	0.113	.91	0.412	0.064	0.129	1.06	0.619
Protection Motivation	0.912	0.243	2.49	0.000***	1.22	0.294	3.39	0.000***
SEX					-1.99	0.574	0.136	0.000***
2LL		172.15				147.67		
Nagelkerkes R square			66% 72%					
Hosmer and Lemeshow Test		P = 0.25				P = 0.51		

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Discussion

This study aimed to investigate the effect of protective motivation Theory constructs on the prediction of physical activity behaviour in patients with type 2 diabetes. Results of physical activity behaviour showed that physical activity was low despite repeated recommendations. In the case of women, about half of the population was in the inactive group. These results are in line with the results of other studies similar to those recommended for diabetic patients [10][19][20]. Studies on the level of physical activity in diabetic patients in Iranian population showed low levels, especially in women with type 2 diabetes [21]. There are several elements regarding the low level of physical activity, including social, cultural and personal factors. The chronic nature of the disease, and physical, as well as cultural and social constraints, for women, in particular, can play a role in the low level of physical activity in this group. Nevertheless, it is necessary to adopt strategies to increase the level of physical activity.

Regarding demographic factors, the results showed that the level of physical activity was related to the level of education and income, while the age and history of diabetes did not correlate with it. These findings are consistent with the results of similar studies in this area. Norouzi et al. showed the level of education as a predictor of physical activity behaviour in people with diabetes [22]. Although awareness does not necessarily change the behaviour of individuals, people with a higher education level can access and understand more information provided to diabetic patients, and this can be effective in improving the behaviour of physical activity in this group. In our study, the effect of age was not significant on physical activity which was consistent with Costanzo study [23]. Given that behaviour such as physical activity has evolved, ageing may not affect the physical activity of patients. On the other hand, our age range was up to 65 years, and if higher ages are entered into the study, mixed results may be attained due to a limitation in the elderly.

The results of our study showed that there was a significant relationship between physical activity intention and construct scores of protection motivation Theory. However, the correlation between structures and intention was different, so that the highest relationship was between intention and self - efficacy and the lowest was the perceived severity. Regarding self - efficacy, the results of this study are in line with the majority of similar studies. Most studies have considered self-efficacy as one of the most important constituents of effective physical activity [24][25]. In a systematic review and meta-analysis, self - efficacy was one of the most promising structures of predicting behaviour [26]. Research has shown that patients with type 2 diabetes, in addition to having a low level of physical activity, have a lower level of self - efficacy for exercise than normal people [27].

In the present study, there was a weak correlation between perceived severity and physical activity behaviour in diabetic patients. This finding was not consistent with the results of Courneya [28]. They considered perceived severity as one of the most important predictors of intention. In justifying this contradiction, it can be pointed out that in the study of Courneya, the target group was those who were exercising and the increase in perceived severity led to increasing in intent, while in our study, people had a less physical activity or did not intend to do so. On the other hand, the nature of diseases is different in these studies.

In the present study, the relationship between the threat appraisals with the intention to do physical activity was weaker than the relationship between the coping appraisal and the intention to do physical activity. According to the results of Purdie (2002), coping appraisal is a stronger predictor of motivation [29]. The research done to identify persuasive methods suggests that older people, compared to younger people, respond less to messages with the content of fear (perceived sensitivity and severity) [30]. Given that the majority of participants in our study were over 50 years of age, it could justify the results.

Overall, the results of this study were consistent with the overall structure of the theory, so that self - efficacy, response efficacy and perceived severity and Susceptibility had a positive relationship

with intention, while the costs of behavior and rewards of lack of perceived physical activity was inversely related to the intention to do physical activity.

The results of regression test indicated that the constructs of protection motivation theory predicted 60% of the behavioral variance, while, after adjusting the confounding variables, two constructs, self - efficacy and costs had the highest predictability of the intention of physical activity. In interventional studies in this field, self - efficacy in combination with response efficacy has been the most important predictors of physical activity[31]. The importance of self - efficacy in explaining the intention of physical activity can be considered in two directions: *First* is prevalence the psychological problems such as stress and depression in this group which has a negative effect on self - efficacy, and the second is the nature of exercise, which requires careful planning and support from the family and community. In this way, promotion self - efficacy to perform the recommended physical activity can have a significant effect on the decision to do so. Self - efficacy was also the only construct that predicted both the intention and the physical activity behaviour. In the present study, the perceived cost construct had a reverse and significant relationship in predicting the intention to perform physical activity. These results were not consistent with the results of Rahaei et al., which showed that the rewards combined with self - efficacy were predictor of behaviour [32]. In justifying this contradiction, we can point to the difference in the nature of behavior in two studies, as it is possible to say that people with diabetes do not feel significant external and internal rewards for not doing physical activity, while the perceived costs such as spending time, cost, tiredness and weakness can be more effective in physical activity intention. Therefore, by adjusting perceived costs, you can take steps to motivate patients to start physical activity.

Our study had some limitations, including the self - report nature of physical activity, which may cause some degree of bias. On the other hand, although the instruments were used based on experts' opinions and after reliability and validity investigations, perhaps designing a questionnaire using qualitative research methods and obtaining patient feedback can provide more accurate results. Despite these limitations, the present study was the first study to investigate the intention and behaviour of physical activity in patients with type 2 diabetes and, in addition to correlation; it investigated the predictive power of protection motivation theory in predicting the intent and behaviour of physical activity. Nevertheless, it is recommended that by conducting precise interventional studies based on the theory of protection motivation, the practical effectiveness and results of the intervention based on this theory should be analysed for patients with type 2 diabetes.

In conclusion, the present study showed that although there was a correlation between all the constructs of the PMT with the intention to do physical activity, only self - efficacy and cost were able to predict the intent of behaviour. Also, behaviour was also predictable by intention and self - efficacy. Thus, it seems that intervention based on the constructs of this theory emphasising self - efficacy and reducing perceived costs can increase the incentive to initiate physical activity and Promote the level of physical activity in Diabetic Patients.

Compliance with Ethical Standards

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The Effect of Diabetes Self-Management Education on Hba1c Level and Fasting Blood Sugar in Type 2 Diabetes Mellitus Patients in Primary Health Care in Binjai City of North Sumatera, Indonesia

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Abstract

AIM: The study aimed to evaluate the effect of short-term diabetes self-management education (DSME) on Hba1and Fasting Blood Sugar in type 2 diabetes mellitus patients attending the Primary Health Care (PHC) in Binjai city of North Sumatera, Indonesia.

SUBJECTS AND METHODS: A quasi-experimental (pretest-posttest) study was conducted in 4 PHCs, involving 80 patients with type 2 diabetes mellitus. The patients in received a 3-months intervention, including an 8 week education on self- management of diabetes mellitus and subsequent 4 weeks of practice of the self- management guidelines.The patients received standard advice on diet management.

RESULTS: There was a significant reduction in Hba1c levels. The statistical analysis using t-test found that there was a significant difference of Hba1c value between pre and post education among type 2 diabetes mellitus patients ($p < 0.005$).

CONCLUSIONS: Diabetes self-management education in PHC of Binjai city can reduce the Hba1c level in type 2 diabetes mellitus patients.

Introduction

Diabetes mellitus, commonly type 2 diabetes mellitus, is an increasing health problem worldwide. It has been estimated that there will be 552 million patients with diabetes and 300 million people with impaired glucose tolerance in 2030 [1]. Diabetes mellitus is associated with various atherosclerotic complications, including cerebrovascular and cardiovascular diseases, causing significant morbidity and mortality [2].

Diabetes self-management education (DSME) is the ongoing process of facilitating the knowledge, skill, and ability necessary for diabetes self-care. This process incorporates the need, goals, and life experiences of the person with diabetes and is guided by evidence-based standards. Therefore, it plays an important role in the clinical management of diabetes [3].

Monitoring of metabolic markers, such as blood pressure, body weight, lipid profile, fasting blood sugar and Hba1c, is essential in the clinical management of patients with diabetes because hypertension, obesity and dyslipidemia are well-known risk factors of atherosclerosis and are common

in diabetic patients [4]. Monitoring of these risk factors also helps in evaluation of treatment response of the patients [5]. As a long-term disease, diabetes mellitus needs lifetime care and management. However, 50-80% of patients with diabetes did not have enough skills and knowledge for self-care of the disease [6]. Previous studies have shown that DSME improves homeostasis of metabolism of the patients, and healthy lifestyle prevents the development of atherosclerosis in patients with type 2 diabetes [7][8].

This study was undertaken to evaluate the effect of DSME on Hba1c and Fasting Blood Sugar.

Methods

In this study, a total of 80 patients with type 2 diabetes mellitus were recruited from four Primary Health Care in Binaji city, North Sumatera, Indonesia. This is quasi-experimental research with One Group Pretest-Posttest Design. This was conducted from April to September 2017. From each PHC 20 samples were taken. The patients were recruited in accordance with the inclusion criteria, age > 40 years old-cooperative patients and willing to join the research and exclusion criteria (using diuretic and the middle of cancer therapy). Among the 80 patients, two of them did not complete the DSME programme or did not attend the follow-up examination and thus were excluded from the study. This research was approved by Health Research Ethical Committee, Medical Faculty of University Sumatera Utara/H. Adam Malik General Hospital number 591/TGL/KEPK FK USU-RSUP HAM/2016. Patients were informed of the detail of the study, and written consent was obtained from the patients before they participated in the study.

Blood samples were collected (using syringe) twice before and after interventions and transferred to the laboratory immediately to conduct glycosylated haemoglobin test by Alere Afinion as 100 Analyzer. Fasting blood sugar of samples, we examined by using a portable measuring instrument (Gluco DR). We examine blood pressure, weight, and height and waist size as well.

A questionnaire interview method was adopted to collect information about the family history of the disease, education level and type of the job.

The patients in the intervention participated in a DSME programme in which the patients were required to attend a two-hour lesson weekly for eight weeks and to follow the self-management guidelines of the education programme in the daily activities within the study period. For the DSME programme, all the lessons were focused on the skill and knowledge for healthy eating, being active, monitoring, taking medication, problem-solving, reducing risks, and healthy coping [9]. For the samples blood test (fasting blood sugar and HBa1c), height, weight waist size examinations were performed before the commencement of the DSME programme as the baseline and one month after the completion of the DSME programme.

Table 1: The content of the diabetes self-management education programme for the patients in the PHCs of Binjai city

	Contents
Management diet and Healthy eating	Common misunderstanding of diet for self-management of type 2 DM Understanding about healthy food for type 2 diabetes Limiting sugar consumption Appropriate caloric intake for type 2 diabetes Kinds of food, drink, fruits for type 2 diabetes Understanding the effect the heated food over and over again When the time to eat
Exercising DM	Understanding about exercise for self-management of type 2 DM The importance of regular exercise for type 2 DM Understanding what is the right exercise for type 2 DM An individualised plan for regular exercise every day for 30 minutes Self-check and control body weight The wrong belief that all activities are an exercise Understanding that exercise is a regular and rhythmic activity
Monitoring	The importance of regular monitoring of fasting blood sugar. Monitoring of fasting blood sugar by self Self-management of fasting blood sugar
Medication	Pathology and medical treatments for type 2 diabetes mellitus The importance of taking diabetic medications Knowing the type of medication for each patient (drugs, injected) The right time and frequency of taking diabetic medication
Reducing risks	Common complication of type 2 DM (stroke, renal failure, cataract, etc.)) Stopping unhealthy behaviours like smoking, drinking with much sugar, Maintaining a healthy lifestyle Risk factors for type 2 diabetes
Problem-solving	Avoid unhealthy foods Regular medication Appropriate time and frequency of exercise every day for 30 minutes

The continuous data were expressed as the mean ± standard deviation (SD). Shapiro-Wilk test was used for checking the normality of distribution. If the data were normally distributed, a t-test was used. Otherwise, a nonparametric test was applied.

Results

The number of the samples from the four PHCs were 80 comprising 52 females (65%) and 28 males (35%), achieving the assigned target of interviews before and after the intervention. The demographic data; about gender, age-group, education level, kinds of job, family history and type of treatment are presented in Table 1. We give the education for 3 months only. Two of samples did not complete the DSME programme or did not attend the follow-up examination and thus were excluded from the study.

Table 2 shows that BMI values had a minimum of 18.20 kg/m^2 and a maximum of 35.25 kg/m^2, FBS values minimum of 76 mg/dL and a maximum of 600 mg/dL and Hba1c values had a minimum of 5.7% and a maximum of 12.50%.

When we did the post-test the number of samples is 78 because two of that are excluded because one of them was is died, and the other did not complete all of the education.

Table 1: Baseline information about study participants

Respondent characteristic		N	%
Gender			
	Male	28	35
	Female	52	65
Age Group			
	Early Elderly (45-55y)	36	60
	Further Elderly (56-65y)	31	35
	Seniors (> 65 y)	13	16.25
Education Level			
	Primary School	27	33.75
	Junior High School	16	20
	Senior High School	23	28.75
	Academy/University	14	17.5
Job			
	Not work	40	50
	Labour		
	Harvester	2	2.5
	Self Employe	9	11.25
	State Employee	19	23.75
	Etc	6	7.5
Family History			
	Maternal history	4	5
	Faternal history	40	50
	Maternal and Paternal	20	25
	History	20	25
Type of treatment			
	Oral drug	79	98.75
	Insulin	1	1.25

We found that there was a significant difference in all variables between pretest and post-test.

Table 2: Baseline characteristics of the samples before intervention (Pretest)

Characteristics	N	Minimum	Maximum	Mean	Std. Deviation
BMI	80	18.20	35.25	24.9944	3.65597
FBS	80	76.00	600.00	218.387	96.98799
Hba1c	80	5.70	12.50	8.6613	1.75640
Waist size	80	107	75	92.0313	8.38541
Valid N	80				

Discussion

This study aimed to evaluate the effect of diabetes self-management education on reducing Hba1c levels among the type 2diabetes mellitus patients by measuring glycaemic indexes before and after intervention in primary health care in Binjai city. One of the reasons for diabetic patients' failure to achieve the desired outcomes is their lack of participants in treatment. This participation is an important factor in the treatment of patients who are willing to follow a treatment plan throughout the life [10].

Table 3: Baseline characteristics of the samples after intervention (Postest)

Characteristics	N	Minimum	Maximum	Mean	Std. Deviation
BMI	78	18.20	32.25	24.03	3.12
FBS	78	98.00	345.00	149.22	52.704
Hba1c	78	5.7	11.90	7.8897	1.28018
Waist size	78	73	105.00	90.3321	8.5802
Valid N	78				

A study by Chuang Yuan ect showed that the patients in the intervention group had significant reduction of the Hba1c level and body weight after receiving the education as compared to the control group, indicating that education had positive effects for improving the health status of patients with type 2 diabetes [11]. Different with us all the patients with type 2 diabetes mellitus we gave diabetes self-management education, and it had a positive effect on improving the health status by reducing Hba1c after the intervention.

Table 4: Statistical analysis

Characteristics	Pretest	Posttes	P value
BMI	24.9944 ± 3.656	24.03 ± 3.12	P<0.0005
FBS	218.39 ± 96.99	149.22 ± 52.704	
Hba1c	8.66 ± 1.76	7.889 ± 1.280	
Waist size			

In spite of the short exposure time to education, our findings suggest that the educational intervention was successful in achieving some significant changes in the lifestyles of participating subjects. Among our target population there was an increase in the number of patients being involved in exercise like bike, aerobic and just walking for 30 minutes, an increase in the proportion of patients using the healthy diet that we suggested, increased vegetable intake, diet soft drinks, and the reason for those changes was the doctor's advice and more personal concern about their health care. This resulted in achieving a good change in the control of diabetes mellitus (mean HbA1c) in the post-educated than the pre-educated group.

Respondents in the two surveys were similar about gender, age, marital status, educational level, the presence of a maternal history of diabetes. On our studying we took the samples at the age > 60 years old is a little compared than < 60 years old, the reason was it make us easy for giving education, for the samples with age>60 years old we gave education besides for the patient we gave to the education for their close family. Most respondents had received some form of health education in the recent past and the follow-up survey. However, the recent health education was well organised and directed mainly to diabetic patients and their close family. All the study participants received the drugs of diabetes mellitus type 2 from PHCs because we included patients diagnosed with type 2 diabetes mellitus. Only one patient received insulin.

The level education was more at the middle level (30 people) than at the low (20 people), the high (25 people) and academic (5 people). We can say that these results are encouraging, considering that the health education intervention was of very short duration and was of limited scope and quality. A large-scale, effective and high-quality health education program is likely to have much better results; such a program is expected to reduce the burden of diabetes mellitus in Binjai city, North Sumatera.

The conclusion of this study is that improving the quality of health education in PHC in Binjai city through well-designed programs will improve the awareness and practices among the population in

general, but particularly among patients with diabetes mellitus, increasing awareness for diabetes mellitus patients about exercising like aerobic in PHC each week and exercise every day at least for 30 minutes. Several studies concluded that lack of knowledge, self -care skills, and correct information about the treatment programs hinder the improvements. One important problem is non-compliance and adherence to treatment plan [12] however, we must not forget emphasis just on knowledge, because in many cases, people know what to do, but do not put their knowledge in action [13].

There are several limitations in our study: Firstly; the time interval between pre and posttest was relatively short. Secondly; the possibility of bias in the end line survey cannot be excluded (patients exposed to health education and/or a similar interview at the end-line are more likely to give 'adequate' answers to the questions). Thirdly, we recorded data only for pretest and post-test time points. Finally, it is expected that the effect of diabetes self-management education intervention may have been short-lived, as observed in several other studies.

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Patterns of Antibiotic Prescription in Children: Tirana, Albania Region

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Abstract

BACKGROUND: Antibiotics (abx) constitute the most prescribed therapeutic agent in the world. There is little data regarding antibiotic consumption by young children in Albania.

AIM: This study aims to evaluate antibiotic prescription in children in quantitative and qualitative terms, and therefore, propose recommendations to improve overall clinical outcomes.

METHODS: A retrospective, cross-sectional drug utilisation study was conducted based on unreimbursed prescriptions collected in 25 pharmacies, randomly selected within the district of Tirana, during the period beginning December 2015 to January 2016. They contain at least one antibacterial therapeutic agent prescribed for children 0-15 years old, for systemic use. The data were analysed using SPSS 20.

RESULTS: A group of 904 prescriptions meet inclusion criteria, 54.1% patient were female, and 45.9% were male. The most exposed age group were 2-6 years old. The most common diagnosis was respiratory tract infections: bronchitis (59.2%), tonsillitis (17%) followed by bronchopneumonia (9.6%). The most prescribed antibiotic classes are Penicillins (33%), Cephalosporins (33.2%) and Macrolides (21,5%). Amoxicillin (19.4%), Azithromycin (14.7%), the combination of Amoxicillin and Clavulanic acid (13.5%) and Cefaclor (11.7%) were the most commonly prescribed. We observed short duration therapies, with a mean duration of 5.21 days and in 17.4 % of cases with a duration of ≤ 2 days.

CONCLUSIONS: We observed a large use of broad-spectrum antibiotics for common respiratory tract infection in children less than 6 years old. We would recommend the creation of an electronic database of patient's record in order to monitor the quality of prescription and education of the healthcare professionals and patient of risks related to antibiotic resistance.

Introduction

Antibiotics (abx) constitute the most prescribed therapeutic agent in the world [1]. Their use has become prolific worldwide, and the rate of prescriptions grown along with [2] [3]. Preschool children are the primary recipients of this medication. [4]. Differences in antibiotic prescription in quantitative and qualitative terms, caries greatly from region to region, country to country [5]. Overuse and misuse of antibiotics have been proven to be the primary cause of antibiotic resistance in patients [6]. It is estimated that almost 50% of antibiotics prescribed for children by primary care physicians are unnecessary [7]. In the USA, almost three-quarters of all antibiotics are prescribed for acute respiratory infections, and 44% of children with common colds were reported to be treated with antibiotics [8] [9]. In Canada, 74% of preschool children seeking care for respiratory infections received antibiotic prescriptions [10]. Prescribing broad-spectrum antibiotics, instead of the narrow spectrum (when appropriate) is also a common type of inappropriate usage [11]. There is little data regarding antibiotic consumption by young children in Albania.

This study aims to evaluate antibiotic prescription in children in quantitative and qualitative terms and to, therefore, propose recommendations and counter-measures to improve overall clinical outcomes.

Patients and Methods

A *retrospective, cross-sectional* drug utilisation study was conducted based on unreimbursed prescriptions collected in 25 pharmacies, randomly selected within the district of Tirana. Drugs were prescribed by physicians and dispersed by pharmacies during the period beginning December 2015 to January 2016. They contain at least one antibacterial therapeutic agent prescribed for children 0-15 years old, for systemic use. The aim of this study is: to evaluate the most prescribed antibiotics; to discover the most common diagnosis they are prescribed for and the mean duration of therapy for each diagnosis; and finally, to compare possible similar patterns of prescription dissemination with other countries. To define each drug, we referred to the Anatomical Therapeutic Chemical Classification System (ATC). According to this system, antibiotics for systemic use are defined as the *J01* main therapeutic group [12]. The data were analysed using SPSS 20.

Results

We collected a total number of 904 prescriptions, 54.1% patient were female, and 45.9% were male. According to children age, the most exposed age group were 2-6 years old, and preschool children (≤ 6 years old) represent 2/3 of total prescription.

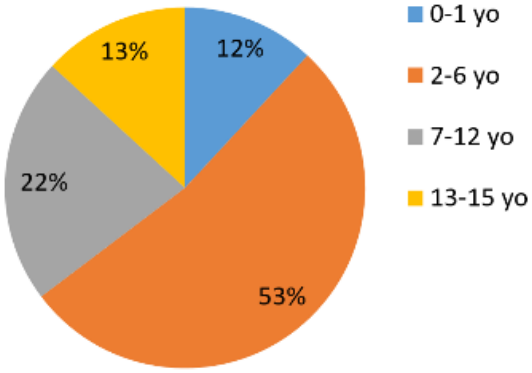

Figure 1: Prescription of antibiotics among age groups

Antibiotic therapy was given to children for 1 - 10 days, with a mean therapy duration of 5.21 days. In 17.4% of cases, antibiotic are given to children for ≤ 2 days. Most common diagnosis for prescribing an antibiotic was respiratory tract infections: bronchitis (59.2%), tonsillitis (17%) followed by bronchopneumonia (9.6%).

Table 1: Diagnosis for prescribing an antibiotic and mean duration of therapy

Diagnose	%	Mean Duration (days)	Std. Deviation	95% Confidence Interval for Mean	
				Lower Bound	UpperBound
Tonsilitis	17	5.15	2.309	4.78	5.52
Bronchitis	59.2	5.22	2.291	5.02	5.41
Bronchopneumonia	9.6	5.63	1.472	5.32	5.95
Gastroenteritis	4.1	5.05	2.438	4.24	5.87
Otitis	2	5.89	1.875	4.96	6.82
Urinary tract infection	0.7	6.83	1.941	4.80	8.87
Synositis	3.4	5.35	2.039	4.60	6.00
Dental infection	2.5	4.17	2.188	3.23	5.12
Parasitic infection	1.4	3.00	2.000	1.79	4.21

The most prescribed antibiotic classes are Penicillins (33%), Cephalosporins (33.2%) and Macrolides (21.5%) followed by Aminoglycosides with 3.3% of cases.

Table 2: Systemic antibiotics prescription

Chemical group	Drug	%
J01C Penicillins	J01CA04 Amoxicillin	19.4
	J01CR02 Amoxicillin and Clavulanic acid	13.5
J01DB First-generation cephalosporins	J01DB01 Cefalexin	5
	J01DB04 Cefazolin	3
J01DC Second-generation cephalosporins	J01DC02 Cefuroxime	2.2
	J01DC04 Cefaclor	11.7
J01DD Third-generation cephalosporins	J01DD02 Ceftazidime	0.2
	J01DD04 Ceftriaxone	6.4
	J01DD08 Cefixime	4.8
J01FA Macrolides	J01FA01 Erythromycin	0.2
	J01FA09 Clarithromycin	6.5
	J01FA10 Azithromycin	14.7
J01G Aminoglycoside	J01GB03 Gentamicin	3.4
J01EE Combinations of sulfonamides and trimethoprim	J01EE01 Sulfamethoxazole and trimethoprim	5.3
J01XD Imidazole derivatives	J01XD01 Metronidazole	3.7

Amoxicillin (19.4%), Azithromycin (14.7%), the combination of Amoxicillin and Clavulanic acid (13.5%) and Cefaclor (11.7%) were the most commonly prescribed. Penicillins were the most prescribed drugs overall, while Cephalosporins were more prescribed for Bronchitis, Bronchopneumonia and Otis. For dental infections, Penicillins remained the first pharmaceutical treatment choice. Macrolides are usually prescribed for Bronchitis and Bronchopneumonia.

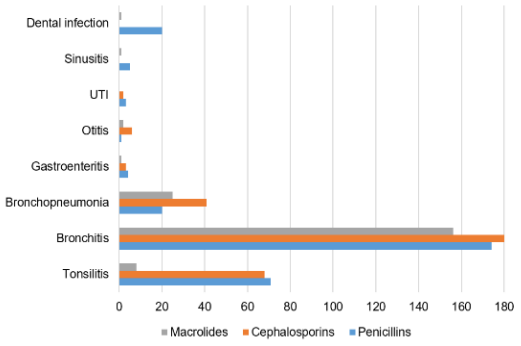

Figure 2: Prescription of Penicillins, Macrolides and Cephalosporins According to Diagnosis

Syrups were the most commonly used pharmaceuticals dosage forms (53.2%), followed by injections (29.8%) and tablets/capsules (15%).

Discussions

From 902 prescriptions, 64.4% were dispensed for children under 6 years old. In point of fact, the general worldwide trend is that preschool children are the most exposed to these drugs [4] [13]. Other studies have evidenced that antibiotics are often prescribed without likely therapeutic effects (e.g. for viral respiratory infections). This phenomenon is particularly frequent amongst children [9] [10] [14] [15] [16]. Most of the prescriptions in our study were for respiratory tract infections and bronchitis, as the most frequent diagnosis. According to the pediatric treatment recommendations of the Center for Disease Control and Prevention (CDCP) antibiotics are not helpful and should not be used for bronchitis [17]. Because the risk of antibiotic resistance and *Clostridium difficile* infection, antibiotics should not be routinely used in the treatment of acute bronchitis, especially in younger patients in whom pertussis is not suspected [18]. From this study, we discovered that broad spectrum abx are far more preferred pharmaceuticals in the Tirana region, with Penicillins being the most prescribed drug class, followed by Cephalosporines and Macrolides. Amoxicillin, Azithromycin, the combination of Amoxicillin and Clavulanic acid and Cefaclor are usually prescribed and dispersed for respiratory tract infection. The trend of using newer broad-spectrum agents in pediatric outpatients is spreading globally. In the Netherlands [13], UK [19], Italy [20], and in the US [21] [22], the use of broad-spectrum abx has increased disproportionately. The use of broad-spectrum agents for viral infections is also a serious concern regarding antibiotic resistance [23] [24]. In this study, we observed short duration therapies, with a mean duration of 5.21 days and in 17.4 % of cases with a duration of ≤ 2 days. This use is in contrast with general guidelines of antibiotic use where in most cases it is recommended that a therapy continues 7-10 days for diagnosis such as Tonsillitis and Pneumonia [25]. Nevertheless, according to the pharmacokinetic characteristics of some antibiotics [26], and some studies in which 5 days duration therapies, showed that these could be equally effective as therapies with a duration of more than 7 days, authors of the studies recommended to be cautious about the results [27]. We observed the widespread use of injections for outpatient children (almost 30% of all patients). Oral dosage of pharmaceutical forms is more appropriate and less traumatic for children. This study gives information about the patterns of antibiotic prescriptions in children for the mentioned region of Tirana. However, it has some limitations. First, it is limited only to unreimbursed prescriptions. However, a previous study of antibiotic consumption in Albania showed that the unreimbursed abx use is almost seven times higher than the reimbursed use [28]. Secondly, the location of the study is restricted to the region of Tirana, and this is a random sampling study. The absence of clinical electronic health record databases for drug prescription outside the reimbursement scheme presents a major difficulty in collecting data to evaluate antibiotic prescriptions for outpatients on a national scale. This program could help to monitor pharmaceuticals like antibiotics which are frequently prescribed and dispersed outside this scheme, increase the responsibilities of appropriate antibiotic use, including warnings, and finally, to decrease costs for disease management for Albanian families.

In conclusion, this study demonstrated a large use of broad-spectrum antibiotics for common respiratory tract infection in children less than 6 years old. Guidelines recommend that for some of these infections, antibiotics can be avoided or at least delayed, as the situation is monitored. Short duration therapies were observed, raising other concerns such as antibiotic resistance. This study has a limited number of prescriptions, and it is nor conducted on a national scale. We would recommend the creation of an electronic database of patients record to monitor the quality of prescription and education of the health care professionals and patient of risks related with antibiotic resistance.

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Job Demands, Burnout, and Teamwork in Healthcare Professionals Working in a General Hospital that Was Analysed At Two Points in Time

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Keywords: Job demands; Burnout; Teamwork; Longitudinal study; Healthcare professionals

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Abstract

AIM: The purpose of the paper was to assess job demands, burnout, and teamwork in healthcare professionals (HPs) working in a general hospital that was analysed at two points in time with a time lag of three years.

METHODS: Time 1 respondents (N = 325) were HPs who participated during the first wave of data collection (2011). Time 2 respondents (N = 197) were HPs from the same hospital who responded at Time 2 (2014). Job demands, burnout, and teamwork were measured with Hospital Experience Scale, Maslach Burnout Inventory, and Hospital Survey on Patient Safety Culture, respectively.

RESULTS: Significantly higher scores of emotional exhaustion (21.03 vs. 15.37, t = 5.1, p < 0.001), depersonalization (4.48 vs. 2.75, t = 3.8, p < 0.001), as well as organizational (2.51 vs. 2.34, t = 2.38, p = 0.017), emotional (2.46 vs. 2.25, t = 3.68, p < 0.001), and cognitive (2.82 vs. 2.64, t = 2.68, p = 0.008) job demands were found at Time 2. Teamwork levels were similar at both points in time (Time 1 = 3.84 vs. Time 2 = 3.84, t = 0.043, p = 0.97).

CONCLUSION: Actual longitudinal study revealed significantly higher mean values of emotional exhaustion and depersonalization in 2014 that could be explained by significantly increased job demands between analysed points in time.

Introduction

Burnout is most often analysed as a psychological syndrome, resulting from exposure to chronic emotional and interpersonal workplace stressors [1] [2] [3]. This syndrome is represented by three dimensions - emotional exhaustion (an overwhelming exhaustion, as well as feelings of being overextended by the demands of the job and depleted of one's emotional and physical resources), depersonalization or cynicism (a negative, callous, or excessively detached response to various aspects of the job, as well as cynical response to the recipients of care), and reduced personal accomplishment (feelings of incompetence and a lack of achievement and productivity at work) [3]. Since reduced personal accomplishment correlates weakly with both emotional exhaustion and depersonalization and with detected burnout correlates [4] [5], recent studies focused mostly on emotional exhaustion and depersonalization [6] [7] [8].

It is clearly shown that workplace stress and burnout are important issues for a wide range of occupations. For example, the teaching profession is becoming more and more stressful due to increased responsibilities and demanding deadlines [9] [10] [11]. Healthcare professionals (HPs), while providing healthcare services to the patients, are exposed to different psychosocial hazards that originate from the workplace demands and conditions [12] [13]. These hazards are particularly detrimental when

requirements of the work do not match the capabilities, resources or needs of the worker [14]. Hospital HPs in everyday practice is dealing with different physical (e.g., responsibility for too many patients, very fast work), social, emotional, cognitive, and organisational factors (job demands) that require prolonged physical and/or psychological (cognitive and emotional) efforts. Job demands are associated with certain physiological and/or psychological costs in workers (leading to overtaxing and emotional exhaustion) when the invested personal efforts are high [3] [6] [15] [16].

On the other hand, job resources represent those aspects of the job that reduce both job demands and changes in workers related to the job demands. Additionally, job resources help workers in achieving work goals; they stimulate personal growth, learning and development of employees [15]. Job resources are present at the organizational (e.g., good salary, job security) or interpersonal (e.g., teamwork, supervisor and co-worker support) level, at the specific job position (e.g., participation in decision making), and at the level of the task (e.g., autonomy, performance feedback) [16].

According to the job demands/resources model of stress (JD - R model), burnout develops through two types of psychological processes: demanding aspects of work (high job demands) resulting in overtaxing and exhaustion (energetic process); and lack of resources leading to withdrawal behaviour (depersonalization) and disengagement (motivational process) [6] [16]. When the organizational context is represented by adequate job resources, such as proper feedback, adequate supervisor and co-worker support, and appropriate teamwork, in employees could be detected high levels of job engagement as well as low levels of depersonalization. On the contrary, in the context of reduced job resources (e.g., inappropriate performance feedback, low salary, job insecurity, inadequate supervisory coaching and teamwork), job demands are particularly effective and detrimental [6] [16] [17].

Teamwork, as a particular job resource, is a specific cooperative process between team members that allows them to develop effective, mutual relationships in achieving team goals through sharing knowledge and skills [18] [19]. Teamwork is an extremely important resource in hospital settings, and HPs are coordinating their activities to deliver healthcare services to the patients that should be safe, effective, patient - centred, timely, efficient, and equitable [20]. Also, teamwork could protect workers from emotional exhaustion, depersonalization, and disengagement [15]. However, research in this field has demonstrated that teamwork had stronger effects on the relationship job engagement - job satisfaction than on the relationship job demands - burnout. Those findings support theoretical and empirical data about the existence of two psychological processes: 1. energetic process, in which job demands lead to poor health via burnout, and 2. motivational process, in which job resources result in higher job satisfaction via job engagement [15] [21] with job resources having weaker effect on the relationship job demands - burnout.

Despite numerous studies on burnout in HPs, either cross-sectional or longitudinal [22] [23] [24] [25] [26] [27], mainly analysing its predictors and consequences, to date there is poor pool of data in the region of South - East Europe (SEE) on changes in burnout levels over time. In this study we analysed HPs working in a general hospital in Skopje, Macedonia that is an educational base of the Faculty of Medicine in Skopje, providing health care to the general population at secondary and tertiary level.

The health care system in the Republic of Macedonia faces continuous reforms oriented towards improvement of safety, effectiveness, patient-centeredness, timeliness, efficiency, and equity of patient care, as the key attributes of high quality health care [28] [29]. Within aforementioned reforms, followed by reorganisation of hospital settings, HPs from the study hospital are referring increased job demands (ex., significantly increased number of patients served, low salaries of HPs, complex administrative requirements, etc.) [28]. On the other hand, previous qualitative and quantitative studies [28] [29] [30] demonstrated protective workplace factors (job resources) within analysed hospital context (ex., appropriate working conditions, support from superiors and co-workers, teamwork, independence in decision - making, excellent interpersonal relationships, etc.). But, although hospital management continued to implement good organizational and management standards, high level of discipline, teamwork, good communication between team members, HPs working in this hospital still complain about the listed job demands.

The actual paper analyses the concepts of job demands, burnout, and teamwork taking into consideration the national context and specifics of the hospital in focus. The purpose of the present study was to assess job demands, burnout, and teamwork in HPs working in a general hospital that was analysed at two points in time with a time lag of three years.

Methods

The study was conducted in a general teaching hospital from Skopje, Macedonia, an educational base of the Faculty of Medicine. The most important criteria for selecting the hospital were to have stable management and to represent the most typical hospital organisational system, providing

inpatient and outpatient (specialist healthcare with specialist diagnostics) health care and laboratory tests. The total number of employees in the hospital in 2011 was about 420, and it had about 500 beds. Before the research, ethical approval was obtained by the hospital's ethics committee.

The research design included two cross-sectional studies. The hospital employees were studied at two points in time during hospital reorganisations that were conducted in line with country health care reforms. The reforms were followed by higher job demands in HPs, represented mainly by the increased workload, increased number of patients served, and complex administration procedures. The surveys were performed at two points in time (2011 and 2014) with a time lag of three years. On both occasions, the hard copies of the questionnaires together with a letter explaining the objectives of the study and assuring participants' anonymity and confidentiality were distributed in envelopes to all HPs working in the Hospital. Questionnaires were returned anonymously in sealed envelopes to protect participants' privacy. Participation in the study was voluntary.

Participants were HPs working in general hospital that was subject to analysis. Time 1 respondents (N = 325) were those who participated during the first wave of data collection (2011) and who had completed the questionnaires. Time 2 respondents (N = 197) were HPs from the same hospital who responded at Time 2 (2014) and who had also completed the questionnaires. Job demands, burnout, and teamwork were measured with Hospital Experience Scale, Maslach Burnout Inventory, and Hospital Survey on Patient Safety Culture, respectively.

Hospital Experience Scale (HES), which was constructed and developed for FP7 ORCAB Project (http://orcab.web.auth.gr/), was applied for the assessment of job demands within the actual study. The items were categorised into four subscales: physical workload (seven items, e.g., I am responsible for too many patients in hospital rounds), organisational (six items, e.g., The roles in my department are not clear/ambiguous), emotional (six items, e.g., I have to deal with verbally abusive patients) and cognitive (five items, e.g., I have to make decisions when I don't have all the information I need) job demands. Participants indicated their level of agreement with the items on a 5 - point Likert scale (1 = never to 5 = always), and points for statements relating to each of the job demands types were averaged to derive the four types of job demands. The higher mean score means, the higher perceived level of particular job demands type.

Burnout was examined with the Maslach Burnout Inventory (MBI) [3]. Emotional exhaustion (nine items) and depersonalisation (five items) subscales were applied and measured using a 7 - point Likert scale (0 = never to 6 every day). Emotional exhaustion refers to the feelings of overwhelming exhaustion, lack of energy and depletion of emotional resources and the person feels used up. Depersonalization is the interpersonal dimension of burnout that refers to the feelings of frustration, anger, and cynicism. It is described as an excessively detached response to other people. Emotional exhaustion was assessed through items such as "I feel emotionally drained from my work" and "I feel burned out by my work", and depersonalization with items such as "I feel I treat some patients as if they were impersonal objects". Responses are added to form a score for each subscale, thus giving each participant scores for the two components of burnout. The higher the score in one dimension delineates the higher level of burnout.

Teamwork (four items, e.g., when one area in this unit gets busy, others help out) was measured with the Hospital Survey on Patient Safety Culture, developed by the US Agency for Healthcare Research and Quality (http://www.ahrq.gov/qual/patientsafetyculture/hospcult1.htm). Participants indicated the level of agreement with the items (1 = strongly disagree, 2 = disagree, 3 = neither agree nor disagree, 4 = agree, 5 = strongly agree) and the mean score was calculated. The higher mean score represents a higher level of perceived teamwork.

To keep participants' anonymity and confidentiality, the questionnaires were returned anonymously in sealed envelopes and data were made completely anonymous before being analysed and published. Therefore, there was no possibility to follow one person from one point of time to the next. Afterwards, data were analysed as if they were independent.

Independent samples *t*-test was used to compare data obtained at two points in time (2011 and 2014). Pearson's correlation coefficients were calculated to examine relationships between continuous variables (burnout dimensions, different types of job demands, and teamwork). *P* values < 0.05 were considered statistically significant, and the main results are given with 95% confidence intervals (CI). The Statistical Package for the Social Sciences (SPSS) statistics (Chicago USA 2011, version 20) was used for the statistical analyses.

Results

Completed surveys were returned by 325 HPs (70.2% females, 29.8% males) at Time 1. Participants had an average age of 38.12 (SD = 10.58) years, and they worked for an average of 130.55 (SD = 121.01) months at the same hospital and 105.18 (SD = 106.03) months within the same unit. They worked for an average of 42.14 (SD = 11.21) hours per week.

At Time 2, 197 HPs (79.9% females, 20.1% males) returned completed surveys. They had an average age of 42.36 (SD = 9.29) years, and they worked for an average of 157.3 (SD = 115.27) months at the same hospital and 115.25 (SD = 108.19) months within the same unit. They worked for an average of 43.73 (SD = 8.41) hours per week. Similar frequencies of participants were married or lived together with their partner and reported full - time contract as a type of employment at Time 1 and Time 2.

Means, standard deviations, internal consistency coefficients (i.e., Cronbach's alphas), and correlation coefficients of analysed variables at Time 1 and Time 2 are presented in Table 1.

Table 1: Means, standard deviations, internal consistency coefficients (i.e., Cronbach's alphas), and correlation coefficients of variables in Time 1 and Time 2

Variable	Mean	St. dev.	α	1	2	3	4	5	6
Time 1									
1. Emotional Exhaustion	15.37	11.46	0.88	/					
2. Depersonalization	2.75	4.3	0.75	0.623**	/				
3. Job demands Physical demands	3.29	0.67	0.71	0.283**	0.146**	/			
4. Job demands Organizational demands	2.34	0.77	0.78	0.229**	0.21**	0.431**	/		
5. Job demands Emotional demands	2.25	0.61	0.75	0.285**	0.263**	0.367**	0.58**	/	
6. Job demands Cognitive demands	2.64	0.71	0.71	0.27**	0.161**	0.422**	0.502**	0.449**	/
7. Teamwork	3.84	0.79	0.84	-0.249**	-0.25**	-0.161**	-0.435**	-0.291**	-0.206**
Time 2									
1. Emotional Exhaustion	21.03	12.78	0.91	/					
2. Depersonalization	4.48	5.42	0.78	0.639**	/				
3. Job demands Physical demands	3.3	0.64	0.73	0.379**	0.315**	/			
4. Job demands Organizational demands	2.51	0.73	0.72	0.342**	0.346**	0.362**	/		
5. Job demands Emotional demands	2.46	0.65	0.75	0.293**	0.306**	0.355**	0.605**	/	
6. Job demands Cognitive demands	2.82	0.73	0.72	0.221**	0.245**	0.309**	0.586**	0.546**	/
7. Teamwork	3.84	0.86	0.91	-0.182**	-0.173*	-0.163*	-0.28**	-0.172*	-0.23**

** Correlation is significant at the 0.01 level (2 - tailed); * Correlation is significant at the 0.05 level (2 - tailed); *Note*. First row numbers (1 - 6) indicate the same respective; variables as shown in the first column (eg., 1. Emotional Exhaustion, 2. Depersonalization, 3. Job demands - Physical demands etc.)

The reliability of the scales at both Time 1 and Time 2 was above 0.7 which was adequate for further statistical analyses. Using bivariate analyses, we found significant positive correlations of burnout dimensions with all job demands types at both times. On the other hand, teamwork was negatively correlated with both burnout dimensions and all types of job demands at Time 1 and Time 2 (see Table 1). Figure 1 and Figure 2 show mean emotional exhaustion and depersonalization scores in 2011 and 2014.

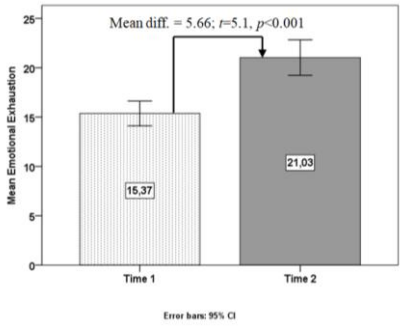

Figure 1: Mean emotional exhaustion scores in 2011 (Time 1) and 2014 (Time 2)

Figure 1 and Figure 2 data demonstrate that significantly higher scores of emotional exhaustion (21.03 *vs*. 15.37, Mean diff. = 5.66, t = 5.1, p < 0.001) and depersonalization (4.48 *vs*. 2.75, Mean diff. = 1.72, t = 3.8, p < 0.001) were detected at Time 2 (2014).

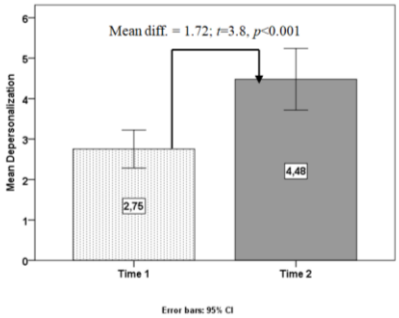

Figure 2: Mean depersonalization scores in 2011 (Time 1) and 2014 (Time 2)

Descriptive statistics of job demands and teamwork and differences between 2011 (Time 1) and 2014 (Time 2) are shown in Table 2.

Table 2: Descriptive statistics of job demands and teamwork and differences between 2011 (Time 1) and 2014 (Time 2)

		Mean	SD	*t*	Mean diff. (95% CI)	*p*
Job demands - Physical demands	2011	3.29	0.67	-0.043	0.003 (-0.11, 0.12)	0.965
	2014	3.3	0.64			
Job demands - Organizational demands	2011	2.34	0.77	-2.38	-0.16 (-0.3, -0.03)	0.017
	2014	2.51	0.73			
Job demands - Emotional demands	2011	2.25	0.61	-3.68	-0.21 (-0.32, -0.1)	<0.001
	2014	2.46	0.65			
Job demands - Cognitive demands	2011	2.64	0.71	-2.68	-0.17 (-0.3, -0.07)	0.008
	2014	2.82	0.73			
Teamwork	2011	3.84	0.79	0.043	0.003 (-0.14, 0.15)	0.965
	2014	3.84	0.86			

The data obtained represent significantly higher scores of organizational (2.51 *vs*. 2.34, Mean diff. = 0.16, t = 2.38, p = 0.017), emotional (2.46 *vs*. 2.25, Mean diff. = 0.21, t = 3.68, p < 0.001), and cognitive (2.82 *vs*. 2.64, Mean diff. = 0.17, t = 2.68, p=0.008) job demands at Time 2. Despite aforementioned significant differences, teamwork levels were similar at both points in time (Time 1 = 3.84 *vs*. Time 2 = 3.84, Mean diff. = 0.003, t = 0.043, p = 0.97) (see Table 2).

Discussion

Emotional exhaustion (15.37) and depersonalization (2.75) mean scores in 2011 within the actual study were lower than it was found in previous studies analysing burnout in hospital HPs. For example, Vahey DC et al. registered an average score of emotional exhaustion - 24.3 and an average score of depersonalization - 7.4 in nurses from 40 units in 20 urban hospitals across the United States [31]. Similarly, low mean values of both burnout dimensions were also registered in HPs in other

studies conducted in R. Macedonia [29][30][32][33]. In a study conducted in HPs from seven South and South - Eastern European countries, adopting a cross-national approach, Macedonian HPs demonstrated levels of emotional exhaustion (EE) and depersonalization (DP) in the lower end of the average EE and DP scores [34].

On the other hand, job demands in 2011 showed medium level (physical - 3.29, organisational - 2.34, emotional - 2.25, and cognitive - 2.64 job demands) with the relatively high level of perceived teamwork - 3.84. The average score of teamwork with the added value of the standard deviation - 0.79 becomes over 4.5, the level that demonstrates teamwork as a significant protective factor within analysed hospital, taking into consideration that teamwork is found to be one of the most important job resources, together with good interpersonal relationships, support from superiors, adequate feedback, and independence in decision making.

The implementation of JD - R Model within analysed hospital context in 2011, references to both medium level job demands and high level teamwork that could boost the compensatory efforts in HPs in order to maintain high level of performance (high job engagement) and to reduce physiological and psychological costs in HPs associated with their work efforts (low exhaustion and depersonalization) [16]. Indeed, job demands may lead to the depletion of energy (emotional exhaustion), but in our case job resources (e.g., high level of teamwork) with their protective and motivational potential have led to low levels of exhaustion and depersonalization. The protective function of teamwork was demonstrated elsewhere. Different studies have shown that teamwork predicted lower levels of burnout. The more that HPs experienced teamwork, the less they felt burnt out [15] [17] [32].

To our knowledge, similar studies analysing changes in burnout levels over time in the SEE Region are very rare. The present study was aimed to assess job demands, burnout, and teamwork in HPs, working in a general hospital, at two points in time (2011 and 2014). We have found that both burnout dimensions were positively correlated with all types of job demands, while teamwork was negatively correlated with burnout dimensions and job demands at both occasions (2011 and 2014). Similarly to our findings, studies have shown that HPs who perceive their job demands as reasonable and who have more support from colleagues (higher levels of teamwork) have higher levels of psychological well - being [35]. Additionally, equivalent results of bivariate analyses were also registered in other studies conducted in R. Macedonia [29] [30].

The actual study demonstrated that in 2014, the mean values of emotional exhaustion and depersonalization were significantly higher than in 2011. This increase in emotional exhaustion and depersonalization levels between 2011 and 2014 has to be explained in a hospital context where job demands also significantly increased between analysed points in time, while perceived teamwork levels remained relatively high and constant from 2011 until 2014. Namely, our data suggested that in HPs, in the period between 2011 and 2014, the feelings of more strict hierarchy in the hospital, ambiguous roles, and problematic communication have become more intense, they became more concerned about the negative influence of media on the work of HPs, together with an increase in emotional (e.g., emotional involvement in work, dealing with verbally abusive patients) and cognitive (e.g., inadequate feedback, time pressures) job demands.

According to the JD - R model, demanding aspects of work (high job demands) as well as involvement in jobs with chronic job demands (either work overload or emotional demands or others) exhaust employees' mental and physical resources and may, therefore, lead to the depletion of energy [16]. It was scientifically proven that these job demands predict overtaxing and exhaustion in workers. Additionally, in Macedonian context, it was detected that ageing of HPs positively predicted both emotional exhaustion and depersonalization [30]. Having in mind this knowledge, we further performed bivariate analyses between burnout dimensions and age within the actual study. These analyses showed a positive correlation of both burnout dimensions with an age of HPs in either 2011 or 2014, meaning that the older HPs experienced more emotional exhaustion and depersonalization. Therefore, higher burnout scores in 2014 than in 2011 could be a result not only of higher job demands but also an effect of the ageing of HPs.

The other component of JD - R model, besides job demands, is job resources. Hospital context is, usually, characterised by one of the most important job resources, defined as teamwork. It is well established that teamwork could protect workers from emotional exhaustion, depersonalization, and disengagement [15]. Research has shown that teamwork demonstrated a buffering effect (i.e., increasing the teamwork would decrease either the effect of the job demands on burnout or the effect of age on burnout) [30].

Since the level of perceived teamwork was constantly high between 2011 and 2014 (over 4.5, together with the added value of the standard deviation), we expected that the high level of this job resource would boost the compensatory efforts in HPs to reduce exhaustion and depersonalization. However, the levels of burnout significantly increased from 2011 to 2014. These findings could be explained the results of mediation and moderation analyses showing that teamwork had stronger effects on the relationship job engagement - job satisfaction than on the relationship job demands - burnout [32]. In other

words, in the analysed hospital context, teamwork has failed to decrease the effect of the job demands on burnout. Finally, this study again confirms the existence of two processes in the psychosocial functioning of workers: 1. Energetic process (job demands - burnout - poor health), and 2. Motivational process (job resources - job engagement - job satisfaction) [15] [21].

Findings of the actual study should be interpreted with caution as answering bias could rise because it is possible that more affected HPs tended to answer. Also, a “healthy worker effect” may have underestimated the levels of burnout. Additional limitations also include the fact that the analyses were based on self - reporting from questionnaires.

As a conclusion, this study shows differences in job demands and burnout in HPs working in a general hospital that was analysed at two points in time with a time lag of three years. In 2011, emotional exhaustion and average depersonalization scores were lower than it was found in previous studies analysing burnout in hospital HPs. On the other hand, job demands in 2011 showed medium level, while teamwork demonstrated relatively high level. The actual study revealed significantly higher mean values of emotional exhaustion and depersonalization in 2014 that could be explained by significantly increased job demands between analysed points in time. The perceived teamwork levels remained relatively high and constant from 2011 until 2014. Although, scientific data demonstrated that teamwork negatively predicted burnout and it can boost compensatory efforts in HPs to reduce exhaustion and depersonalise zation, this study supports the findings that teamwork had stronger effects on the relationship job engagement - job satisfaction than on the relationship job demands - burnout.

Obtained data can be used in the implementation of specific organisational interventions in the hospital setting through providing adequate JD - R interaction to prevent burnout in HPs. The interventions should be guided by the findings that job demands have a key role in the increase of exhaustion and depersonalization levels over time. The most importantly, this study highlights the importance of job demands in burnout development, even in the context when teamwork has constantly high levels. Specific strategies should be implemented in the hospital towards minimising competitiveness between colleagues, giving clear roles to workers, improvement of communication, giving adequate feedback about completed workplace tasks, and lowering time pressures. Further keeping the teamwork on high levels can be used towards the improvement of job engagement, job satisfaction, as well as good health and well - being in HPs since it has effects on the energetic and motivational psychosocial processes.

Physical job demands should not be overlooked since there level over time was constantly higher than the levels of other job demands. Reducing workload and time pressure through new employment as well as by purchasing new medical equipment and other supplies should be taken into account. Raising awareness in policymakers and key stakeholders should be strengthened. Finally, it should be stressed that providing adequate job demands-resources interaction can lead to the prevention of job burnout in HPs, as well as to improve the quality of care for the final consumers, or patients.

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Review Article

Stevens - Johnson Syndrome and Toxic Epidermal Necrolysis; Extensive Review of Reports of Drug-Induced Etiologies, and Possible Therapeutic Modalities

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Keywords: Stevens-Johnson Syndrome; Toxic Epidermal Necrolysis; Drug hypersensitivity; Adverse drug reaction; Skin; Mucous membranes

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Abstract

Stevens - Johnson Syndrome and Toxic Epidermal Necrolysis are adverse hypersensitivity reactions that affect the skin and mucous membranes. They are characterised by erythematous macules and hemorrhagic erosions of the mucous membranes. Epidermal detachments of varying degrees of severity also occur in these conditions. Various aetiologies are associated with these conditions, with adverse drug reaction being the most common. Though the worldwide incidence of these conditions is recorded as low, diverse types of medication are being observed to lead to these conditions. This review compiles information on the details of Stevens-Johnson syndrome and Toxic Epidermal Necrolysis, the pathophysiology, therapeutic management, and largely considers the drug-induced etiologies associated with these conditions.

Introduction

Stevens-Johnson syndrome (SJS) is a dermatological condition with the more severe form being toxic epidermal necrolysis syndrome (TEN) or Lyell's syndrome. These two syndromes are said to exist at the two ends of the spectrum of an adverse skin reaction occurring from severe epidermolysis [1]. They present as severe exfoliative reactions affecting mainly the skin and mucous membranes [1]. The characteristic clinical presentation includes mucocutaneous tenderness, hemorrhagic erosions, and erosion of the mucous membrane, erythematous macules, blisters and denuded skin occurring as a result of the severe separation of the epidermis from the dermis [2]. The Bastuji-Garin et al. criterium is used in the diagnosis of SJS/TEN in which patients are classified into three categories based on the degrees of skin detachment [2], while the international classification uses the affected body surface area (BSA); SJS involves lower than 10% of the BSA, while TEN affects greater than 30% of the BSA. The definition of SJS and TEN incorporates an overlap which shall be highlighted in Table 1 below. It has been shown that SJS and TEN have mortalities in the range of 10 to 50 percent [3].

SJS/TEN can present in any age group but occurs more frequently in women, HIV-Infected patients, and the elderly. The global incidence rate associated with TEN is low, estimated in 2005 between 0.4 and 1.2 or 1.3 per million persons yearly. An epidemiologic study of TEN in France gave a similar incidence of 1 to 1.3 cases/million/ annum [4].

However, as the years' advance, the numbers of incidence seem to be increasing in other parts of the world. Literature shows a correlation between the incidence and increasing age. The incidence increases sharply with increasing age, as does the use of drugs with ageing [5]. Females are most commonly affected represented by a female-male ratio of 3:2. The average age of patients reported is between the ages of 46 and 63, while the proportion of females is estimated between 61.3% and 64.3%, respectively [4]. Reports have also been linked to patients with Human Immunodeficiency Virus (HIV)-1 infection, recipients of bone-marrow transplants and systemic lupus erythematosus (SLE) [5].

Drug hypersensitivity has been associated with relatively complex genetic factors, which have been studied in diverse populations as well as in a variety of ethnic background. Chung et al., demonstrated a uniquely strong correlation between drug hypersensitivity (Carbamazepine triggered SJS), ethnic background (Hans Chinese) and Human Leucocyte Antigen (HLA)-B*1502 [3]. This strong association resulted in a further investigation into a similar cohort of Hong Kong Han Chinese having severe adverse cutaneous reactions to anticonvulsant drugs [6].

In another study of a Thai population, the susceptibility of individuals with HLA-B*1502 to the anticonvulsant carbamazepine was confirmed [7]. However, a weak association between carbamazepine and HLA-B*1502 was only demonstrable in an Indian-based study. While no genetic correlation could be established in Europeans and Japanese [8][9].

To further corroborate this non-genetic association in Europeans, a large study (RegiSCAR) carried out an HLA-B genotype on patients who suffered severe adverse cutaneous reactions triggered carbamazepine, lamotrigine, sulfamethoxazole, allopurinol, and NSAIDs of oxicam-type regarded as high-risk drugs. The study showed that HLA-B*1502 is not a confirmatory marker for any of the studied high-risk drugs known to cause SJS/TEN, and hence cannot be authoritatively labelled the cause of pathology in Europeans [10][11]. Therefore, it can be concluded that for SJS/TEN in individuals exposed to Carbamazepine, "the genetic constellation of HLA-B*1502 is not a population independent marker" [6].

HLA-B*5801 is another genotype which has been highly correlated with SJS/TEN in Han Chinese patients exposed to allopurinol. The study showed a 100% correlation of allopurinol exposure to HLA - B*5801 positive genotype in patients who presented with the adverse drug reactions [12]. Subsequent studies further revealed a high correlation between HLA-B*5801 and SJS/TEN in Thai patients [7], Japanese patients [9], and too much lower degree patients of European descent (about 55% of cases) [11].

Epidermal necrosis that occurs as a part of the TEN disease process is mainly associated with massive keratinocyte apoptosis [4]. This is mediated by surface receptors such as tumour necrosis factor (TNF) receptor (Fas), and when coupled with the Fas ligand causes disassembly of DNA and cell death by the induction of apoptosis [7]. Cell death could also be modulated by death receptor-independent mechanisms that include the release granzyme-B and perforins from cytotoxic T lymphocytes, thus activating the caspase-dependent or caspase-independent mechanism [12].

Clinical Presentation

The initial presentation of SJS/TEN may include non-specific symptoms such as fever, discomfort with swallowing, and stinging eyes. Typically, the cutaneous manifestations of SJS/TEN are usually preceded by these non-specific symptoms [13] [14]. Early locations for skin involvement include the presternal truncal region, the face, and could also involve the palms and the soles. In about 90% of patients, there is involvement of the mucosa of the mouth, genital and/or gastrointestinal tracts visible as erythema and erosions [13] [14]. Other frequent presentations at the beginning of the pathology is eye related and this ranges from acute conjunctivitis, erythema, edema of the eyelid, ocular discharge and crusting, to corneal erosion, the formation of conjunctival membrane or pseudomembrane, and in severe cases, to corneal ulcerations, cicatrizing lesions, fornix shortening, and symblepharon [15] [16]. However, late complications of SJS/TEN cannot be predicted by the severity of acute ocular manifestations [17]. Erythematous and livid macules typify the morphology of early cutaneous lesions.

Table 1: Clinical manifestations distinguishing SJS, SJS-TEN overlap, and TEN [2]

Clinical entity	SJS	SJS-TEN overlap	TEN
Primary Lesions	Dusky red lesions Flat atypical targets	Dusky red lesions Flat atypical targets	Poorly delineated erythematous plaques Epidermal detachment Dusky red lesions Flat atypical targets
Distribution	Isolated lesions Confluence (+) on face and trunk	Isolated lesions Confluence (++) on face and trunk	Isolated lesions (rare) Confluence (+++) on face, trunk, and elsewhere
Mucosal involvement	Yes	Yes	Yes
Systemic symptoms	Usually	Always	Always
Detachment (%body surface area)	< 10	10-30	> 30

The second phase is characterised by the development of wide-spread areas of epidermal separation. If no epidermal separation is observed, it warrants more detailed skin examination during which a tangential mechanical pressure is exerted on many erythematous areas, called Nikolsky sign. If the

mechanical pressure causes and epidermal detachment, Nikolsky sign is positive. However, Nikolsky sign is not only defined for SJS or TEN, as it can equally be positive in conditions like the autoimmune bullous cutaneous pathologies like the pemphigus vulgaris [15] which can be utilised as a distinguishing feature from a similar autoimmune condition, bullous pemphigoid.

A major prognostic factor is the degree of cutaneous involvement. The evaluation of the degree of skin involvement should only include the already detached necrotic skin or detachable skin, that is, those that are Nikolsky positive [17].

Magina and colleagues [16] reported the following presentations for the late phase of TEN: Cutaneous hypo and hyperpigmentation (62.5%), nail dystrophies (37.5%), and the rest being eye complications. In another study, Yip et al. reported late ocular complications in about 50% of patients with TEN and reported them by ranking them in decreasing frequencies; “severe ocular dryness (46% of cases), trichiasis (16%), symblepharon (14%), distichiasis (14%), visual loss (5%), entropion (5%), ankyloblepharon (2%), lagophthalmos (2%), and corneal ulceration (2%)” [18]. Hypertrophic scars have only been reported in a handful of patients [19]. Reports have shown that 73% of patients with acute phase mucosal involvement subsequently presented with long-term complications with mucosal sequelae involving the oral and oesophagal mucosa majorly, and to a lesser degree, the genital and pulmonary mucosa [20]. Similarly, a nine-patient SJS/TEN study showed seven of the patients presenting with either keratoconjunctivitis or xerostomia or both, with a resemblance to Sjogren-like syndrome [21]. Furthermore, another report revealed a patient with “Sjögren-like pluriglandular exocrine insufficiency”, which is also resulted in an impairment of the exocrine pancreas [22].

Drug etiologies associated with SJS and TEN

A major percentage of the disease is caused by medications, while the remaining is due to upper respiratory infections such as HIV [23], Hepatitis virus, Herpes virus, Mycoplasma pneumoniae [24] [25]. Others include malignancies, as well as idiopathic. A substantial number of medications have been implicated in the aetiology of SJS/TEN. This article has attempted to review as many as possible of the published reports. The table 2 below summarises some drug categories that have been implicated in the aetiology of SJS/TEN.

Table 2: Reported cases of drug-induced sjs/ten

Drug Classification	References
Antibiotics	23-34
Anticonvulsants	35-41
Sulfonylureas	42
Diuretics	43-44
Analgesics	45-48
Antidepressants	49-50
Tyrosine Kinase Inhibitors	51-54
Xanthine Oxidase Inhibitors	55
Androgenic hormones	56-57
Antineoplastic drugs	58-60
Antiviral drugs	61-63
Combination drug(Aggrenox)	64
Immunosuppressant/modulators	65-67
Antihistamines	68
Angiotensin-converting enzyme inhibitors	69
Anti-osteoporotic agent	70
Contrast agent	71
Insecticide	72-73

Antibiotics

Sulfonamides: Among the antibiotics, the most implicated high-risk cause of SJS/TEN are the sulfonamides especially *Trimethoprim - Sulfamethoxazole* which accounts for about 69% of cases. The other non-sulfonamide antibiotics are considered low risk [23] [24]. Figure 1, is a female patient with adverse cutaneous reaction from Trimethoprim-Sulfamethoxazole prescribed for an upper respiratory tract infection.

Figure 1: Patient with SJS/TEN caused by Trimethoprim - Sulfamethoxazole, before the commencement of therapy

Aminopenicillins: Aminopenicillins have been shown to be the most frequent causes of SJS when compared to the other antibiotics. This could be due to how frequently they are prescribed [25]. *Amoxicillin/clavulanic* acid (Co-amoxiclav) even resulted in SJS in an 18-month-old child treated post-caustic poisoning and esophagogastric necrosis [26].

Fluoroquinolones: Ciprofloxacin induced SJS in a patient treated for otitis media reported in Sweden. [27] *Norfloxacin* induced SJS may appear similar to pemphigus, hence making early diagnosis a bit difficult [28].

Tetracyclines: Doxycycline has been implicated in the aetiology of SJS in the systemic use of ophthalmologic eyelid and ocular surface disorders [29]. *Minocycline* has been shown to induce both SJS and concurrent bilateral Parotitis in a young boy [30].

Macrolides: Azithromycin has been shown to cause SJS after a five-day outpatient completion [31].

Cephalosporins: Cefotaxime has been implicated in causing SJS when administered to an elderly lady for treatment of upper urinary infection [32], likewise *Cefepime* [33].

Metronidazole: Metronidazole induced SJS tends to start off with neurological manifestations before mucocutaneous and skin eruptions. This is worth noting, as patients should be advised of the early symptoms to prevent this rare adverse effect [34].

Anticonvulsants

Phenytoin: There is a possible association between the HLA-B*1502 allele and phenytoin-induced SJS in Asian patients. This is still under review by the FDA. This could mean a possible genetic predisposition to getting SJS in certain populations as opposed to others [35].

Lamotrigine: A potential rare side effect of SJS/TEN has been implicated regardless of appropriate dosing and adjustments; Concurrent use with Valproic acid increases risk [36].

Carbamazepine: Increased frequency of its use for pain control has further increased its implication in causing SJS/TEN [37].

Oxcarbazepine: A case was reported in India after use for treatment of epilepsy in a 21 – year-old male. SJS occurred 2 weeks during treatment despite accurate titrations [38].

Phenobarbital: Risk increases within the first 2 months of treatment. Genetic predisposition has been associated with this medication in conjunction with SJS/TEN [39].

Sodium valproate: A potential cause of SJS/TEN, though lower risk than the rest of the anticonvulsants. Increased risk when used together with other anticonvulsants. When used as monotherapy, it rarely causes SJS. However, if it occurs, it seems to be restricted to the involvement of only the oral mucosa [40].

Levetiracetam: It has been implicated in hypersensitivity syndrome reactions as well as SJS. Although rare it can be probably dose related [41].

Sulfonylureas

Glipizide: A study showed the increase in dosage from 5mg to 10 mg in a certain patient triggered a complex immune reaction that resulted in SJS the following day, it was postulated it could be due to the certain delayed immune reaction and possibly due to hapten hypothesis [42].

Diuretics

Furosemide: A potential adverse effect is SJS, especially when used as an additive with other sulfa-containing drugs [43].

Acetazolamide: A commonly used drug in ophthalmology. It is also a sulfonamide as well as a carbonic anhydrase inhibitor. It's been associated with fatal SJS in patients of Korean and Japanese descents. HLA-B59, which is specific to Japanese descents, is a risk factor [44].

Analgesics

i) Non-steroidal anti-inflammatory drugs (NSAIDS):

Diclofenac: It could cause SJS especially in the elderly; caution should be applied when prescribing this drug [45].

Ibuprofen: SJS occurred in a Nepali male after taking 400mg of Ibuprofen every 8 hours for 2days. It could be due to genetic predisposition by HLA type or some inflammatory mediators causing epithelial damage [46].

Rofecoxib: A selective COX-2 inhibitor that has decreased gastrointestinal side effects was shown to cause SJS after three weeks of administration to a patient with systemic arthralgia [47].

ii) Paracetamol (Acetaminophen):

Paracetamol was shown to cause SJS/TEN despite its fair safety margin. It was shown to be dose-dependent in causing SJS [48].

Antidepressants

Mirtazapine: A patient with Systemic lupus erythematosus (SLE) who took mirtazapine for depression presented with SJS after 15 days of use. Though a very rare cause of SJS. The presence of the autoimmune disease led to a dilemma between either SLE or mirtazapine as the cause. The history and resolution of the disease eventually pointed to Mirtazapine as the culprit [49].

Duloxetine: The study showed 0.01% of patients treated with this medication could potentially cause SJS. An adolescent was affected in this study; so far it had only been adults involved [50].

Tyrosine kinase inhibitors

Afatinib: SJS can be seen in patients treated with Afatinib for Non-small cell lung cancer (NSCLC) [51].

Vandetanib: Also used in the treatment of NSCLC, was shown to cause SJS in certain patients

[52].

Imatinib: SJS occurred in a patient treated with Imatinib for chronic myeloid leukaemia after treatment for 2 days. Caution should be taken in the prescription of this medication, as SJS is a potential adverse effect [53].

Sunitinib: A patient was treated with Sunitib for renal cell carcinoma with metastasis to the lung. On day 14 of treatment, the patient presented with SJS [54].

Xanthine oxidase inhibitor

Allopurinol: Commonly used in the treatment of chronic gout. It is usually considered a safe drug, and due to its frequent administration, increased risk for SJS/TEN is possible, also common in genetically predisposed patients especially in the Han Chinese population [55].

Androgenic hormones

Danazol: A patient diagnosed with systemic lupus erythematosus was prescribed danazol for treatment of autoimmune hemolytic anaemia. It has been approved as a second line agent in SLE related haematological disorders including thrombocytopenia [56].

Androgenic anabolic steroids: An athlete involved in the illicit use of steroids presented with SJS immediately after injecting drostanolone propionate, danazol, and metenolone enanthate [57]. Considering the common use of steroids for body performance, patients should be alerted to this rare side effect.

Antineoplastic drugs

Paclitaxel: An antineoplastic agent used to treat several cancers; Though SJS could be a rare complication, caution is advised when prescribed. A 53 – year-old male treated with Paclitaxel manifested symptoms after administration of second dose [58].

Docetaxel: Due to its strong toxicity, a patient presented with SJS after its use as a chemotherapeutic agent. Skin eruptions erupted after the first cycle of chemotherapy [59].

Tegafur/gimeracil/uracil (TS-1): A 78-year-old Japanese male presented with SJS eight days after treatment for carcinoma of the oral floor. Further tests showed an association between drug eruptions and antinuclear antibodies and positive drug-induced lymphocyte stimulation test [DLST] [60].

Anti viral drugs

i) Neuraminidase inhibitor (Oseltamivir); the medication is popularly known as Tamiflu and is indicated for prevention and treatment of Influenza. Considering the increased use of this drug, there are concerns about an increased risk for SJS [61].

ii) Nucleoside reverse transcriptase inhibitor (Adefovir): Commonly used in the treatment of Hepatitis B and Herpes simplex virus. A case of SJS was reported due to adefovir use [62].

iii) Non-nucleoside reverse transcriptase inhibitor (Nevirapine): Commonly used in the combination treatment for HIV. Patients infected with HIV-1 are more prone to SJS [63]. Figure 2 shows a healing adverse cutaneous reaction from Nevirapine (a component of the HAART) for a patient diagnosed with HIV.

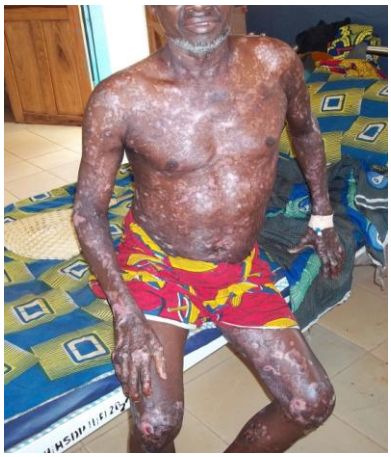

Figure 2: Patient with SJS/TEN caused by Nevirapine responding to therapy

Aggrenox

This is a combination of Aspirin and Dipyridamole, it is mainly used for stroke reduction in high-risk patients. It caused SJS in an elderly Chinese woman with transient ischemic attack who was recently switched from aspirin to Aggrenox [64].

Immunosuppressants/immunomodulators

i) Immuno-modulatory imide drugs (IMiDs):

Thalidomide: This is approved for use in the treatment of multiple myeloma. It inhibits Interleukin 6 (IL-6), a vital component in the proliferation of myeloma cells. A study once showed thalidomide could be used to treat SJS/TENS. However a case of SJS was reported with its use. This contradiction is worth noting [65].

Lenalidomide: Though similar to thalidomide, it has shown to have lesser adverse effects. A rare adverse effect is SJS [66].

ii) Imidazole nucleoside:

Mizoribine: An immunosuppressant used in

renal transplants, lupus nephritis and rheumatoid arthritis. A 32-year-old Japanese woman diagnosed with SLE was started on mizoribine for lupus nephritis. She desired pregnancy hence cyclophosphamide was not used. Mizoribine induced SJS 6 months later despite its known safety margin [67].

Antihistamines

Fexofenadine: Telfast-D a drug containing both fexofenadine-pseudoephedrine was prescribed to a patient due to unrelenting allergic rhinitis. It further resulted in SJS. It was confirmed by skin testing (prick, intradermal and patch) which showed a positive reaction 96 hours later [68].

Angiotensin-converting enzyme inhibitors

Ramipril: Ramipril resulted in SJS after being newly prescribed for a patient for hypertension. ACE inhibitors are known to interfere directly with cell cohesion causing bullous eruptions, which are similar to pemphigus vulgaris or bullous pemphigoid. These reactions are usually non-immunological [69].

Antiosteoporotic agent

Strontium ranelate: It is known as a dual action bone agent (DABA) because of increases deposition of bone by osteoblasts and decreases resorption of bone by osteoclasts. A 67-year-old Chinese woman was diagnosed with SJS 3 weeks after starting this medication for treatment of post-menopausal osteoporosis [70].

Other reported non-therapeutic agents/chemicals in the aetiology of SJS

Iopentol: This is a contrast medium. A 6year old diagnosed with Hodgkin's disease was injected with iopentol to undergo CT scan to explore his lymphadenopathy. Three days later he presented with SJS [71]. Knowing how common CT scans are used, it is worth noting the potential adverse effect of contrast medium.

Carbamate: This is an insecticide. A 63 – year-old farmer was exposed to the insecticide two days before presenting with SJS. He wasn't on any medication. He admitted to contact of the carbamate with his skin [72]. There has been a reported case of TENS associated with oral ingestion of carbamate as a suicide attempt [73].

The therapeutic approach to SJS and TENS

The treatment modality employed in patient management is dependent on the aetiology of the disease which as previously mentioned could be; infectious, drug-induced, as well as malignancies or idiopathic [74].

The first step to the treatment of SJS and TENS is to eliminate the causative factor. In cases of SJS/TENS caused by infections with organisms, the patient is treated with the appropriate antimicrobial. For drug-induced SJS/TENS, the offending drug is withdrawn immediately [75].

The next step is the provision of supportive care for the patient. This includes administration of intravenous fluids as well as parenteral or nasogastric feeding. Patients are also to be kept in a warm environment [76].

The final step is symptomatic treatment. Several methods have been employed in symptomatic treatment (Table 3**).**

Table 3: Various approaches in the management of SJS and TEN

Therapy	Mechanism of Action	Advantages	Disadvantages	Reference
Systemic Corticosteroids	They decrease immune response to an exogenous agent.	Since SJS/TEN is thought to be as a result of an immune response of the body to an exogenous agent, corticosteroids may decrease the severity of this response.	Corticosteroids are possible causative factors for SJS/TENS. Some studies show that they can increase the risk of infections.	[77, 78]
Human Intravenous Immune Globulin (IVIG)	In addition to a combination of immunoglobulins, IVIG contains autoantibodies against Fas receptors. Fas receptors are on the surface of keratinocytes. When bound to by the Fas ligand they mediate the Fas-Fas ligand-mediated apoptosis. The autoantibodies in IVIG bind to Fas receptors to prevent this apoptotic process	Autoantibodies in IVIG are believed to reduce complications of TEN. It can be used in combination with corticosteroids as management therapy resulting in a better chance of decreasing mortality rate. It was discovered that when compared to supportive care only, early intervention with IVIG appeared to significantly improve the ocular involvement of SJS/TEN.	Some studies record higher mortality rates with the use of IVIG when compared to supportive care or corticosteroids	[77, 78]
Cyclosporine	It inhibits calcineurin and thus decreases T cell activity. It acts as an immunosuppressant. It can also prevent the process of apoptosis through the downregulation of NF-kB.	Patients treated with cyclosporine completed re-epithelization more quickly than with other treatments. Fewer numbers of patients treated developed organ failure and died than with other treatments.	Leukoencephalopathy, neutropenia, pneumonia, and nephropathy.	[77, 79, 80]
Plasmapheresis	This process involves the filtration the patient's blood. -The cellular component is separated, and the plasma is discarded. -Artificial plasma and albumin is added to the filtered cellular components and then re-transfused back into the patient. -This is done to eliminate the non-dialyzable pathogens in the plasma.	- It is a safe procedure. -it has yielded favourable results with survival rates of 77-100% after 1 to 8 exchanges.		[78]
Granulocyte Colony Stimulating Factor	-It increases neutrophil counts.	-It can reduce the risk of infection in neutropenic patients with SJS/TEN.		[77]

Other potential therapeutic measures like the TNF-alpha inhibitor, thalidomide and

Cyclophosphamide have been associated with increased mortality [77].

In conclusion, SJS and TEN are both life threatening adverse hypersensitivity reactions. Proper understanding of the etiology as well as the progression of these conditions is necessary for early diagnosis as well as treatment. It is expected that the investigation of the mechanism of action of drugs associated with SJS and TEN will improve the current understanding of the condition with aim of eliminating its incidence. There are existing treatment modalities for these conditions, however, there is no therapeutic measure defined as superior to others. It has however been observed that the earlier these conditions are diagnosed and managed the better the prognosis.

Authors Contribution

AOJ, PO, PA, FA, PE, DO and EO wrote the manuscript; AOJ and DO reviewed the manuscript; all the authors approved the manuscript for publication.

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Review Article

Pain Relief as an Integral Part of the Palliative Care

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Abstract

BACKGROUND: Palliative therapy represents active care for patients whose illness has such nature that is not responding to the curative treatment. The palliative care aims to provide comfort and prevention from the suffering of the patients at the end of their life. Treatment of the pain presents an important integral part of palliative care.

AIM: This article aims to discuss and answer to some of the analgesic regimes and therapeutic dilemmas.

RESULTS: Pain control, in addition to the other treatments such as alleviation of psychological, sociological and spiritual problems, has a priority. The proper pain management can achieve a better quality of life for the patients and their families.

CONCLUSION: It can be concluded that because of the different origin of the pain, the use of analgesic therapy should be individualised and adapted to the real need of every person. Finally, only a good organisation and institutionalisation of the palliative care in one society could permit better prevention of suffering at the end of the life.

Introduction

The complexity of the management of the patients suffering the pain is a challenge for professionals, particularly for the patients at the end of the life. Most of the terminal patients, especially those with malignancy have a poor quality of life. The persistence of the pain is a result of the terminal illness itself, or of the therapeutic approach, such as chemotherapy, neuropathic, or from concomitant diseases such as osteoarthritis, spondylolisthesis, migraine, etc.

Pain evokes unpleasant sensation to the patients. This is a result of stimulations which hurt or destroy tissues and commonly is associated with a pathological event in the body. Pain is a subjective phenomenon, and its objective evaluation is difficult. This is especially difficult in palliative care because of the pain complexity. The reason for the pain, especially in malignant diseases, is very often neural compression and infiltration, bone metastasis, obstructions and infiltration of the soft tissues. In approximately 70–90% of the patients, during the palliative phase, the pain is present in one moment [1]. The patients on palliative care had fear of the pain with a physical, emotional and psychological component of the pain. In early 1900, Sherrington attempted to define the pain and he accentuated the two components of the pain: sensitivity and affection. Later, Henry Head found a double reaction in acute sensation. Today it is accepted to speak about pain as a complex perception experience with plasticity [2].

The reaction to the pain is complex and individual. The magnitude, the severity of the illness and the degree of patients' discomfort, all take part in the formation of individual experience to pain. Different physiological and psychological phenomena in the body produce modifications of the quality of the pain. The memories of pain episodes, the patients' reactivity to pain, families and friends support, religions, personal defence skills, and therapeutic

strategies are the most frequent reasons for these modifications. The level of education, culture and tradition take an important part in the formation of the pain experience. The threshold to the pain is individual. Severe pain produces mental and physical torture of the body. The person is exhausted, fatigue and without energy. Fatigue is one of the leading symptoms of the terminal states and often concomitant symptom of the malignancy, producing a poor quality of life [3]. The pain experience is unique, stretch individual and leads to changes in the personality. It has social implications; it disturbs the sleep, appetite, lowers the tolerance to the stress and is often the reason for depression [4].

In the strategy for pain management, and in palliative care, there are two known approaches: evaluation of the pain, and the treatment (management) of the pain.

Even though pain is an individual complex experience, it is supported by different physiological, psychological and spiritual factors. During the evaluation of the quality and quantity of pain, all additional factors and their interactions must be taken into account. The detailed anamnesis is essential. It orients the clinician for the patient's needs and determines whether an aggressive and sufficient therapy for pain relief is necessary or not [5].

Evaluation of the pain

Since pain is an individual experience, symptoms should be converted to measurable magnitudes in purpose to make an appropriate assessment. Taking anamnesis is the first step which helps tremendously, and also pain must be described in details. This is not so easy in palliative care!

To assign the appropriate management, it is important to discover: the origin of the pain, the states in which the pain is more intensive, the quality of the pain, the route of propagation of the pain, and the degree and the intensity of the pain. There are several methods that help in the evaluation of the severity of the pain. The approach is as follows:

1st: Where? (Location and propagation of the pain)
2nd: When? (Constant: intermittent: night pain)
3rd: How? (Description of the pain)
4th: How much? (Intensity of the pain)

Methods for the evaluation of the pain are Type I and Type II (Figure 1). They are based on the physiological, neurological and neuro-pharmacological findings or the patient's subjective experience [6].

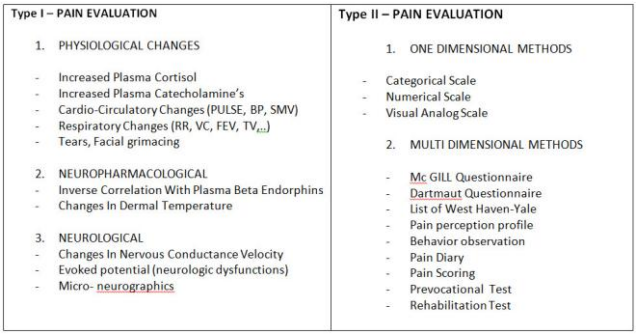

Figure 1: Methods for the evaluation of the pain

Pain control

In general in palliative care, the pain therapy primary contains a general analgesic approach and secondary, use of additional alternative therapy.

The primary analgesic management starts with management of the nociception pain (by WHO as "step by step" approach), combined with the management of the neurogenic pain (anticonvulsive and antidepressants - gabapentin, carbamazepine, phenytoin, and Amitriptyline, Nortriptyline) and management with adjuvants (corticosteroids, sedatives, antiemetic, Alfa - 2 agonists, local anaesthetics, NMDA receptor antagonists etc.) [4] [5] [6].

Today a flexible approach to pain relief therapy for palliative care provides the best results. It consists of the use of more therapeutic abilities at the same time frame at different time intervals. The main characteristic of this therapy is a continuum of analgesic management. As an addition to the standard analgesic therapy is specific therapeutic operations (ex: anti-tumour therapy, neuroaxial therapy, parenteral PCA, neurolytic the etc.) [7].

The secondary, alternative approach in pain control is very popular in palliative care. It compromises of the use of several methods which are cheap, effective and popular for patients at the end of their life. Those are interventional, rehabilitation, stimulation, psychological and complementary approaches [8] [9].

The organisation of the pain management in palliative care

Most of the patients undergoing palliative care are hospitalised in different institutions. Some of them are in geriatric centres or specialised clinics as surgery, oncology, intern medicines or others. This

approach is very expensive to the society. In developed countries, this category of patients (at the end of their life) is lodged in “hospices”. In this type of institutions, the quality of care is at a very high level, where the pain relief is performed by specialised professional team [9] [10] [11].

The second organisational model is “home care”. This type of palliative care is more practical and cheaper. A mobile team of one GP and one nurse (educated for assessing and treating the pain) visits the patients at their homes. This type of care needs big efforts from the families and their maximal support [12].

Ethical dilemmas in palliative care

In terminal patients, the occurrence of ethical issues and questions is very frequent. Common response to these questions is to take measures that will help the sufferers. But when the conventional medical methods of management in palliative care are spent, the last choice is the use of alternative methods of treatment. Most of them must offer comfort to the patient and prevention from suffering [13].

In conclusion, pain management is an integral part of the palliative care. Pain relief is a very important part of improving the quality of life in terminal patients. Because of unpleasant sensations, experiences and fear of pain, the treatment must be complex and multidisciplinary. Good organisation and institutionalisation of the palliative care in one society could only permit better prevention of suffering at the end of the life.

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Review Article

Advanced Computational Methods in Bio-Mechanics

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Keywords: Biomechanics; Finite Difference Method; Finite Element Method; Finite Volume Method; Applications; Computational Techniques; Computational Methods

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Abstract

A novel partnership between surgeons and machines, made possible by advances in computing and engineering technology, could overcome many of the limitations of traditional surgery. By extending surgeons' ability to plan and carry out surgical interventions more accurately and with fewer traumas, computer-integrated surgery (CIS) systems could help to improve clinical outcomes and the efficiency of healthcare delivery. CIS systems could have a similar impact on surgery to that long since realised in computer-integrated manufacturing. Mathematical modelling and computer simulation have proved tremendously successful in engineering.

Computational mechanics has enabled technological developments in virtually every area of our lives. One of the greatest challenges for mechanists is to extend the success of computational mechanics to fields outside traditional engineering, in particular to biology, the biomedical sciences, and medicine. Biomechanics has significant potential for applications in orthopaedic industry, and the performance arts since skills needed for these activities are visibly related to the human musculoskeletal and nervous systems.

Although biomechanics is widely used nowadays in the orthopaedic industry to design orthopaedic implants for human joints, dental parts, external fixations and other medical purposes, numerous researches funded by billions of dollars are still running to build a new future for sports and human healthcare in what is called biomechanics era.

Introduction

The term biomechanics had been developed during the early 1970s. Biomechanics has been defined as the study of the movement of living things using the science of mechanics [1], while Biomedical Engineering is the engineering branch that is concerned with solving problems in biology and medicine. Biomedical engineers use principles, methods, and approaches drawn from the more traditional branches of electrical, mechanical, chemical, materials, and computer engineering to solve this wide range of problems. On the other hand, numerical methods are mathematical techniques for performing accurate, efficient and stable computations, using a computer, to solve mathematical models of biomedical systems.

Governing equations, material properties, mechanisms, etc., may lead to nonlinearities. Simulating, and analysing such complicated and challenging problems may be impossible to be done analytically. Where, the analysis is done in an iterative process of hypothesis and verification, including several steps of modelling, computer simulation and experimental measurements.

Branches and Applications of Biomechanics

As illustrated in Figure 1, human beings, Biomechanics can be divided into three major branches of applications; Sports Biomechanics

(kinematics), Orthopedic Biomechanics (kinetics) and Anthropometry.

Biomechanics is widely used in orthopaedic industry to design orthopaedic implants for human joints, dental parts, external fixations and other medical purposes. Biotribology is a very important part of the study of performance and function of biomaterials used for orthopaedic implants. It plays a vital role to improve the design and produce successful biomaterials for medical and clinical purposes.

Biomechanics is also applied to studying human musculoskeletal systems. Such research fields utilise force platforms to study human ground reaction forces and infrared videography to capture the trajectories of markers attached to the human body to study human 3D motion [2]. Research also applies electromyography systems to study the muscle activation. By this, it is feasible to investigate the muscle responses to the external forces as well as perturbations.

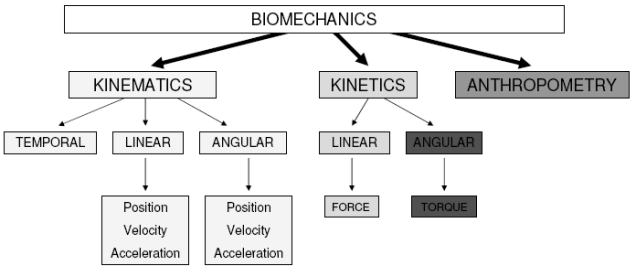

Figure 1: Major branches of mechanics used in most biomechanics studies [2]

Body displacements often lead to strains within the body. Strains are deformations that result in changes of shape within the body. These strains, in turn, lead to stress, the interior forces due to the stretching or compression of atomic bonds. These forces result in the acceleration of the material, affecting the motion and hence the evolution of the strains.

The equations of elasticity consist of Newton's law relating force to acceleration together with stress-strain relations (depends on the material) describing the force that results from a given strain. For sufficiently small strains the stress may be assumed to vary linearly with strain, resulting in the equations of linear elasticity. On the other hand, Rigid-body motions (translations and rotations) in which the body is simply moved as a rigid entity do not lead to any internal strain or stress.

Biomedical engineers start with a continuous mathematical model to explain an observed phenomenon in a biological system. Simulating and modelling such system is too difficult or impossible to be solved analytically. Therefore, a numerical analysis that used mathematical theories leads to algorithms for solving mathematical models approximately on computers. Continuous functions are approximated by finite arrays of values, and algorithms approximately solve the mathematical problem efficiently, accurately, and reliably.

For example, the application of imaging methods to assess solid and fluid biomechanical events is becoming increasingly powerful and important. Imaging methods have classically been used for assessing the relative motion of objects, such as blood cells in the microcirculation, or markers on tissues [3]. With the advent of CT, MRI and other molecular tagging methods, both solid and solute mechanics can be assessed. Case studies include simulation of tumour compression, experimental compression and ultrasound imaging, in vitro high - resolution breast tumour imaging and cardiac imaging are representing typical cases nowadays [3].

Biomedical engineers use/develop computer programs, which control precision and accuracy of the measurements and computations as an integral part of solutions to real-world bioengineering problems. There are tons of biomedical problems require the solution of nonlinear equations. Most nonlinear equations cannot be explicitly solved using analytical methods, however. Unlike linear equations, whose roots can be found using analytical methods, solvers need to be formulated to determine the roots of nonlinear equations.

Systems that have one independent variable can be modelled by ordinary differential equations, whereas systems with two or more independent variables require partial differential equations. The great majority of differential equations, especially the nonlinear ones and those that involve large sets of simultaneous differential equations, do not have analytical solutions but require the application of numerical techniques for their solution. Several numerical methods for differentiation, integration, and the solution of ordinary and partial differential equations are based on the concept of finite differences.

The calculus of finite differences enables to take a differential equation and integrate it numerically by calculating the values of the function at a discrete (finite) number of points. Another very useful application of the calculus of finite differences is in the derivation of interpolation/extrapolation formulas, the interpolating polynomials, which can be used to represent experimental data when the actual functionality of this data is not known [3]. Three basic formulations can be used in the representation of the PDE terms; Backward, Forward, and Central Finite Differences. On the other hand, a huge number of different schemes can be found in the literature for scanning time, or spatial domain(s).

Finite difference method changes the set of governing PDE into another set of algebraic equations, once this set is formulated in matrices form can be solved. Direct or iterative root finding, and/or

matrix inverse can give solution(s) for the algebraic equations. On the other hand, drawbacks of using finite difference method appear in irregular domain boundaries, selecting spatial and time steps, and its effect on solution stability. Poor selection of these steps may alter the solution convergence rate and may lead to diverged or no solution.

The Finite element method (FEM) is a powerful computational technique for approximate solutions to a variety of engineering problems having complex domains subjected to general boundary conditions. Analyses by finite element become an essential step in the design or modelling of a physical phenomenon in various engineering disciplines. A physical phenomenon usually occurs in a continuum of matter (solid, liquid, or gas) involving several field variables. The field variables vary from point to point, thus possessing an infinite number of solutions in the domain.

FEM relies on the decomposition of the domain into a finite number of sub-domains (elements) for which the approximate systematic solution is constructed by applying the variational or weighted residual methods. FEM reduces the problem to a finite number of unknowns by dividing the domain into elements and by expressing the unknown field variable regarding the assumed approximating functions within each element. These functions (also called interpolation functions) are defined regarding the values of the field variables at specific points, referred to as nodes (connect adjacent elements). The ability to discretise the irregular domains with finite elements makes the method a valuable and practical analysis tool for the solution of the boundary, initial, and eigenvalue problems arising in various engineering disciplines [3].

Finite volume methods are closely related to finite difference methods. That finite volume method can often be interpreted directly as a finite difference approximation to the differential equation. However, finite volume methods are derived on the basis of the integral form of the conservation law, a starting point that turns out to have many advantages. Classical finite difference methods, in which derivatives are approximated by finite differences, can be expected to break down near discontinuities in the solution where the differential equation does not hold. Finite volume methods are based on the integral form instead of the differential equation. In one space dimension, a finite volume method is based on subdividing the spatial domain into intervals (the “finite volumes,” also called grid cells) and keeping track of an approximation to the function integral over each of these volumes. In each time step these values using approximations to the flux through the endpoints of the intervals to be updated. Rather than point-wise approximations at grid points, that break the domain into grid cells and approximate the total function integral over each grid cell, or the cell average of the function, which is this integral divided by the volume of the cell [4].

Instrumentation such as the blood pressure (BP) monitor, computed tomography (CT) or magnetic resonance imaging (MRI), and the microscope; have a common structure. In all cases, the computer controls the electronic interface (the user interface and data collection), which is connected to the subsystems that sense the tissue properties and control the sensor. The computers embedded in the instruments have helped to standardise the operation. Thus non-trained users can operate the BP monitor and other medical devices while being in better control of their healthcare. Computerized systems of sensor and controllers are undergoing continuous development for several goals like increasing accuracy, reducing the cost of testing and inspection, etc. [5].

Element elastic modulus values from the CT images were determined via two main steps. First, averaging element brightness values (B), based on the CT image pixels brightness data were calculated. The relationship between image brightness of normal and osteoporotic bone and density was used to estimate the density of each element.

The finite element model [6] was used to examine the mechanical response of normal and osteoporotic L5 human vertebral body under compressive stress. Results showed that during the elastic range of the vertebral bone there is a linear relationship between applied load and displacement, but with a different slope for normal and osteoporotic vertebral body. Further increase in the applied load increases the displacement especially for the osteoporotic vertebral body reaching 50% increase in the deformation more than the normal one. The proposed model can expect the maximum fracture risk in the L5 as well as detecting the beginning of osteoporosis.

A similar complex biomechanical analysis of the human lumbar spine was performed [7], aimed to improve the efficiency of conservative traction therapies and to prevent osteoporosis. The former concerns tension, the latter compression of the lumbar spine. As for tension, time-related in vivo elongations were measured during the regular traction hydrotherapy of patients. Based on the experimental results, parameter-dependent viscoelastic numerical tensile models of the lumbar functional spinal units were created for numerical simulation and parameter identification.

A research team from Katholieke Universiteit, Leuven, Belgium, studied the biomaterial surface characteristics modulate the outcome of bone regeneration around oral implants. Experimental investigations have demonstrated the importance of platelets and their activation for bone regeneration around implants. A higher amount of bone-to-implant contact has been observed on “micro rough” sandblasted/acid-etched versus “smooth” turned implant surfaces.

The team performed numerical demonstration [8] for the key role of activated platelets which is controlled by implant surface characteristics.

Dental implantology problems were numerically studied [9] [10] [11] from different perspectives. Results of these studies showed that implant stability depends on implant design parameters [12]. For example, increasing implant diameter and length generates better stress distributions on spongy and cortical bones. Approximate implant design equations and curves were obtained [9]. Bone stresses increase as bone level decreases with varying values depending on implant parameters.

Increasing value of the ratio between dental implant side area and its cross-sectional area reduces stresses transferred to cortical and spongy bones. Therefore, using implants with a higher ratio of side area to cross-section area, especially with the weak jaw bone, is recommended [11].

Recent numerical studies of rotary and reciprocating instruments proved that modelling of the instrument with equivalent circular cross-sectional area did not affect results quality, while the cross-sectional shape and its cutting angles could affect instrument cutting efficiency [13] [14]. The reciprocating system has great advantages over other root therapy instruments.

There are many examples of how applying biomechanics in changing equipment designs had improved sports performance. When improved javelin designs in the early 1980s resulted in longer throws that endangered other athletes and spectators, redesigns in the weight distribution of the “new rules” javelin again shortened throws to safer distances [15].

Aiming to break world records, many biomechanics studies aimed to improve performance in exercise and conditioning programs. Biomechanical studies of exercise movements and training devices serve to determine the most effective training to improve performance. Strength and conditioning professionals can better apply the principle of specificity when biomechanical research is used in the development of exercise programs. Computer-controlled exercise and testing machines are another examples of how biomechanics contributes to strength and conditioning [2].

Movement safety, or injury prevention/treatment, is another primary area where biomechanics can be applied. Those biomechanical studies of auto accidents had resulted in measures of the severity of head injuries, which has been applied in biomechanical testing, and in the design of many kinds of helmets to prevent head injury. Sports medicine professionals have traditionally studied injury data to try to determine the potential causes of disease or injury (epidemiology). Engineers and occupational therapists use biomechanics to design work tasks and assistive equipment to prevent overuse injuries related to specific jobs. Combining biomechanics with other sports sciences has aided in the design of shoes for specific sports [16], especially running shoes [17].

In conclusion, biomechanics is used in a diverse range of disciplines including biology, ergonomics, engineering, physiology, medicine, dentistry, and mechanical physics. It may be the major area of concern in some instances (e.g. artificial joints, prosthetics and orthoses, mechanisms of physical injury) or it may be a vital adjunct to another area (e.g. design of an implantable pacemaker or specialist surgical tools).

Many professionals, engineers, designers, physical therapists, oral and orthopaedic surgeons, cardiologists, and aerospace engineers use practical applications of biomechanics. That biomechanics helps the physical therapist prescribe rehabilitative exercises, assistive devices, or orthotics.

Biomechanical research is a powerful ally in the sports medicine quest to prevent and treat the injury. Biomechanical studies help prevent injuries by providing information on the mechanical properties of tissues, mechanical loadings during movement, and preventative or rehabilitative therapies.

Numerical solution of physical phenomena's governing equations using finite techniques is a vital step in many case studies. That it helped in better understanding of the effect of many oral/dental devices and materials.

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