ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1555-1560.
https://doi.org/10.3889/oamjms.2018.318
eISSN: 1857-9655
Basic Science

HER-2 Immunohistochemical Expression in Bone Sarcomas: A New Hope for Osteosarcoma Patients

Sahar A. Tabak, Sara E. Khalifa*, Yasmine Fathy

Pathology Department, Faculty of Medicine, Cairo University, Cairo, Egypt

Citation: Tabak SA, Khalifa SE, Fathy Y. HER-2 Immunohistochemical Expression in Bone Sarcomas: A New Hope for Osteosarcoma Patients. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1555-1560. https://doi.org/10.3889/oamjms.2018.318

Keywords: HER-2; Bone sarcomas

***Correspondence:** Sara E. Khalifa. Pathology Department, Faculty of Medicine, Cairo University, Cairo, Egypt. E-mail: sarah.mekawy@kasralainy.edu.eg

Received: 20-Jun-2018; **Revised:** 10-Jul-2018; **Accepted:** 27-Jul-2018; **Online first:** 04-Sep-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Osteosarcoma and chondrosarcoma, remain the most common primary bone tumours. Questions have been raised about the prognostic influence of HER-2 in bone sarcomas, but so far the results have been debatable. The her-2 expression is possibly a predictor of chemotherapy response.

AIM: In this study, we investigated the extent of HER-2 expression in bone sarcomas, and attempted to correlate it with pertinent variables that will help to provide better treatment options, especially for metastatic ones.

MATERIAL AND METHODS: Fifty-two cases of bone sarcomas (32 osteosarcoma cases and 20 chondrosarcoma ones) were studied for HER-2 immunohistochemical expression then correlation with all available clinicopathologic features was done.

RESULTS: Most of the osteosarcoma cases exhibited membranous staining (78.1%). Strong staining was observed (score 3+) in 34.4%; while 21.9% showed moderate staining (score 2+); and 21.9% displayed weak staining (score 1+), on the other hand, no staining was detected in 7 out of 32 cases (21.9%) (score 0). As regards chondrosarcoma, the absence of staining in all examined cases was noted. Immunohistochemical HER-2 overexpression correlated significantly with osteosarcoma site with P value = 0.004, with variation relating HER-2 intensity score to the site of osteosarcoma (P = 0.051). A statistically significant negative correlation was detected between HER-2 expression and the presence of metastasis at time of diagnosis (P = 0.006), A significant correlation was also found regarding HER-2 score and presence of metastasis with P value = 0.046 as more than half of cases with no metastasis at diagnosis (17/28 cases, 60.7%) showed positive intensity score. A statistically significant correlation was detected between HER-2 expression and patients' age (P = 0.044). Also, HER-2 expression significantly correlated to histopathological detection of fibrous tissue, with P value = 0.033. Higher scores of HER-2 expression were associated with a significantly better differentiation (P = 0.038) since detection of wide areas of osteoid were associated with higher HER-2 scores.

CONCLUSION: Further research would still be needed to delineate HER-2 role being a new hope for therapeutic targeting in bone sarcoma patients, mainly osteosarcoma in contrast to chondrosarcoma that didn't express HER-2 at all.

Introduction

Osteosarcoma and chondrosarcoma, remain the most common primary bone tumours [1]. Osteosarcoma is a primary skeletal malignancy, signified by malignant spindle cells of mesenchymal origin with deposition of the immature osteoid matrix [2] [3]. Histologically, osteosarcoma subtypes can be separated into high and low-grades. Treatment of low-grade tumours involves surgery alone and bears a favourable prognosis. High-grade osteosarcoma should be regarded as micrometastatic at diagnosis and thereupon treated with both surgery and systemic chemotherapy [4].

Surgery and chemotherapy are relatively successful treatment modalities for localised disease, yet metastatic disease continues to be a challenging issue [5]. Nearly 15-20% of patients have evidence of metastases at diagnosis. Moreover, patients having the metastatic disease have a very poor prognosis, with approximately only 20-30% of them being long-term survivors, as compared to 65-70% of patients having localised disease [6]. The ability to stratify patients at diagnosis is imperative to identify prognostic factors, to select patients who might benefit from more intensive forms of therapy. Recognition of select malignant cellular features could make up the keystone of targeted therapy interferences [7].

Concerning chondrosarcoma, it may arise de novo and hence called primary, whereas those

developing on top of preexisting benign cartilaginous tumours are itemised as secondary. Chondrosarcomas being heterogeneous, can be categorised by anatomic location into central or peripheral. Besides conventional chondrosarcoma that exhibits hyaline cartilage differentiation, other types include dedifferentiated, mesenchymal, and clear cell ones [8].

Chondrosarcoma is typically considered to be resistant to conventional chemotherapy and radiotherapy [9]. Accordingly, surgical resection has been the primary form of treatment. Thus, identification of prognostic factors is of dire necessity, not only to predict patients' outcome but also to shape decisions as regards treatment. Novel therapeutic approaches have been appraised in an experimental study [10]. Human epidermal growth factor receptor 2 (HER-2) has been viewed as a protein of potential prognostic importance in addition to being a therapeutic target. Additionally, it is fundamentally involved in the pathogenesis of several human cancers. Through some pathways, it regulates cell growth, survival, cellular proliferation and differentiation [7] [11].

Questions have been raised about the prognostic influence of Her-2 in bone sarcomas, but so far the results have been debatable. Meanwhile, the HER-2 expression is possibly a predictor of chemotherapy response [12] [13]. In this study, we investigated the extent of HER-2 expression in bone sarcomas and attempted to correlate it with pertinent variables that will help to provide better treatment options, especially for metastatic ones.

Material and Methods

This retrospective study was conducted on 52 cases of bone sarcomas (32 osteosarcoma cases and 20 chondrosarcoma ones). Formalin-fixed paraffin-embedded tumour (FFPE) blocks of patients, diagnosed as osteosarcoma or chondrosarcoma were collected from Pathology Department, Kasr Alainy Hospital, Faculty of Medicine, Cairo University, after receiving approval from the institutional research ethics committee.

Four μm sections from each submitted tumour paraffin block were stained with hematoxylin and eosin, to assess pertinent histologic findings. Exclusion criteria for tumour blocks included scanty tumour tissue, poor fixation and overt necrosis. Serial sections (4 μm) were prepared and float-mounted on adhesive-coated glass slides for HER-2 staining. Primary antibodies included rabbit antihuman c-erbB2 (HER-2) oncoprotein antibody (DAKO, Dako Corporation, Carpinteria, CA, USA) at 1:200 dilution for HER-2. The DAKO Envision system (DAKO Envision labelled polymer, peroxidase) was used as the detection system for HER-2. For all cases, a negative control was the adjacent non-tumorous part of the specimen and the positive control was a known HER2/*neu*-overexpressing breast carcinoma.

Hematoxylin and eosin stained sections were evaluated by light microscopy along with Her-2 immunohistochemically stained slides were then scored. To determine the score of HER-2 expression, both the membrane and cytoplasmic staining patterns were estimated, and its intensity was scored on a scale of 0 to 3+. A reaction in > 20% of cells in the membrane (focal/linear) or cytoplasm, with an intensity score of 2 or greater, was considered positive, provided that the staining was limited to the tumour cells and did not represent background or artefact [14].

Clinical data were retrieved from patients' files, including the age, gender, site of a tumour, and metastasis presentation. Histopathologic features were assessed as regards type, grade, necrosis, and matrix differentiation. Her-2 immunohistochemical results were recorded. Findings were then tabulated.

Collected data was documented using the Statistical Package for the Social Sciences (SPSS) version 22.0 (IBM Corp., Armonk, NY, USA). Statistical analysis was done using descriptive statistics. Results were expressed as percentages, frequencies, and mean ± S.D. correlations were determined using the Chi-square $\chi 2$ test. A p value of less than 0.05 represented significance.

Results

Fifty two bone sarcoma cases were enrolled in this study. Median age was 40 years, with 27 (51.9%) males and 25 (48.1%) females. Patients ranged in age from 10 to 86 years, with a mean of 40.4 ± 19.888 years. The osteosarcoma series included 32 cases ranged from 10 to 86 years, with a mean of 32.38 ± 16.590 years, while chondrosarcoma cases included 20 cases with a range from 25 to 85 years and a mean of 53.25 ± 14.682 years.

All of the 52 cases were evaluated for HER-2 expression by immunohistochemical staining. Regarding cases of osteosarcoma, the vast majority of specimens exhibited membranous staining (either complete or not) (25 cases out of 32, 78.1%). A strong staining was observed in > 20% of cells in the membrane (focal/linear) or cytoplasm (score 3+, strongly positive cases) in 11 out of 32 cases (34.4%); while 7 cases out of 32 (21.9%) showed moderate staining (score 2+, equivocal or weakly positive); and 7 cases (21.9%) displayed weak staining in more than 20% of the tumor cells (score 1+, negative), on the

other hand, no staining was detected in 7 out of 32 cases (21.9%) (score 0, negative) (Figures 1, 2, and 3).

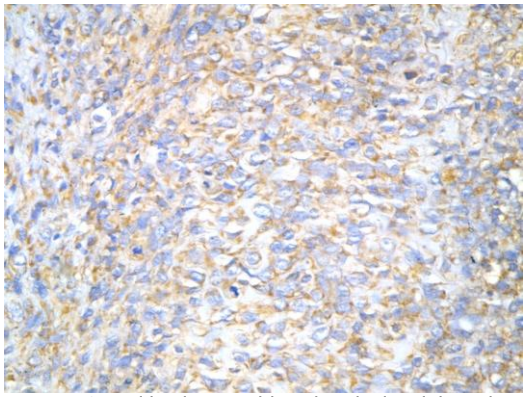

Figure 1: HER-2 positive immunohistochemical staining where the neoplastic cells of osteosarcoma showed staining in part of their membrane (low power)

As regards chondrosarcoma, the degree of immunohistochemical expression of HER-2 was similar in all cases given the absence of staining in all of the 20 examined cases (Figure 4).

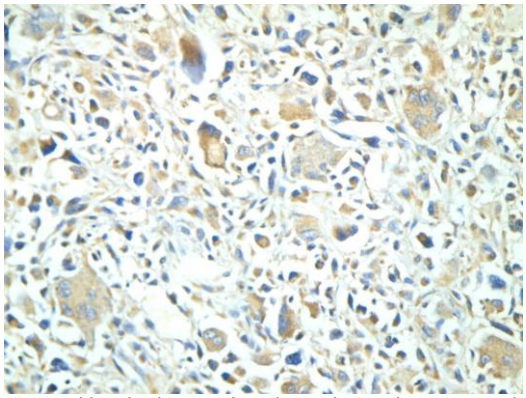

Figure 2: positive both cytoplasmic and membranous staining of HER-2 in a case of osteosarcoma (high power)

Clinicopathologic characteristics of cases and the results of HER-2 immunostaining are listed in Tables 1, 2, and 3.

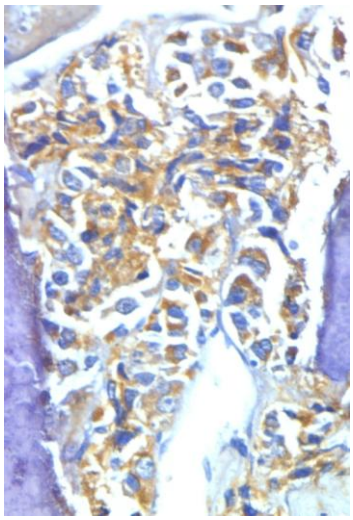

Figure 3: cytoplasmic staining of HER-2 without membrane staining in osteosarcoma case (high power)

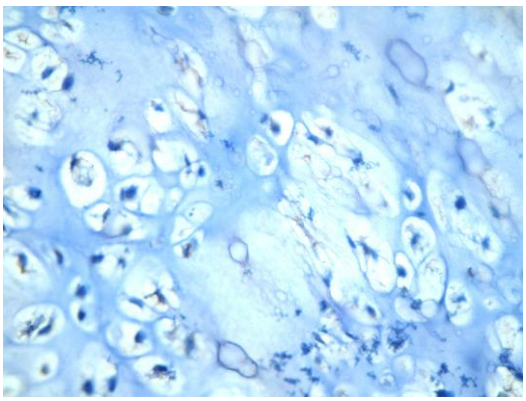

Figure 4: Chondrosarcoma with negative her-2 staining (high power)

Immunohistochemical HER-2 overexpression correlated significantly with osteosarcoma site with P value = 0.004, where 43.7% of the cases showed positive membranous staining, located in an extremity. Also, variation was found relating HER-2 intensity score to the site of osteosarcoma, and this difference nearly reached statistical significance, with P = 0.051.

Table 1: HER-2 immunohistochemical expression in correlation to osteosarcoma patients' clinicopathologic characteristics

		HER- 2 membranous staining			
		No membranous staining	Positive for any membranous staining	Total	P value
Gender	Female	1 (3.1%)	12 (37.5%)	13 (40.6%)	0.108
	Male	6 (18.8%)	13 (40.6%)	19 (59.4%)	
Site	Axial	3 (9.4%)	11 (34.4%)	14 (43.8%)	0.004
	Extremity	4 (12.5%)	14 (43.7%)	18 (56.2%)	
Presence of metastasis at the time of diagnosis	No metastasis	4 (12.5%)	24 (75.0%)	28 (87.5%)	0.006
	+ve for metastasis	3 (9.4%)	1 (3.1%)	4 (12.5%)	
Age group	Less than 20 yr	0 (0.0%)	10 (31.3%)	10 (31.3%)	0.044
	20 yr or more	7 (21.9%)	15 (46.9%)	22 (68.8%)	
Grade	High	7 (21.9%)	19 (59.4%)	26 (81.3%)	0.150
	Low	0 (0.0%)	6 (18.8%)	6 (18.8%)	
Presence of necrosis	Absent	2 (6.3%)	12 (37.5%)	14 (43.8%)	0.360
	Present	5 (15.6%)	13 (40.6%)	18 (56.3%)	
Presence of cartilage	Absent	5 (15.6%)	14 (43.8%)	19 (59.4%)	0.463
	Present	2 (6.3%)	11 (34.4%)	13 (40.6%)	
Presence of fibrous tissue	Absent	6 (18.8%)	10 (31.3%)	16 (50.0%)	0.033
	Present	1 (3.1%)	15 (46.9%)	16 (50.0%)	
Osteoid tissue detection	Focal	7 (21.9%)	19 (59.4%)	26 (81.3%)	0.150
	Wide	0 (0.0%)	6 (18.8%)	6 (18.8%)	

A statistically significant negative correlation was detected between HER-2 immunohistochemical expression and the presence of metastasis at the time of diagnosis (P = 0.006), as 85.7%, (24/28 cases) with the absence of metastatic deposits at the time of diagnosis showed HER-2 membranous staining. A significant correlation was also found regarding HER-2 score and presence of metastasis with P value = 0.046 as more than half of cases with no metastasis at diagnosis (17/28 cases, 60.7%) showed positive intensity score.

A statistically significant correlation was detected between levels of HER-2 immunohistochemical expression and patients' age (P

= 0.044) where HER-2 was expressed more in the younger age group (<20 years) in which all cases (10/10) showed membranous staining.

Table 2: Intensity scoring for HER-2 expression in correlation to clinicopathologic data of osteosarcoma cases

		HER-2 score				Total	P value
		0	1+	2+	3+		
Gender	Female	1 (3.1%)	3 (9.4%)	4 (12.5%)	5 (15.6%)	13 (40.6%)	0.403
	Male	6 (18.8%)	4 (12.5%)	3 (9.4%)	6 (18.8%)	19 (59.4%)	
Site	Axial	3 (9.4%)	3 (9.4%)	2 (6.3%)	6 (18.7%)	14 (43.7%)	0.051
	Extremity	4 (12.5%)	4 (12.5%)	5 (15.6%)	5 (15.6%)	18 (56.3%)	
Presence of metastasis at time of diagnosis	No metastasis	4 (12.5)%	7 (21.9%)	7 (21.9%)	10 (31.3%)	28 (87.5%)	0.046
	+ve for metastasis	3 (9.4%)	0 (0.0%)	0 (0.0%)	1 (3.1%)	4 (12.5%)	
Age group	Less than 20 yrs	0 (0.0%)	3 (9.4%)	2 (6.3%)	5 (15.6%)	10 (31.3%)	0.197
	20 yrs or more	7 (21.9%)	4 (12.5%)	5 (15.6%)	6 (18.8%)	22 (68.8%)	
Grade	High	7 (21.9%)	4 (12.5%)	5 (15.6%)	10 (31.3%)	26 (81.3%)	0.145
	Low	0 (0.0%)	3 (9.4%)	2 (6.3%)	1 (3.1%)	6 (18.8%)	
Presence of necrosis	Absent	2 (6.3%)	3 (9.4%)	4 (12.5%)	5 (15.6%)	14 (43.8%)	0.758
	Present	5 (15.6%)	4 (12.5%)	3 (9.4%)	6 (18.8%)	18 (56.3%)	
Presence of cartilage	Absent	5 (15.6%)	4 (12.5%)	4 (12.5%)	6 (18.8%)	19 (59.4%)	0.906
	Present	2 (6.3%)	3 (9.4%)	3 (9.4%)	5 (15.6%)	13 (40.6%)	
Presence of Fibrous tissue	Absent	6 (18.8%)	3 (9.4%)	3 (9.4%)	4 (12.5%)	16 (50.0%)	0.197
	present	1 (3.1%)	4 (12.5%)	4 (12.5%)	7 (21.9%)	16 (50.0%)	
Osteoid tissue detection	Focal	7 (21.9%)	4 (12.5%)	7 (21.9%)	8 (25.0%)	26 (81.3%)	0.038
	Wide	0 (0.0%)	3 (9.4%)	0 (0.0%)	3 (9.4%)	6 (18.8%)	

HER-2 immunohistochemical expression significantly correlated to histopathological detection of fibrous tissue, with P value = 0.033. Osteosarcoma cases showing fibrosis expressed HER-2 more frequently than those without a fibrous tissue.

Table 3: HER-2 immunohistochemical expression in correlation to chondrosarcoma patients' clinicopathologic characteristics

		HER-2 membranous staining			
		No membranous staining at all		Positive for membranous staining	
		Count	%	Count	%
Age group	Less than 52 yrs	10	50.0	0	0
	52 yrs or more	10	50.0	0	0
Gender	Female	12	60.0	0	0
	Male	8	40.0	0	0
Site	Axial	10	50.0	0	0
	Extremity	10	50.0	0	0
Biopsy	Excision	13	65.0	0	0
	Incision	7	35.0	0	0
Grade	I	16	80.0	0	0
	II	4	20.0	0	0
Cartilage	Focal	6	30.0	0	0
	Wide	14	70.0	0	0
Myxoid	No present	12	60.0	0	0
	Present	8	40.0	0	0
Necrosis	No	19	95.0	0	0
	Wide areas	1	5.0	0	0
Histological type	Chondrosarcoma, conventional	14	70.0	0	0
	Mesenchymal, Chondrosarcoma	1	5.0	0	0
	Myxoid Chondrosarcoma	5	25.0	0	0

Higher scores of HER-2 expression were associated with a significantly better differentiation (P = 0.038) since detection of wide areas of osteoid were associated with higher HER-2 scores.

There were no statistically significant differences in the other clinicopathologic features as patient gender, tumour grade, the presence of necrotic foci or cartilaginous areas between osteosarcomas cases expressing or lacking HER-2.

Overexpression of HER-2 has not noted in any of the chondrosarcomas studied cases with similar absence of expression in all cases regardless of diverse clinicopathologic characters.

Discussion

Bone tumours', mainly osteosarcoma and chondrosarcoma prognostic factors at diagnosis other than clinical stage have not been identified, and this is the only method that determines prognosis and therapy of patients. Therefore, there is a critical need for feasible prognostic and possibly therapeutic methods.

HER-2 has been applied as being a promising marker for targeted biological therapy. The development of trastuzumab, a humanised monoclonal antibody that binds specifically to HER's-2 prompted an opening to make use of this targeted therapy [15]. Thus, clinically the demand for HER-2 assessment is rapidly rising.

The HER-2 immunohistochemical expression has been detected in tissues derived from all three germ layers. Its expression has been observed in fetal tissue as well. In embryos, the expression of HER-2 was observed in the placenta, genitourinary tract epithelium, gastrointestinal tissue, pulmonary tract and the adrenal medulla. In contrast, HER-2 was not detected in liver, nervous tissue including brain, striated and smooth muscle, endothelium or fibroblasts of the embryo [16]. Adult tissue expression levels are lower than those in the early stages of development. These expression patterns ascertain the prospect for the involvement of HER-2 in a range of human neoplasms. The HER-2 expression has been recognised in some tumour types mainly breast, gastric, oesophageal, pancreatic, and many others. Even though there are agreement about antibodies and tyrosine kinase inhibitors targeting HER-2 in the breast, gastric and oesophagal adenocarcinomas, still there are other evident prospects in other tumour types [17].

In this study, we examined HER-2 expression in the commonest primary malignant bone tumours namely osteo and chondrosarcomas, immunohistochemically, as being a practical and cost-effective method available in most laboratories. Accordingly, immunohistochemistry is the most widespread method of HER-2 assessment.

In the current study, HER-2 has been identified as an important possible prognostic, therapeutic factor in osteosarcomas. First, we illustrated the immunohistochemical expression of HER-2 in osteosarcomas, where 78.1% of the studied cases (25/32) exhibited membranous staining (whether complete or not). Our results were higher than those of Gorlick et al., [18] who demonstrated somewhat high levels of HER-2 expression in 42.6% of their cases and Japanese investigators [19] who reported HER-2 overexpression in 42% of their patients. Moreover, Ma et al., [20] assured that 60.3% of their samples were HER-2 positive (ranging from low- to high-positivity). Additionally, Ebb et al., [21]

reported that around half of their cases showed positive HER-2 membranous staining. This high expression incidence was greater than what was previously demonstrated by Thomas et al., [22] and Kilpatrick [23] who both reported lack of membranous HER-2 immunoreactivity in their samples.

Moreover, Zhou et al., [24] revealed that the vast majority of their cases exhibited immunopositivity with focal to diffuse cytoplasmic reactivity yet without any membranous staining. This inconsistency could be explained by geographic and genetic variability between patients, sample size, differences in immunohistochemical processing (either decalcified or not and the duration of fixation), and variability in scoring methods with different thresholds or tumour heterogeneity [25]. Furthermore, the results are also contingent on the antibody used where Press et al. evaluated a panel of 28 antibodies (7 polyclonal and 21 monoclonal antibodies) on 187 cases previously shown to be HER-2 positive and demonstrated that the ability to detect HER-2 positive cells varied vastly from 6% to 82% [26].

In this research work, overexpression of HER-2 correlated significantly with the presence of osteosarcoma in an extremity rather than an axial site with P value = 0.004, also variation was noted relating the HER-2 intensity score to the osteosarcoma site, and this difference nearly reached statistical significance, with P value = 0.051. Also, a significant correlation was detected between the levels of HER-2 immunohistochemical expression and the patients' age (P = 0.044) where it was expressed more in the younger age group. Besides, a significant negative correlation was detected between HER-2 membranous immunohistochemical expression and absence of metastasis at the time of diagnosis (P = 0.006). This significant correlation was also attained as regards HER-2 score and the presence of metastasis with P value = 0.046, as more than half of cases which failed to show metastasis at diagnosis (60.7%, 17/28 cases) revealed positive intensity score. These findings support the belief that HER-2 level of expression may have a role in the early development of osteosarcoma, and it may act as a marker of good prognostic significance as it is down-regulated in tumors with metastatic deposits at the time of diagnosis. This is in agreement with the conclusions drawn by some previous investigators as Akatsuka and his colleagues [27], who demonstrated low levels of expression of HER-2 in metastatic lesions relative to the primary tumour. The majority of the earlier research studies demonstrated the poor prognostic effect of HER-2 overexpression mainly in the presence of metastasis as proclaimed by Gorlick et al., Ma et al., Onda et al., and Li et al., [18] [19] [20] [28] as they showed overexpression of HER-2 with poor prognostic factors mainly the presence of metastasis.

In this work, we reported that osteosarcomas with considerable fibrosis expressed HER-2 more frequently than osteosarcomas without, achieving a statistically significant relationship with a P value of 0.033. Also, higher scores of HER-2 immunohistochemical expression were associated with significantly better differentiation (P = 0.038) since detection of wide areas of osteoid were associated with higher HER-2 scores. Similar patterns of expression were also observed in the study by Zhou et al., [24] where they reported cytoplasmic staining more in undifferentiated spindle cells, while both membranous and cytoplasmic staining was detected in differentiated, malignant-appearing chondroblastic foci.

Regarding chondrosarcoma, the level of immunohistochemical expression of HER-2 was similar in all cases as we didn't find membranous staining in any of the examined cases. Our findings were just identical to those reported by Nelson et al. and Park et al., [17] [29] who couldn't detect immunohistochemical staining of HER-2 in their chondrosarcoma cases. Therefore, it is unlikely that targeted therapy against HER-2 overexpression could be used effectively in the treatment of such miserable patients also experiencing well-known resistance to most chemotherapeutic regimens.

In conclusion, Her-2 has expressed in bone sarcomas specifically osteosarcoma, and it is probably related to tumour prognosis and outcome. It may act as a marker of good prognostic significance as it is down-regulated in tumours with metastatic deposits at time of diagnosis.

Further clinicopathologic research work would still be needed to delineate its role being a new hope for therapeutic targeting in bone sarcoma patients, mainly osteosarcoma in contrast to chondrosarcoma that didn't express HER-2 at all.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1561-1570.
https://doi.org/10.3889/oamjms.2018.322
eISSN: 1857-9655
Basic Science

Evaluation of an Improved Chitosan Scaffold Cross-Linked With Polyvinyl Alcohol and Amine Coupling Through 1-Ethyl-3-(3-Dimethyl Aminopropyl)-Carbodiimide (EDC) and 2 N-Hydroxysuccinimide (NHS) for Corneal Applications

Mohamed Ali Seyed[1*], Kavitha Vijayaraghavan[2]

[1]Department of Clinical Biochemistry, Faculty of Medicine, University of Tabuk, Tabuk 71491, Saudi Arabia; [2]Department of Chemical Engineering, Agni College of Technology, Old Mahabalipuram Road, Thalambur, Chennai, Tamil Nadu 600130, India

Citation: Ali Seyed M, Vijayaraghavan K. Evaluation of an Improved Chitosan Scaffold Cross-Linked With Polyvinyl Alcohol and Amine Coupling Through 1-Ethyl-3-(3-Dimethyl Aminopropyl)-Carbodiimide (EDC) and 2 N-Hydroxysuccinimide (NHS) for Corneal Applications. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1561-1570. https://doi.org/10.3889/oamjms.2018.322

Keywords: Tissue engineering; Biocompatibility; Chitosan; Poly (vinyl alcohol); PVA; Corneal cells

***Correspondence:** Mohamed Ali Seyed. Department of Clinical Biochemistry, Faculty of Medicine, University of Tabuk, Tabuk, Saudi Arabia, Tabuk 71491, Saudi Arabia. E-mail: m.mohamed@ut.edu.sa

Received: 04-Jun-2018; **Revised:** 26-Jul-2018; **Accepted:** 27-Jul-2018; **Online first:** 18-Sep-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Corneal blindness resulting from various medical conditions affects millions worldwide. The rapid developing tissue engineering field offers design of a scaffold with mechanical properties and transparency similar to that of the natural cornea.

AIM: The present study aimed at to prepare and investigate the properties of PVA/chitosan blended scaffold by further cross-linking with 1-Ethyl-3-(3-dimethyl aminopropyl)-carbodiimide (EDC) and 2 N-Hydroxysuccinimide (NHS) as potential in vitro carrier for human limbal stem cells delivery.

MATERIAL AND METHODS: Acetic acid dissolved chitosan was added to PVA solution, uniformly mixed with a homogenizer until the mixture was in a colloidal state, followed by H_2SO_4 and formaldehyde added and the sample was allowed to cool, subsequently it was poured into a tube and heated in an oven at 60°C for 50 minutes. Finally, samples were soaked in a cross-linking bath with EDC, NHS and NaOH in H_2O/EtOH for 24 h consecutively stirred to cross-link the polymeric chains, reduce degradation. After soaking in the bath, the samples were carefully washed with 2% glycine aqueous solution several times to remove the remaining amount of cross-linkers, followed by washed with water to remove residual agents. Later the cross-linked scaffold subjected for various characterization and biological experiments.

RESULTS: After viscosity measurement, the scaffold was observed by Fourier transform infrared (FT-IR). The water absorbency of PVA/Chitosan was increased 361% by swelling. Compression testing demonstrated that by increasing the amount of chitosan, the strength of the scaffold could be increased to 16×10−1 MPa. Our degradation results revealed by mass loss using equation shows that scaffold degraded gradually imply slow degradation. In vitro tests showed good cell proliferation and growth in the scaffold. Our assay results confirmed that the membrane could increase the cells adhesion and growth on the substrate.

CONCLUSION: Hence, we strongly believe the use of this improved PVA/chitosan scaffold has potential to cut down the disadvantages of the human amniotic membrane (HAM) for corneal epithelium in ocular surface surgery and greater mechanical strength in future after successful experimentation with clinical trials.

Introduction

The cornea is a clear, avascular, multi-laminar structure plays an important role in vision [1]. The World Health Organization (WHO), an agency of the United Nations has recognised corneal diseases as a major cause of blindness in the world, next to cataract, which affects more than 10 million people [2] [3]. At present, the corneal transplantation is the only existing therapy of choice [4] [5]. Besides, a severe scarcity of fresh donor corneas [6] and an unknown threat of immune rejection had seen with routine heterograft; hence, it is very imperative and crucial to construct a corneal equivalent to replace pathologic corneal tissue.

Corneal tissue engineering has appeared as a viable approach to developing corneal tissue alternates [7] [9]. The design of a scaffold with mechanical properties and transparency similar to that of the natural cornea is significant for the regeneration of corneal tissues but also able to resist the culture conditions, flexible to the shape of the cornea and quite strong for surgical manipulation including the suturing [10]. Currently, the substratum commonly used is the human amniotic membrane (HAM) [11], which includes denuded HAM over an intact

membrane [12]. However, many inherent problems still exist like the thinness, wrinkling nature, sterile storage and early degradation, its possible danger for the spread of pathogens and the risk of immune-mediated graft rejection etc. [13]. Since HAM has many disadvantages, there has been a substantial amount of research to find a good alternative source for replacement.

The key challenge in tissue engineering is the designing of an artificial extracellular matrix (ECM) component because it can support cell growth and allow deposition of the natural ECM proteins over it during the initial stages [14]. Although various biomaterial scaffolds are available for many applications like sutures, bone plates, heart valves and screws [15] [16], recent years had also witnessed tremendous research attention towards the improvement of few other naturally derived biopolymers like silk [17] and purified ECM based molecules like collagen, elastin and glycosaminoglycan (GAGs) [18] [19]. Besides the above, polylactic acid (PLA), polycaprolactone (PCL) [20] [21], and the PVA membranes as well as their blends, have been widely used in the production of scaffolds for various biomedical applications [20] [21].

The development of chitosan-based biomaterial attracted much attention [22] recently for various applications because of its novel potentials like minimal foreign body reactions and intrinsic antibacterial property [23]. Also, the biocompatibility, biodegradability and chitosan's ability to mould into various forms and geometries make it to suitable for cell ingrowth and conduction [24] [25]. Since chitosan alone is not sufficient to support cell growth, enhancing its mechanical strength needs another partner like polyvinyl alcohol (PVA), a biodegradable polymer/or its blends often used in tissue engineering applications. The addition of chitosan to the PVA solution has an effect of thickener, increasing the viscosity and giving rise to uniform nanofibers, even for low PVA concentration [26]. These favourable intermolecular interactions between PVA and chitosan influence the culture of corneal epithelial stem cells. Chitosan containing hydroxyl and amine groups has, therefore, the potential to miscible with PVA due to the ability to form hydrogen bonds.

On the other hand, stem cells provide a potentially boundless source of cells for treating a plethora of human diseases [27] [28]. The corneal limbus, located at the corneoscleral junction, believed to harbour the cornea stem cells in the basal layer of the epithelium [29] [30]. These limbal epithelial stem cells (LESCs) possess all of the properties of an adult stem cell population [31] and are responsible for maintaining and regenerating the corneal epithelium throughout the life. Also, limbal stem cells also act as a "barrier" to conjunctival epithelial cells and normally prevent them from migrating on to the corneal surface [32]. Extensive studies performed to investigate the feasibility of explant culture method of cultivating corneal epithelial cells and their characteristics in comparison to the limbal explant culture [33]. Hence, the present study employed cultured corneal epithelial cells (HCEC), as an ideal substitute to test the ability of PVA cross-linked chitosan together with amine coupling through EDC-NHS scaffold to facilitate their growth.

The present study objectives were as follows: (i) to develop a biodegradable and non-toxic PVA cross-linked chitosan scaffold by further cross-link with EDC and NHS; (ii) to characterize its physiochemical properties to support the growth of HCEC, so that it had the ability to facilitate enhanced adhesion, expansion and proliferation of HCEC; while maintaining its mechanical properties (iii) to investigate the corneal epithelial marker and antimicrobial peptide expression in the HCEC. With consideration of the ultimate goal to use the methods in clinical applications, we were mindful of the potential risks of using culture media containing defined or undefined animal derivatives. Such components have the potential to transmit communicable diseases and provoke immunological problems during transplantation. To reduce the potential harmful complications and to minimise any risk for future patients, we used a culture medium that was free of supplements containing non-human animal derivatives.

Subjects and Methods

Polyvinyl alcohol (87-89% hydrolysed), the average molecular weight of 72000 gmol−1), acetic acid (AA 35% pure), and glutaraldehyde (GA) (25% aqueous solution) were purchased from Merck (Merck Specialities Pvt Limited Mumbai, India). Chitosan [poly (β-(1-4)-2-amino-2-deoxy-D-glucopyranose)] (75% degree of deacetylation) (medium molecular weight of 190,000–310,000) was purchased from Himedia, Mumbai, India.

The chitosan powder was separately dissolved in 1% acetic acid (20 mL) at room temperature. The PVA (4 g) was dissolved completely in Milli-Q water (20 mL) by heating. The chitosan was added to the PVA solution and mixed uniformly with a homogeniser at 300 rpm and 90°C for 30 minutes until the mixture was in a colloidal state. After adding H_2SO_4 (10 mL) and formaldehyde (5 mL) and stirring, the sample was cooled to room temperature. Finally, the sample was poured into a tube and heated in an oven at 60°C for 50 minutes. Finally, samples were soaked in a cross-linking bath with EDC, NHS and 0.1 M NaOH (4 mg/mL) in H_2O/EtOH 2:1 for 24 h consecutively stirred to cross-link the polymeric chains, reduce degradation, and enhance the biomechanical properties of the scaffolds for delivery or tissue repair. After soaking in the bath, the samples

were carefully washed with 2% aqueous glycine solution several times to remove the remaining amount of cross-linkers, followed by washing with water to remove residual agents. The present study experimented on trial base with few scaffolds sterilised with either ethylene oxide gas or alcohol by complete immersion in 75%, 50%, 25%, 5% and 1% alcohol solution with an incubation time of 10min. Eventually, the scaffold was washed twice with water and incubated for 10 min. Each, followed by dried, separated and used for plating of cells. The scaffold preparation and related experiments were carried out at the Polymer Nanotechnology Center of B.S Abdur Rahman Crescent University (BSA), Vandalur, Chennai, India.

The viscosity of solutions was measured by Brookfield Model DV-III viscometer (Brookfield Engineering Laboratories Inc, Stoughton, MA) before the cross-linking process was begun.

The samples were examined by FT-IR analysis with a Perkin Elmer, model 2000 spectroscopy. For IR analysis, 2-6 mg of the scraped samples (about 10 μm thick) were carefully mixed with 500mg of KBr (infrared grade) and pelletized under vacuum. Then, pellets between 4000-400 cm^{-1} were analyzed with 120 scans averaging 4- cm^{-1} resolution in attenuated total reflection (ATR) mode. The FT-IR analysis was used to characterise the presence of specific chemical groups of PVA and chitosan, chemical interactions and the crosslinking effect in the polymeric scaffolds and to identify the effects of the above process on functional groups.

The optical clarity of the scaffolds is a major pre-requisite for the scaffold platform as they serve the purpose of an artificial extracellular matrix for the cornea, whose primary role to participate in the visual activity [34]. Hence, the scaffold samples were examined for optical clarity by using a Beckman DU-800 spectrophotometer and scanning was done within the visible range of wavelengths (400-800 nm).

Three dumbbell-shaped specimens of 4mm wide and 10mm length were punched out from each scaffold using a dying instrument. Mechanical properties such as tensile strength (MPa) and percentage of elongation at break (percentage) were measured using a universal testing machine (INSTRON model 1405) at an extension rate of 5 mm/min.

The quantity of water imbibed by a material is an important property, as it greatly contributes to the biocompatibility of the end material and decides if the material may be useful for biomedical purposes. To access the water sorption potential of the prepared scaffold, the PVA/Chitosan nanofibrous scaffolds were oven dried at 50°C and placed in a 24-well plate. Each well-contained 1mL of a phosphate buffered solution (PBS; pH 7.4). The scaffolds were incubated *in vitro* at 37°C for different periods (1, 3, 7, and 10 days) [35]. After immersion of the scaffolds in PBS solution for these different periods, excess PBS was wiped from the swollen saturated PVA/Chitosan scaffold, the amount of fluid uptake was determined by careful removal of samples from the medium after wiping off excess fluid with filter paper. The swelling ratio value (S) was calculated using the following formula 1:

$$S = (Ww-Wd)/Wd\times100 \quad (1)$$

For this test, the samples were weighed for determination of the wet weight (Ww) as a function of immersion time and dried weight (Wd) of the samples.

The degradation study of the scaffolds was carried out *in vitro* by incubating the samples in PBS at pH 7.4, 37°C for different periods. After each degradation period, the samples were washed and subsequently dried in a vacuum oven at room temperature for 24 hours. To find out the degradation index (Di), the weight of the samples (Wt) and the degradation index was calculated before and after the degradation test using the mass loss using equation 2:

$$Di = (W0-Wt)/W0\times100 \quad (2)$$

Human corneal epithelial cells (HCEC) were obtained from the commercially available source as primary corneal epithelial cells (Normal, Human (ATCC® PCS-700-010). On every passage, cells obtained by trypsinisation using 0.5% trypsin™ were cryopreserved as secondary cells. The in vitro cytotoxicity of the prepared scaffolds was tested using both NIH3T3 fibroblasts cell line and HCEC. Cells (10^5) were seeded into each well of 24 wells plate. The culture liquid contained DMEM (Dulbecco's modified Eagle's medium), 10% fortified bovine calf serum (FBS), and 1% penicillin-streptomycin solution. The cell culture of PVA/Chitosan scaffold cycles lasted for three days. After 72 hours of incubation, 3-(4,5-dimethylthiazol-2-yl) - 2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/mL) (Sigma, Munich, Germany) was added into each well and incubated for 90-120 minutes. Then, all the media was discarded and 600 μL DMSO was added to each well. An ELISA reader at 590 nm measured the optical density (OD) values after 30 minutes with a reference filter of 620 nm.

Cells were formalin fixed and paraffin embedded for routine histological processing and stained with hematoxylin and eosin (H&E) to visualise the cell attachment and proliferation on the scaffold. The same procedure was followed for cells plated in the Petri plate, which was used as a control. The processed samples were observed by using a light microscope with specific image analysis software from Zeiss [36].

The scaffold washed thrice with PBS, followed by washed with Dulbecco's Modified Eagle's Medium (DMEM) twice and incubated in a CO_2 incubator. After thorough checking HCEC cells viability, they were seeded onto the scaffold with 4 ml of Epilife medium. The same procedure adopted for control.

After the plated cells reached confluency, they were trypsinised (0.02%), and RNA isolation was done (using a QIAGEN kit method) for further expression studies. With a housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), an an internal control, the mRNA expression of different molecular markers for corneal epithelial stem cells and antimicrobial peptides were analyzed by semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR) as described by previous reports [37] [38]. PCR amplification of the first-strand cDNAs was performed with specific primer pairs, designed from published human gene sequences (Table 1) for different markers in a GeneAmp PCR System 9700 (Applied Biosystems) and resultant product of amplification and documented in BioRad gel documentation system; Bio-Rad Laboratories, UK.

All experiments were performed in triplicate. Summary of data was reported as-as mean ± standard deviation (SD). For statistical analysis, SPSS version 12.0 was used. To compare the different groups, the statistical Student's t-test was used, due to the small sample size, considering a significance level at $p < 0.05$.

Table 1: Human primer sequences used for semi-quantitative RT-PCR

Gene Name	Primer sequence - 3'-5'	Annealing temperature (°C)	Base pair size (bp)
Corneal Epithelial Stem Markers			
ABCG2	FP: 5' AGTTCCATGGCACTGGCCATA 3' RP: 5' TCAGGTAGGCAATTGTGAAGG 3'	62	379
Cytokeratin 3	FP: 5' GGCAGAGATCGAGGGTCTC 3' RP: 5'GTCATCCTTCGCCTGCTGTAG 3'	64	145
Connexin 43	FP:5 CCTTCTTGCTGATCCAGTGC 3 RP 5 ACCAAGGACACCACCAGCAT 3	63	150
Antimicrobial peptide – AMP			
hBD-1	FP: GCCTCCAAAGGAGCCAGCGT RP: CTTCTGGTCACTCCCAGCTCA	54	287
hBD-2	FP: CAGCCATCAGCCATGAGG RP: TGGCTTTTTGCAGCATTTT	55	204
hBD-3	FP: AGCCTAGCAGCTATGAGGATC RP: CTTCGGCAGCATTTTCGGCCA	61	205

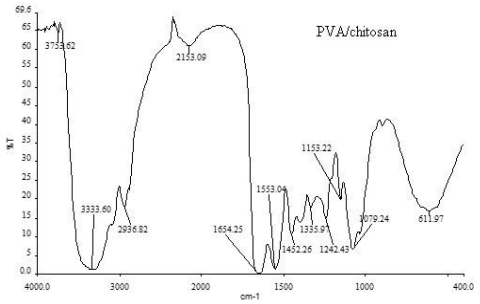
Results

The viscosities of the solutions were measured by Brookfield Model DV-III viscometer. The viscosity of the PVA solution was 557 centipoise, and that of the PVA/chitosan solution (with the weight ratio of 90/10) was 1726 centipoise. This is in line with the results by Paipitak et al., [39], who reported a linear increase in the viscosity of the PVA solution after blending with increasing amounts of chitosan. The high viscosity increases the interaction of two polymers, mainly through hydrogen bonding, and decreases the effects of surface tension. This will result in the formation of fibres with uniform morphology [40].

The optical clarity of the scaffold was done with the wavelength in the visible range of 400-800 nm. Our results showed that PVA/chitosan scaffold was found to be highly transparent with 88% of optical transparency compared with standard cornea as a positive control that showed a range of 72-82% of transparency, whereas, the human amniotic membrane (HAM) has an optical transmission of 78% only.

FT-IR spectroscopy was used to assess the chemical groups of the polymers. Figure 1a and 1b show the FT-IR spectra of PVA/chitosan and HAM. Typical FT-IR spectra of PVA/chitosan blended films having various absorption bands compared to IR spectra of denuded HAM. The specific intensity of absorption bands of chitosan/PVA blend and HAM are indicating the similar presence of protein. In Figure 1a, for the chitosan sample, the major characteristic peaks around 611 and 1152 cm^{-1} related to the saccharide structure (as the repeating unit of chitosan) are observable [41] [42]. Also, the strong absorption peaks at 1653, 1553 and 1346 cm^{-1} are shown, which are characteristic of chitosan and have been reported as amide I, II, and III peaks, respectively. The sharp peaks at 1335 and 1452 cm^{-1} could be assigned to the CH_3 symmetrical deformation mode. Also, the broad peaks at 1079 and 1152 cm^{-1} indicate the C–O stretching vibration in chitosan, and another broad peak at 3333.60 cm^{-1} is caused by amine N–H symmetrical vibration. The peak observed at around 2936 cm^{-1} is due to the typical C–H stretch vibrations [43]. Besides the above, all major peaks related to hydroxyl and acetate groups are shown in the FT-IR spectrum of PVA. More specifically, the broadband observed at 3753.62 cm^{-1} is associated with the O–H stretch from the intermolecular and intramolecular hydrogen bonds.

(a)



(b)

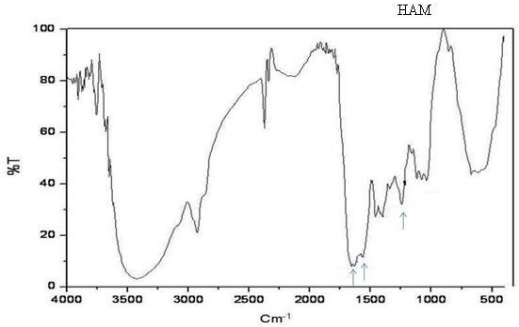


Figure 1: FTIR spectra of the PVA/chitosan cross-linked (a) and HAM scaffolds (b)

The vibrational band observed between 2936.82 and 2153.09 cm^{-1} is the result of the C–H stretch from alkyl groups and the peaks between 1653 and 11553 cm^{-1} are due to the C═O and C–O stretches from the remaining acetate groups in PVA (saponification reaction of polyvinyl acetate) [44] [45] [46]. These observations indicate the existence of good miscibility between chitosan and PVA and this is most likely due to the formation of intermolecular hydrogen bonds between the amino and hydroxyl groups in chitosan and the hydroxyl groups in PVA.

Figure 1b shows the FT-IR spectra of HAM. The absorption band around 1600–1640 cm^{-1} corresponds to amide-I protein absorption band and is mainly attributed to C=O stretching mode, and the other absorption band around 1510–1560 cm^{-1} corresponds to amide-II protein absorption band which attributed to N–H bending mode and C–N stretching mode [47]. The peaks at around 1210–1300 and 1070–1080 cm^{-1} attributed to protein (amide III) and also to the phosphodiester group of nucleic acids, glyco- and phospholipids. The amide III bands resulted from an in-phase combination of C–N stretching and N–H in-plane bending, with some contribution from C–C stretching and C=O bending vibrations [48]. Compared with FT-IR spectra of HAM, the peak intensity of the PVA/chitosan blend related to the amide groups tends to decrease, suggesting the formation of intermolecular hydrogen bonds between the polymer chains [23]. Such a result may explain the high stability of the cross-linked PVA with chitosan, during at least 1-2 weeks of immersion.

Fluid uptake is an important parameter, which influences the chemical and physical characteristics of the scaffolds after and before cell seeding. Herein, swelling experiments were performed after cross-linking of PVA and PVA/chitosan and immersed in phosphate buffered saline (PBS) for a defined period, taken out and gently pressed in between the filter papers and weighed. A representative result of fluid uptake behaviour is shown in Figure 2 for PVA and PVA/Chitosan cross-linked scaffolds. Our results revealed that chitosan strongly influences the swelling volume of the scaffold and increases it from 440% to 1590% over the period (1-24 h). The increased swelling volume could be attributed to a more flexible or relaxed network formed by the inter- and intra-polymer reactions and also to the more of hydrophilic groups in PVA/Chitosan blend. The results may be attributed to the fact that chitosan is a cationic biopolymer, and its content in the scaffold results in loosening of the network chains due to existing repulsion between the cationic chains of chitosan. This observation is in agreement with previous studies which reported that chitosan increases the swelling rate when blended with PVA; however the degree of swelling rate increase or reduction depends on factors such as weight ratio of the components, pH, temperature, and so on [35] [43] [44] [45] [46].

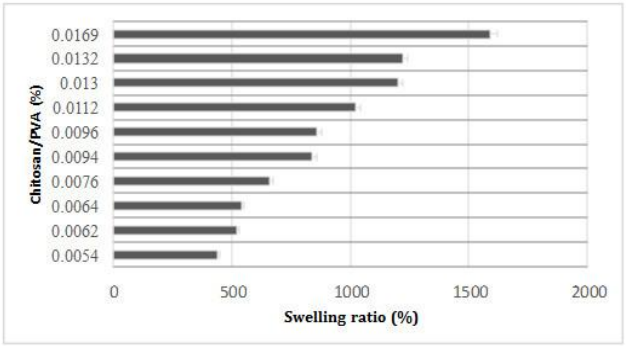


Figure 2: Swelling as a function of PVA and chitosan scaffold at various composition percentages (%) over the period (1-24 h). Graphical data are presented as mean (n = 3) ± standard deviation of three independent experiments

Degradation is the process through which useful physicochemical properties of the polymers are lost. This can include loss of polymer mass through mechanisms such as solvation and depolymerisation. Degradation behaviour of PVA and PVA/Chitosan scaffold using the PBS immersion method was shown in Figure 3. It was observed that the degradation rate of PVA/Chitosan scaffold was much slower than of PVA samples. PVA/Chitosan scaffolds started to degrade from 6th day onwards, and this slow degradation was continued until day 16. This could be due to the higher density of chemical cross-linking between cross-linkers and amine groups of chitosan and leads to slower depolymerisation [49] [50].

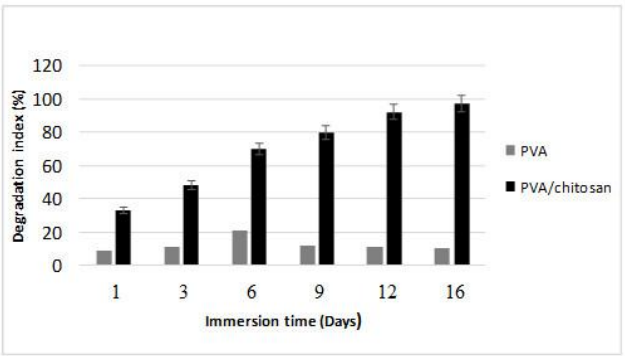


Figure 3: Measurement rate of breakdown of PVA/chitosan scaffolds. Dry weight ratio or degradation behaviour (%) of PVA and chitosan/PVA hydrogels was measured using PBS at different time points. Graphical data are presented as mean (n = 3) ± standard deviation of three independent experiments

The mechanical properties (Young's modulus, tensile strength and elongation at break percentage (%) of PVA/Chitosan blend scaffold was investigated in dry and wet states, and the observation was shown in Table 2. The mechanical properties of a scaffold used for tissue engineering are very important due to the need for the structural stability to oppose the various stresses incurred during culture in vitro or implantation in vivo while the surgeon is handling the membrane.

Table 2: Mechanical properties of PVA/Chitosan blend

S. No	Scaffold	Tensile strength MPa	Elongation at Break (%)	Tearing maximum strength load (N)
1.	PVA/Chitosan	4.38 ± 0.09	20.58 ± 0.36	2.87 ± 0.02
2.	Positive control HAM	1.68 ± 0.08	10.09 ± 0.8	Nil

The cytotoxicity of the pure PVA, Chitosan, PVA/chitosan blend scaffolds have been evaluated by MTT assay. This assay is based on the conversion of MTT to blue formazan by mitochondria in living cells. The amount of formazan formed indicates the level of cell metabolism. However, it does not accurately represent the number of living cells.

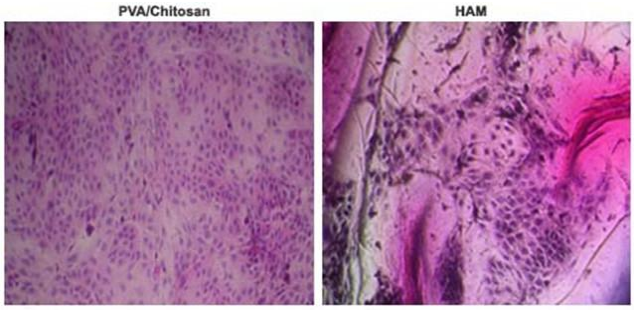


Figure 4: H&E staining of cultured HCEC in HAM and PVA/Chitosan scaffolds

Each experiment was repeated three times, and a low standard deviation of assay results was found. The optical density of formazan at 570 nm was measured for 24h, 48h and 72 h of incubation, respectively. The MTT assay indicated that both NIH3T3 and the cultured human corneal epithelial cells (HCECs) viability, was highest from days 3 to 5, and was not affected by the concentration of PVA used to prepare membranes. The viability of NIH 3T3 (90%) and HCECs cultured on PVA/chitosan (91%) was higher compared with either NIH3T3 or HCECs cultured on PVA alone (78% and 80%). However, it was less when compared with non-treated control cells (98%). H & E stained cells further confirmed the cell viability (Figure 4).

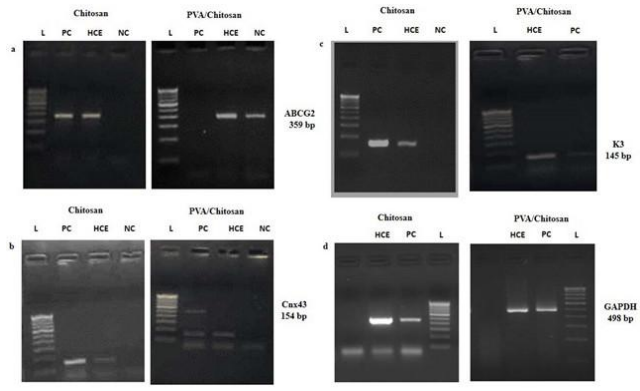


Figure 5: Semi-quantitative RT-PCR for SC-associated markers ABCG2 (379 bp), differentiation-associated markers, K3 (145 bp), and connexin 43 (154 bp) expressed by corneal epithelial cells (a, b, and c); A 100 bp DNA ladder is shown in the first left lane. GAPDH, a housekeeping gene, was used as an internal control (d)

Therefore, these results clearly showed that the PVA/Chitosan blend scaffolds are not deleterious for cell activity and may be safe for their use as a delivery substrate, wound dressing or soft tissue repair [51] [52] [53].

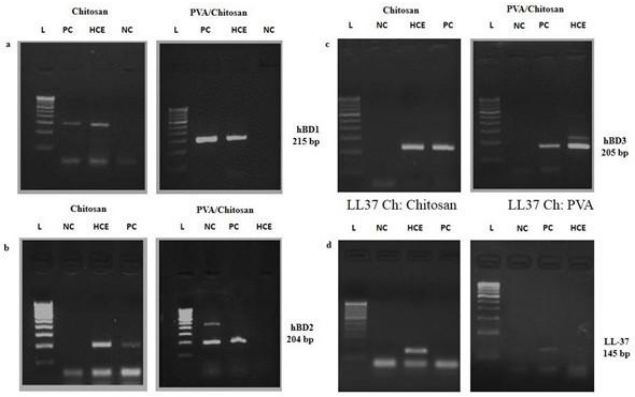


Figure 6: PCR amplification for AMPs expression by human corneal epithelial cells. Semi-quantitative RT-PCR for AMP-associated markers hBD1 (215 bp), hBD2 (204 bp) and hBD3 (205 bp) expressed by corneal epithelial cells (a, b, and c); A 100 bp DNA ladder is shown in the first left lane. GAPDH, a housekeeping gene, was used as an internal control (d)

Besides the above, the present study have also investigated the expression of different molecular markers for corneal epithelial stem cells (ABCG2, connexin43 and cytokeratin 3) (Figure 5, a, b, and c) and antimicrobial peptides (AMP) such as, hBD 1, 2, 3 and LL37 in cultured corneal epithelial cells (Figure 6, a, b, c, and d). Our results show that culture corneal epithelial cells were expressed hBD 1, 2, 3 and LL37 and the stem cell markers confirming the corneal epithelial nature of the cells.

Discussion

Tissue engineering in the cornea has often maintained the use of a carrier system for delivery of corneal stromal stem cells and corneal endothelial cell progenitors [54] [55]. Natural materials, such as collagen, silk and gelatin, which have excellent biocompatibility, biodegradability and low immunogenicity, have been extensively utilised for corneal tissue engineering [18] [19]. Although, scaffolds are preferentially a biodegradable one while providing a favourable microenvironment for cell adherence and proliferation but also expected to degrade gradually, allowing surrounding tissues to replace and sustain the scaffold function [56] [57]. In this regard, cross-linking reagents commonly used to modify scaffolds for enhancing both physical and chemical stability. For instance, in recent years, fibrin [58], human amniotic membrane (HAM) [59], and cellular feeder layers such as 3T3 fibroblasts [58] [60] have facilitated the expansion of corneal epithelial

cells. However, each of these agents has their own merits and some drawbacks.

In this study, we have modified polyvinyl alcohol (PVA) fibres by blending with chitosan to fabricate a Nanofibrous scaffold by cross-linking with EDC and NHS. The PVA/chitosan scaffolds have been found to exhibit physicochemical and biological properties, which we compared with HAM scaffolds to better meet the requirements of cultured corneal epithelial cells. The FT-IR spectra provide information about the functional groups of constituent polymers present in the scaffold. The IR spectra shown in Figure 1a indicates the presence of poly (vinyl) alcohol and chitosan, as evident from the peaks observed. It is clear from the IR results on the peaks that constituent polymers PVA and chitosan are present in the cross-linked scaffold. Whereas in the spectrum of denuded HAM (Figure 1b), nine characteristic absorption bands at the frequencies of 3306, 2954, 1651, 1548, 1451, 1394, 1241, 1077, and 645 cm^{-1} were observed.

The water absorption capacity of scaffold results clearly shows that water intake capacity constantly increases when the wt. Fraction increases from 1 to 4.0 and after that the equilibrium swelling constantly decreases (data not shown). Thus, an optimum swelling is reached at a PVA/chitosan wt. The fraction of 4.0. The results may be explained as follows, since both the constituent polymers, that is PVA and chitosan are hydrophilic, their increasing wt. Fraction results in enhanced hydrophilicity of the matrix, which results in an increased water sorption capacity. However, beyond 4.0 wt. Fraction of PVA, the water sorption capacity falls, which may be explained by the fact that when the PVA content is high, and the resulting scaffold is enriched in crystalline region of PVA, this accounts for lower water sorption tendency of the PVA. A similar observation was observed with chitosan. The results may be attributed to the fact that chitosan is a cationic biopolymer, and its increasing content in the scaffold results in loosening of the network chains due to existing repulsion between the cationic chains of chitosan.

It is well established that chitosan is a potential scaffold for *in vitro* bovine corneal epithelial cell culture with the ability to preserve the corneal epithelial cell phenotype to maintain biological function to a certain extent [61] [62] [63]. Hence, the cellular behaviour of a biomaterial is an important factor determining the biocompatibility of a biomaterial [64]. After cells contact biomaterials, cells will undergo their morphological changes to stabilise the cell and material interface. In our study, we monitored cell viability on PVA cross-linked chitosan using an MTT assay and observed cell morphology periodically to assess any differences in cell morphology. No obvious difference noticed with HCEC and NIH3T3 morphology in cultured cells using light microscopy. Our result implies that HCEC could favourably attach and proliferate on the PVA/chitosan surface, and cells were able to infiltrate the scaffolds and successfully form a 3D corneal epithelium [65] with appropriate pre-clinical and clinical experimentation in future.

Also, the cultured epithelium displayed a phenotype similar to human corneal epithelium as stem cells have certain unique characteristics, which include longevity, high capacity of self-renewal with a long cell cycle time and a short S-phase duration, increased the potential for error-free proliferation, and poor differentiation [66]. Semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR) was done on the cultured cells at varying intervals of time for expression of ABCG2, connexin43 (Cnx43), and keratin 3 (K3). The cells cultured over PVA/chitosan were able to maintain the expression of putative stem cell markers ABCG2, Cnx43 and K3.

Previous studies have shown that the connexins are gap junction proteins involved in cell-cell communication, and are important cell differentiating factors. To date, Cx-43 and Cx-50 are the only two gap junction proteins that have been identified in the corneal epithelium. Cx-43 is abundant on the basal corneal epithelium but is absent from limbal stem cells; thus, Cx-43 is proposed as a negative corneal stem cell marker [67]. Therefore, according to the phenotype of the HCECs cultured on the PVA/chitosan, they were HCE, but not limbal stem cells.

Antimicrobial peptides (AMPs) form an integral part of the innate immune system and provide defence against a range of pathogens as well as modulating immune responses [68]. This help provides a baseline defence against invading pathogens, and several are up-regulated in response to infection and inflammatory stimuli [69] [70] [71] and play a critical role as a microbial barrier [72] (Alison et al., 2004). The human β-defensins (hBD) and the cathelicidin LL37 [73] are peptides expressed by epithelia throughout the body including epithelia of the oral cavity. There are now 28 known β-defensin genes found in human. However; expression of hBD1, 2, and 3 have been most investigated [74]. Despite the constant threat from pathogenic microbes in the air and foreign objects around in the laboratory, the incidence of infection in the culture condition is expected amazingly low [75]. However, in spite of the presence of antibiotics in the culture medium or having an intact sterile surface condition, our results found the expression of all three defensin group AMPs in oral epithelial cells. This model can serve as a useful basic tool for the study of tissue innate immune responses as a purely epithelial model.

In summary, we modified chitosan by cross-linking its polymers with the naturally occurring cross-linker PVA in a safer and faster way and characterised the phenotypes of HCECs cultured on PVA/chitosan. We demonstrated that the improved development of PVA/chitosan showed good biocompatibility for cell

adhesion, expansion, and proliferation. Besides the above, this polymer scaffold will be promising scaffold alternative to AM for clinical use in the future for the transplantation of cultivated limbal stem cells onto the ocular surface with successful clinical trial and experimentation. Therefore, future applications of safe and rapid development of PVA/chitosan membranes can be considered for reconstruction of the cornea and other tissue engineering applications. About biocompatibility, although PVA/Chitosan scaffold produced no or low toxic tissue response, it is yet to be determined whether they produce any inflammatory response, as they are clinically significant. Therefore, further studies are necessary to investigate to rule out the possibility of any possible concerns in their use.

Acknowledgements

The authors are thankful to Deanship for Graduate Studies and Academic Research, University of Tabuk (UT), Kingdom of Saudi Arabia supported to buy some chemicals and reagents for this research (0121-1438-S).

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1571-1576.
https://doi.org/10.3889/oamjms.2018.269
eISSN: 1857-9655
Basic Science

Expression Rate and *PAX5* Gene Methylation in the Blood of People Suffering from Gastric Cancer

Mozhdeh Khajeh Haghverdi, Elham Moslemi*

Department of Biology, East Tehran Branch, Islamic Azad University, Tehran, Iran

Citation: Haghverdi MK, Moslemi E. Expression Rate and *PAX5* Gene Methylation in the Blood of People Suffering from Gastric Cancer. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1571-1576. https://doi.org/10.3889/oamjms.2018.269

Keywords: Gastric Cancer; *PAX5*; Real-time PCR; MS PCR; Methylation

***Correspondence:** Elham Moslemi. Department of Biology, East Tehran Branch, Islamic Azad University, Tehran, Iran. E-mail: drelhammoslemiphd@gmail.com

Received: 14-Jun-2018; **Revised:** 10-Sep-2018; **Accepted:** 11-Sep-2018; **Online first:** 19-Sep-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Gastric cancer is one of the most important health issues in the world. Considering the lack of plenty of pre-awarenesses, the survival of gastric cancer is still quite disappointing. Methylation of *PAX5* gene promoter is observed in most cancer cells of a human. A study has shown that PAX5 is a new tumoral suppressor in gastric cancer and methylation of the *PAX5* promoter is associated with the survival rate of gastric cancer.

AIM: The present research seeks to study the expression rate and methylation of the *PAX5* gene in the blood of patients who have gastric cancer to be used as a biomarker in this type of cancer.

MATERIAL AND METHODS: Real-time PCR technique was used to assess expression of *PAX5* gene, while the methylation status of *PAX5* gene promoter in the blood samples of people who have gastric cancer versus blood samples obtained from normal Iranian population was studied using MS PCR technique.

RESULTS: The final results pointed to the fact that expression of PAX5 in blood samples obtained from those who have gastric cancer is much less than what is observed in normal blood samples. A significant correlation was also observed between expression of this gene and age and promoter methylation rate. The results of methylation also indicated that 28% of *PAX5* gene promoters among patients were methylated, while all normal samples were non-methylated.

CONCLUSION: Studying the decrease observed in *PAX5* gene expression and the rise in promoter methylation can be utilised as a biomarker to enhance pre-awareness of gastric cancer.

Introduction

Gastric cancer is the result of cancer cell formation on the interior lining of the stomach [1]. The most common form of this cancer is known as Adenocarcinoma [2]. Throughout the world, the gastric cancer is the fifth main cause of cancer and the third major reason for death [3]. Lack of pre-awareness factors and proper diagnosis are the main problems in treating gastric cancer [3]. *PAX* genes (paired box) are a family of genes encoding tissue-specific transcription factors that play a major role in adjusting developmental and evolutional programs [4]. *Pax1, Pax2, Pax3, Pax4, Pax5, Pax6, Pax7, Pax8,* and *Pax9* are the members of *PAX* gene. *PAX5* gene encodes a family of transcription factors that regulate development that play a major and central role in cell differentiation and tissue development [4]. Inappropriate expression of PAX5 has been reported in various types of human tumours [4]. Researchers believe that inappropriate expression of *PAX5* contributes to Carcinogenesis and malignant progression of human cancers such as gastric cancer [5]. *PAX5* suppression is closely linked with the hypermethylation status of the promoter, and it can be reconstructed through demethylation treatments [6]. By knocking down *PAX5* trough short hairpin RNA's, the survivability and reproducibility of cells rise [5]. As a result, PAX5 is a useful tumour suppressor in gastric carcinoma and diagnosis of methylated *PAX5* can be considered an independent pre-awareness factor in gastric cancer.

The present research seeks to study the expression rate and methylation of *PAX5* gene in the blood of individuals who have gastric cancer and compare it against normal blood samples and study their role as pre-awareness and diagonal factors in gastric cancer. We also seek to study the possible link between changes in the expression of this gene and gender, age, and methylation status.

Materials and Method

As many as 70 venous blood samples (35 healthy samples and 35 samples obtained from those with gastric cancer) were obtained from Masoud medical diagnostic laboratory in Tehran, Iran. 18 samples were females, and 52 samples were males.

With due observation of the standard protocol of the kit, RNA extraction kit made by Sinagen Iran Co. (MR7713C) was used to extract Total RNA. Then the quality of purified RNA was studied using a spectrophotometer, and the optical absorption difference of 260 to 280 was taken into consideration. To synthesise cDNA, dNTP 10 Mm (1 µl), Random Hexamer (40 Mm) (1 µl), and Oligo dt (1 µl) obtained from Sinagen Co. and, finally, 10 µl purified RNA was added to the microtube. The resulting mixture was exposed to a temperature of 65℃ for 5 minutes; then it was exposed to a temperature of 4℃ for 1 minute. Next, MMULV 10 X buffer (2 µl) and MMULV (200 U/µl) (0.5 µl) were added to this tube, and the total volume was increased to 20 µl using DEPC water. The tube was exposed to a temperature of 42℃ for 1 hour, and then it was exposed to a temperature of 80℃ for 5 minutes.

To conduct SYBR Green-based Real-Time PCR on cDNA samples, the required primers for *PAX5* and *GAPDH* gene (as internal controls) were designed using the sequences that exist in NCBI and using Annealing Primer Express software. The results are presented in Table 1.

Table 1: The primers used in our research

Primer	Sequence	Tm℃	Amplicon size(bp)
PAX5 (F)	GGACCAGCAGGACAGGTATT	59.02	118bp
PAX5 (R)	TTGGCGTTTATATTCAGCGA	59.31	118bp
GAPDH (F)	ATGGAGAAGGCTGGGGCT	62.05	124bp
GAPDH (R)	ATCTTGAGGCTGTTGTCATACTTCTC	61.62	124bp

The reaction mixture consisted of 10 µl master mix obtained from Sinagen Co., 1 µl gene sample, 0.5 µl forward primer (0.4 millimolar), 0.5 µl reverse primer (0.4 millimolar) and 8 µl distilled water. The following temperature was required in this experiment: 95℃ (1 cycle lasting 10 minutes), 95℃ (40 cycles each lasting 15 seconds), 58℃ (1 minute) for *PAX5* and 59℃ (1 minute) for *GAPDH* gene. Finally, the analysis of the data resulting from Real-time PCR was conducted based on the threshold cycle obtained for the target and reference genes. To study the specificity of primers and make sure about the proliferation of exclusive components, separate melting curve graphs were drawn for *PAX5* and *GAPDH* genes using Step One Real-Time PCR Systems-Applied Biosystems. The RQ of samples were calculated by the device, and the chart of results was drawn by Graph pad software (P-value < 0.0001).

To study the presence of CPG islands in *PAX5* gene promoter and its effect on gastric cancer, methylation-specific PCR (MS PCR) technique was used. Using DNA extraction kit made by Sinagen Co. (DN8118C) and the standard protocol of the kit, the genomic DNA was extracted from the blood of participants. The kit made by Thermo Scientific Co. (EPIJET BISULFITE CONVERSION K1461) was used to modify DNA. Two pairs of primers were designed for MS PCR reaction. One pair was used for the areas containing methylated cytosines beginning with G, and the other pair was used for non-methylated cytosines beginning with A (Table 2).

Table 2: The primers designed for methylated and non-methylated areas in MS PCR reaction

Primer	Sequence	Tm℃	Amplicon Size(bp)
PAX5 MET (F)	TTCGCGTATAGCGTAGAGGGTCG	63	**bp 250**
PAX5 MET (R)	AAACGTAACGAACCCGACCCG	62/2	**bp 250**
PAX5 UNMET (F)	TTTGTGTATAGTGTAGAGGGTTG	54/7	**bp 250**
PAX5 UNMET (R)	AAATGTAATGAACCTGACCTG	52/8	**bp 250**

MS PCR reaction was carried out by adding 12 µl master mix, 1 µl forward primer (0.4 millimolar), 1 µl of reverse primer (0.4 millimolar), 2 µl of modified DNA sample (10 ng), and 9 µl of distilled water to the microtube. The following plan was defined for reaction: exposure to a temperature of 93℃ for 3 minutes for the initial denaturation, forty 30-second cycles of exposure to a temperature of 93℃, exposure to a temperature of 66℃ for 30 seconds, exposure to a temperature of 72℃ for 30 seconds, and, finally, exposure to a temperature of 72℃ for 5 minutes. To validate the product of PCR reaction, as much as 10 µl of it was run in 1% Agarose gel.

Results

A group of 39 samples belonged to people older than 50 years old, while the remaining 31 samples were obtained from people younger than 50.

Having extracted the RNA, the quality of extracted RNA was reported 1.7 by comparing the wavelength of 260 to 280.

Normal samples are used as references to compare changes in the expression rate of each gene compared to ill samples (Figure 1 and 2).

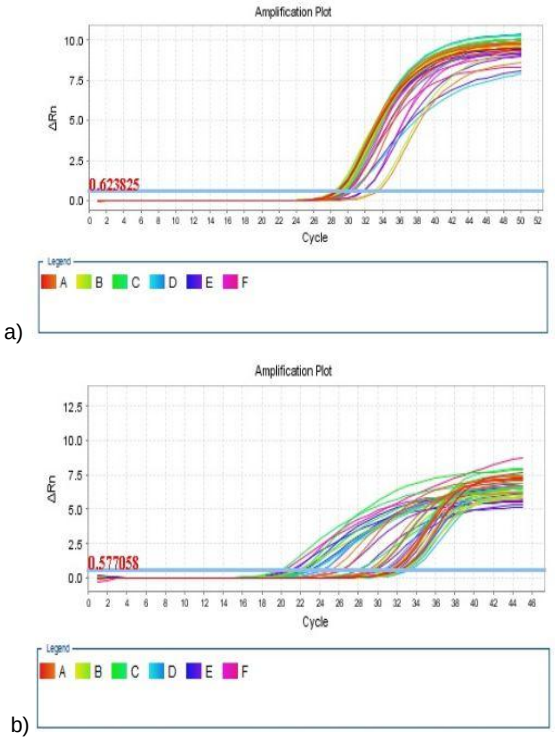

Figure 1: The linear curve of PAX5 gene proliferation in healthy and ill samples (a); the linear curve of GAPDH gene proliferation in healthy and ill samples (b)

The expression rate of sick samples was compared against normal samples. The results represent the expression of the same gene compared to what was observed in the blood of normal people (Figure 3).

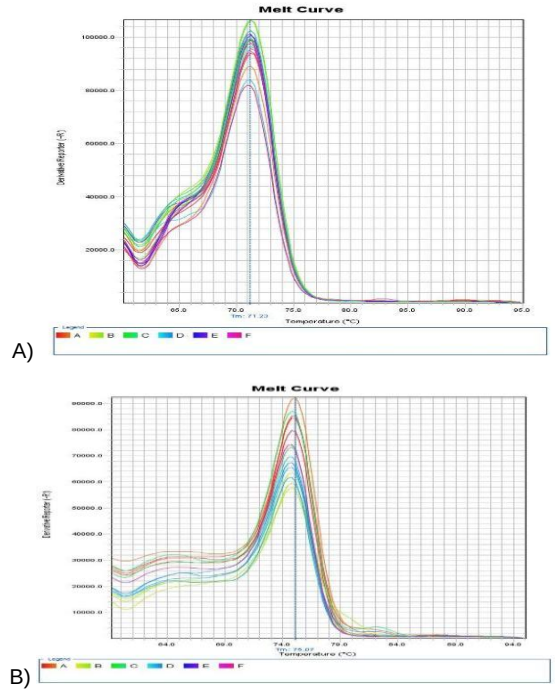

Figure 2: PAX5 gene melting curve in normal and sick samples A); GAPDH gene melting curve in normal and sick samples B)

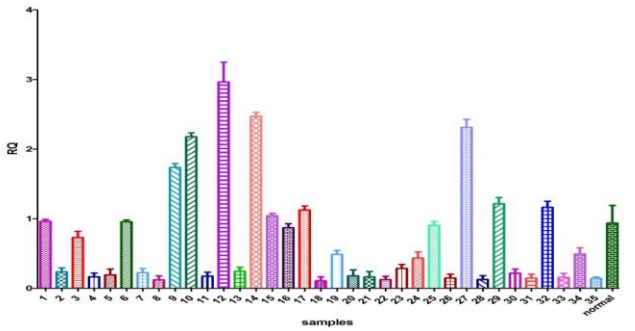

Figure 3: An analysis of PAX5 gene expression in sick samples compared to controls (P-value < 0.0001)

As the chart shows, most samples obtained from patients indicate less expression compared to normal samples. The normal samples are based upon the analysis made by the device, and all samples are compared against the normal samples. The least expression rates are observed in number 2, 4, 5, 7, 8, 11, 13, 18, 20, 21, 22, 26, 28, 31, 33, and 35 samples. As it is expected from PAX5 as a tumour suppressor in gastric cancer, the average expression of this rate in normal samples is more than all samples.

Further analysis showed that the mean expression rate of the *PAX5* gene among patients is less than what is observed among normal participants. What this means for patients is 0.2, while it was 0.55 among normal people. The greater expression may vary based upon gender, age, and methylation status (P-value = 0.0378) (Figure 4).

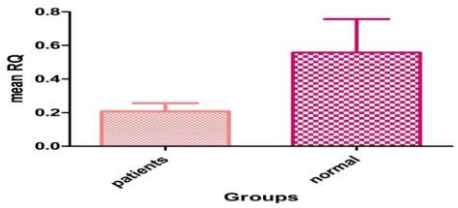

Figure 4: Mean expression of PAX5 gene in among patients and normal participants (P-value = 0.0378)

An analysis of results has shown that the mean expression rate of *PAX5* gene among patients compared to normal participants younger than 50 years old is 1.44, while this value for those older than 50 years old was 0.54 (P-value = 0.0159) (Figure 5).

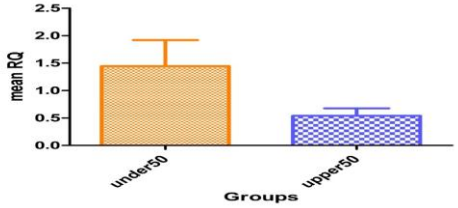

Figure 5: Mean expression rate of the PAX5 gene based upon age (P-value = 0.0159)

The mean expression rate of *PAX5* gene among male patients has been much more than what was observed among the female peers. This average of RQ among men was 73%, while it was 66.8% among women. We may conclude that the expression rate of *PAX5* among male patients was much more than what was observed among female patients (P-value=0.8721) (Figure 6).

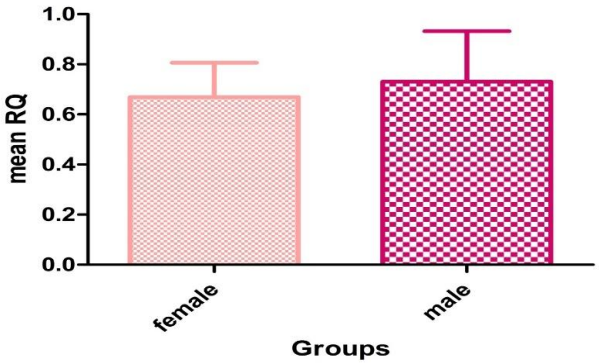

Figure 6: Mean RQ of PAX5 gene based upon gender (P-value = 0.8721)

By conducting MS PCR using methylated primers on 70 samples to study the methylation status of *PAX5* gene promoter, the band was observed in 4, 5, 31, 33, 34, 35, 39, 41, 43, 48 that it indicates methylation of *PAX5* gene promoter in these samples. The band sizes are in the range of 250 bp (Figure 7).

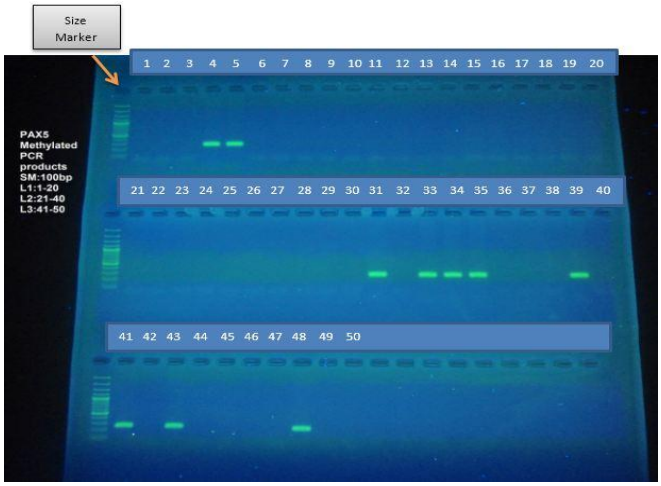

Figure 7: A sample of MS PCR results using methylated primers. Presence of bands with the size of 250 bp indicates methylation of PAX5 gene promoter

By conducting MS PCR using non-methylated primers on 70 samples to study the methylation status of *PAX5* gene promoter, the band was observed in 60 samples out of 70 samples which points to the fact that *PAX5* gene promoter is non-methylated in these samples (Figure 8).

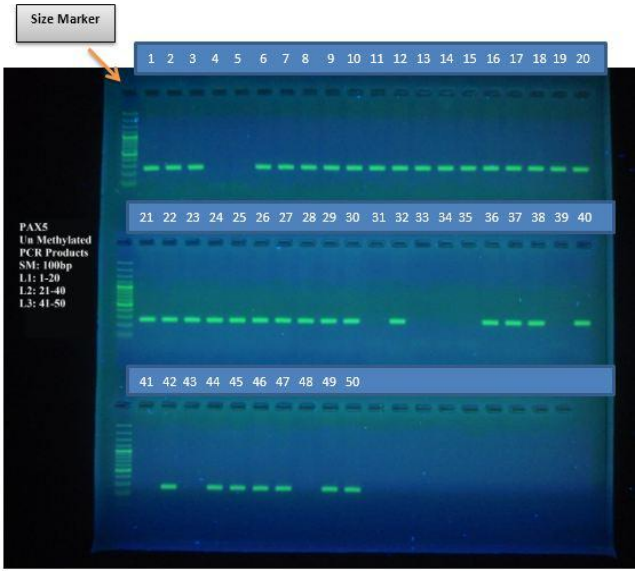

Figure 8: A sample of the results of MS PCR using non-methylated primers. Presence of bands with a size of 250 bp indicates the fact that PAX5 gene promoter is non-methylated

Considering the results of MS PCR test for *PAX5* gene, it was shown that only 10 DNA samples (of the whole 35 DNA samples) of patients are methylated (28%) but all normal samples are non-methylated. Consequently, 28% of samples for *PAX5* gene promoter are methylated. The average expression rate of the *PAX5* gene in non-methylated samples was 99%, while this value in methylated samples is 17%. As a result, the mean expression rate of the *PAX5* gene in non-methylated samples is higher than methylated samples (Figure 9).

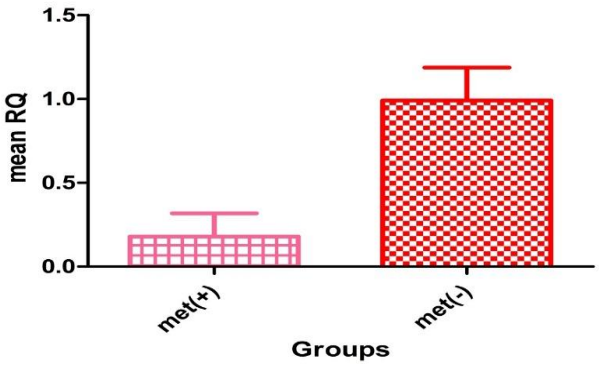

Figure 9: Mean RQ of the PAX5 gene based upon methylation status (P-value = 0.0409)

The P-values obtained through analysis of changes in expression of *PAX5* gene based upon gender, age, and methylation status are 0.8721, 0.0159, and 0.0409 which are respectively insignificant, significant and significant. As a result, no significant correlation is observed between less expression of *PAX5* and gender, but the correlation between less expression of *PAX5* and age and level of promoter methylation is significant.

Discussion

Gastric cancer is still the second and the most common reason of death caused by cancer throughout the world [6]. In Iran, gastric cancer has the greatest prevalence of all cancers among men [7]. Various transcriptional and regulatory factors that play a tumor suppressive role have been diagnosed in gastric cancer. For instance, PAX transcription factor has been diagnosed as a tumor suppressor in gastric cancer [8]. Other researches have pointed to the little or no expression of PAX5 in human cancers which shows PAX5 may act as a potential tumor suppressor in carcinogenesis [8]. Many researches have reported that methylation of the DNA promoter of *PAX5* gene will result in the shutdown of *PAX5* diagnosed in several malignancies [9]. *PAX5* has recently been used in diagnostic predictions of gastric cancer [10]. Using PCR technique for proliferation of methylated areas, Palmisano et al showed that activation of *PAX5* gene can help neoplastic development by suppressing growth regulators through affecting expression of CD19 gene [11]. They suggested that *PAX5* gene methylation is observed in tumors and their surrounding tissues and rarely in natural epithelial cells (assessments confirm them as intermediate markers in breast and lung cancers used to improve sensitivity and exclusiveness for development of risk models in order to diagnose these types of cancer) [11]. Using MSPCR technique and bisulfate genomic sequence, Liu et al., (2011) showed that *PAX5* is a functional tumor-suppressing gene and plays a major role in liver carcinogenesis by direct regulation of P53 signaling path which is usually deactivated through promoter methylation in Primary hepatocellular carcinoma tissues [12]. Using genome-wide methylation screening, Li et al., (2012) reported that suppression *PAX5* is closely associated with promoter hypermethylation and improper expression of *PAX5* in the shutdown cell lines of GC (AGS, BGC823) suppresses formation of colony and survivability of cells, stops cell cycle, induces apoptosis, suppresses cell immigration and invasion, and suppresses tumorigenesis in NUDE mice [13]. Using real-time PCR technique, a study was conducted by Liu et al and the results showed that *PAX5* is suppressed or shut down in most HCC cell lines and primary tumors [12]. In 2014, Deng et al studied the expression and methylation of *PAX5* gene in gastric tumor tissue and normal tissue for the first time. According to this research, mRNA expression of *PAX5* gene was diagnosed in 25 cancerous tissues of the stomach (of the whole 460 cancerous tissues) and 25 healthy mucosal gastric tissues through RT-PCR techniques [14]. Important differences in mRNA expression of *PAX5* gene were found in 25 cancerous gastric tissues. The mean value of the relative expression of mRNA in *PAX5* gene in 25 cancerous gastric tissues was 0.836 ± 0.357, while the mean value of the relative expression of mRNA in *PAX5* gene in 25 normal gastric mucosal tissues was 1.759 ± 0.821. The mean value of the relative expression of mRNA in *PAX5* gene in 25 cancerous gastric tissues was less than the 25 normal gastric mucosal tissues [14]. Considering the importance of *PAX5* gene, the present research studied expression of this gene in the blood samples of people suffering from gastric cancer in Iranian population. This is the first comprehensive research which presents statistical data of *PAX5* gene expression in the blood samples obtained from people suffering from gastric cancer in Iran. Using Real-Time PCR technique, the expression of this gene was compared accurately across the healthy participants and patients. Contrary to other studies and further to investigating the expression rate of *PAX5* gene, the present research studied the methylation rate of *PAX5* gene promoter and the correlation between methylation level and *PAX5* gene expression simultaneously. As the data resulting from this research indicate, the expression rate of *PAX5* in the blood samples obtained from patients is less than what is observed in normal samples. This data is in line with the results of a research by Deng et al., indicating that *PAX5* gene in gastric cancer acts as a tumor-suppressing gene plus less expression of *PAX5* gene in tumor samples obtained from stomach [14]. A review of methylation status of *PAX5* gene promoter showed that 28% of the DNA obtained from patients is methylated and all normal samples were non-methylated. These results are in line with the results of researches conducted on tumor samples of gastric tissue [15]. What's more, there is a significant correlation between the mean expression rate of *PAX5* and age and methylation status of promoter; however, no significant correlation was observed between average expression rate of *PAX5* gene and gender. All these researches were conducted on paraffined tissue samples; however, gastric cancer is not usually diagnosable in its early phases.

As a result, studying the expression and methylation of the *PAX5* gene in the blood of people who have gastric cancer shortly can be used as a diagnostic biomarker for gastric cancer.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1577-1580.
https://doi.org/10.3889/oamjms.2018.400
eISSN: 1857-9655
Basic Science

Performance of 2 Polymerization Protocols Targeting Cloned Toxoplasma Parasites

Nihal A. Hanafy[1,2*], Mohamed S. Badr[3], Ghada M. Nasr[4]

[1]Department of Parasitology, Faculty of Medicine, Cairo University, Cairo, Egypt; [2]Department of Microbiology, International Medical School, Management and Science University, Shah Alam, Malaysia; [3]Department of Molecular Biology, Medical Research Centre, Faculty of Medicine, Ain Shams University, Cairo, Egypt; [4]Department of Molecular Diagnostics, Genetic Engineering and Biotechnology Research Institute, Sadat City University, Sadat City, Egypt

Citation: Hanafy NA, Badr MS, Nasr GM. Performance of 2 Polymerization Protocols Targeting Cloned Toxoplasma Parasites. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1577-1580. https://doi.org/10.3889/oamjms.2018.400

Keywords: *Toxoplasma gondii*; qPCR; cPCR

***Correspondence:** Nihal A Hanafy. Department of Parasitology, Faculty of Medicine, Cairo University, Cairo, Egypt. E-mail: nihal_ahmed@msu.edu.my

Received: 16-Jul-2018; **Revised:** 13-Sep-2018; **Accepted:** 15-Sep-2018; **Online first:** 22-Sep-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: *Toxoplasma gondii* is a common parasitic infection of humans. Infection is usually mild. Serious complications can occur in pregnant and immunocompromised patients.

AIM: The present study aims to investigate the performance of 2 different PCR protocols; real-time quantitative molecular assays (qPCR) and conventional molecular assays (cPCR), using 2 different sets of primers and by using cloned purified Toxoplasma genomic substances to be evaluated as reference samples.

METHODS: The target DNA was provided in 8 different quantities.

RESULTS: Amplification failure was reported only with the cPCR in samples of low concentrations using both primer sets. Quantitative PCR detected the 8 different dilutions of the purified *Toxoplasma gondii* using the 2 sets of primers while cPCR was sensitive to detect only 6 different dilutions.

CONCLUSION: Generally real-time quantitative molecular assays, is easy to use method compared to conventional PCR assay and produces more reliable results within only one hour time but still the possible application of qPCRs in routine diagnosis necessitates analysis of a large number of clinical samples in further studies to make the proper choice.

Introduction

Toxoplasma gondii (*T. gondii*) is a known frequent cause of infection in a lot of warm-blooded animals including humans. This parasitic infection constantly infects between 15 and 85% of the world adult human population depending on geographical location. Fortunately, most cases of human infection are mild, but the severe disease may happen in immunocompromised individuals and congenitally infected fetuses in which serious complications may arise. Early diagnosis is crucial to start treatment that drastically reduces the extent of damage [1] [2]. Diagnosis of active infection using serology is often defective because reactivation of hidden infection is not all the time associated with changes in the levels of antibody, and evolution of latent or dormant toxoplasmosis is highly unpredictable.

Furthermore, IgM detection does not necessarily specify recent infection [3] [4]. Parasite DNA detection by PCR was, therefore, considered in many types of research as a step forward to improve and accelerate toxoplasmosis diagnosis by performing different PCR protocols including quantitative real-time PCR (qPCR) which has been first reported by Bell & Ranford-Cartwright, [5]. These lately described real-time assays allow amplification and concurrent DNA detection in one hour [6] [7] [8]. However, there is no available in vitro diagnostic molecular technique for parasitic infection, unlike many bacterial and viral infections. In general, there are various factors affecting the outcome of PCR including the targeted DNA, the selected primers, the choice of reference control in addition to the optimisation of other reaction condition [9]. Over 25 diverse primer pairs have been used in various assays, most of them aiming for the repetitive 35-copy-number B1 gene [10].

Searching for optimal technique, this work aimed to investigate the performance of 2 different

PCR protocols; qPCR and conventional PCR (cPCR), using 2 different sets of primers and by using cloned purified *Toxoplasma* genomic substances to be evaluated as reference samples.

Methods

A cloned purified *Toxoplasma* DNA (Roche Diagnostics) was prepared according to the manufacturer's recommendations. The target DNA was provided in 8 different quantities to yield from 10 copies/rxn to 10^8 copies/rxn of *Toxoplasma gondii* target molecules in 5 µl once dissolved. Start with the lowest concentration, a hole through the sealing foil was punched, and 45 µl PCR- grade water was added to each vial. The target DNA was then mixed by pipetting the solution up and down 10 times. For quantitative PCR, LightCycler FastStart DNA Master^{PLUS} Hybridization Probes® Kit (Roche Diagnostics, Hoffmann-La Roche Ltd, USA) was used, applying Fluorescence Resonance Energy Transfer protocol (FRET PCR). For amplification, 5 µl of DNA was used for each reaction mixture. The resulting PCR fragment of *T. gondii* was analysed using software data analysis version 3.5.3 which was implemented as stated in the LightCycler® instrument operator's manual. The reaction mixture (20 µl; Master^{PLUS} Hybridization Probes® kit; Roche Diagnostic) contained each primer 0.5 µM, $MgCl_2$ 5 mM and 5 µl template DNA. Capillaries were tightly closed, centrifuged at 500 *g* for 5s, and was then amplified in a LightCycler® instrument. Amplification for 50 cycles was performed: 5s denaturation at 95°C, 10s annealing at 61°C and 15s extensions at 72°C. The overall ramp rate was 20°C/s. One fluorescence reading for each sample was recorded at the extension step. Results were quantitatively demonstrated by determination of the revealing threshold or the crossing point (Cp), which distinct the cycle when the fluorescence of the used sample exceeded the baseline signal significantly. Samples were demonstrated as a fractional cycle number. The two sets of *Toxoplasma* primers were; a) the RE region (GenBank accession number AF146527), a repetitive sequence of 200-300 repeats in the *Toxoplasma* genome, giving an amplified fragment of 134 bp in conventional PCR; b) the commonly used B1 gene producing an amplified product of 432 bp. The adjusted conditions for conventional PCR (cPCR), were; 0.8 µl of every primer (50 pmol/µl), 20 mmol/L dNTPs and 1.25 U recombinant Taq polymerase in 1 × PCR reaction buffer (50 mmol/L KCl and 10 mmol/L Tris-HCl, 1.5 mmol/L $MgCl_2$, 0.1% Triton × 100). The total volume prepared for the reaction was 50 µl, including 5 µl of the individual DNA samples. The Q cycler Quanta Biotech thermal cycler was used for the conventional amplification using: 94°C for 2 min, followed by 30 cycles of 94°C for 1 min, 57°C for 2 min and 72°C for 3 min. For negative control, PCR mixture with no DNA and with DNase-free water was used to detect any cross-contamination.

Results

Quantitative PCR detected the 8 different dilutions of the purified *Toxoplasma gondii* using the 2 sets of primers. Figure 1 shows the results of using the cloned *Toxoplasma* DNA, all concentrations from 10^1 to 10^8 were detected by qPCR. Quantitative genomic estimation of these positive samples in qPCR (Figure 1) ranged from 1.7x 10^1 to 9 x 10^{10}.

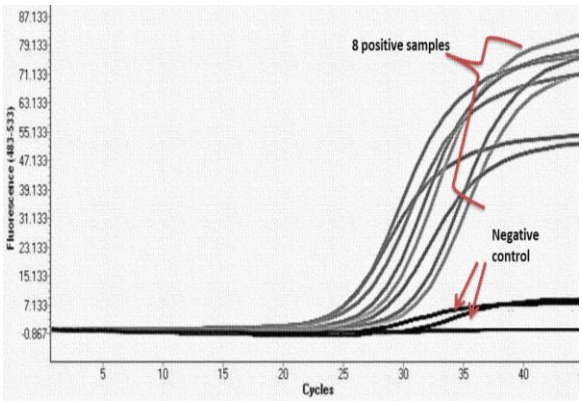

Figure 1: Eight positive samples of qPCR, starting fluorescence emission at different cycles and 2 negative control samples

Crossing points (Cps) showed different values ranged from 29.68 to 12.88 reflecting the different DNA quantities. Regarding cPCR, the amplified fragments were separated by gel electrophoresis at (432 bp for the B1 gene and 134 bp for RE region) as shown in Figure 2. From the 8 serially diluted purified samples included in the present study, conventional PCR was sensitive to detect 10^3 copies while failed to detect the low concentrations of (10^1) and (10^2) with both primers targeting B1 gene or RE region. No PCR contamination was observed during the study, as confirmed by the constant negativity of negative controls.

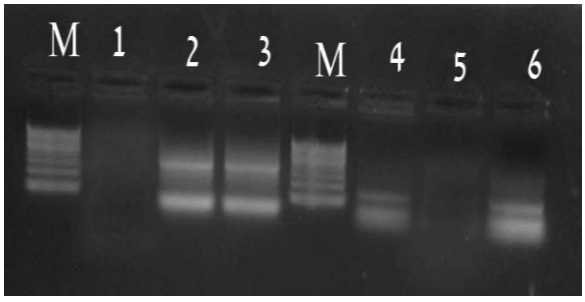

Figure 2: Lane 1 and 5 are negative control samples, M lanes are 100bp DNA markers. Lane 2 and 3 are the positive samples of the B1 gene (432pb), 4 and 6 positive results for RE region (143bp)

Discussion

Almost one-third of humans have been exposed to *T. gondii* parasite**.** Latest estimates of the disease burden rank toxoplasmosis as a foodborne infection at the same level as salmonellosis or campylobacteriosis [1]. These increase the requirement of a sensitive quantitative diagnostic assay to check the severity of such parasitic infection in different clinical situations especially in high-risk groups**.** Lately, molecular techniques using LightCycler® devices have been greatly improved and supported by flexible software technologies which expand their implementation on unique analytic parameters to diagnose a wide range of infectious agents. No molecular kits are available for regular diagnosis of toxoplasmosis, but only for research purposes [6]. In the present study, amplification failure was reported only with the cPCR in samples of low concentrations using the both primer sets. In general, the main advantages of real-time qPCR over cPCR assays as mentioned by the previous authors are as follow; (a) it is less labor-intensive, the test is done in one step, while in cPCR at least two steps are needed; (b) it is performed in a closed system with no need for post-PCR treatment and therefore reduce the risk of any contamination (c) the result of the quantitative PCR (after DNA extraction) can be given in less than one hour for FRET-based assay versus a minimum of 6 hours with the conventional PCR [3] [11]. The LightCycler® device has additional advantages over the thermal cycler used in the conventional PCR, which is the quantitative evaluation of the products. Such numerical exploration of the parasite load may have prognostic implications during follow up after treatment of infected subjects and could thus verify the practicality in comparing different drug regimens. This was also reported in a review done by Lui et al., [11] concerning the different methods used for diagnosis of toxoplasmosis, he recorded that the real-time PCR can be used to evaluate the efficiency of treatment since the intensity of the infection can be estimated. As for all parasitic diseases, molecular diagnosis of toxoplasmosis is still not standardized. Due to the previously mentioned advantages of qPCR over conventional PCR, it seems highly possible that there will be an agreement on real-time PCR assays in the future. In a relatively recent study done by Santos et al., [12], qPCR was able to diagnose *T. gondii* in patients with uveitis, and they recorded the reliability of this assay in diagnosing toxoplasmic active focal necrotizing retinochoroiditis. However, such an agreement on the choice of the most suitable sequence to be amplified will be more challenging. The possible application of qPCRs in routine diagnosis necessitates analysis of a large number of clinical samples in further studies to make the proper choice. Certainly, the cost of the molecular assay–and the reagents needed to extract the DNA template beside the choice between the conventional or qPCR techniques should be cautiously evaluated, excluding the cost of the PCR device instruments which may be bothering, however in the initial phase only

To conclude, real-time quantitative molecular assays are easy to use method compared to conventional PCR assay and produces results more readily. However, the chosen target gene can very much affect the results of the PCR assays, as we checked. Protocols for Real-time PCR should be optimised and properly evaluated in a larger number of samples before being implemented as routine diagnostic methods.

Nevertheless, these molecular tests certainly offer an alternative to conventional or even nested-PCR for a fast, easy and accurate diagnosis of toxoplasmosis. *Toxoplasma* B1 the tandem-arrayed 35-fold-repetitive gene is still a useful target which can be used for detection of *Toxoplasma* strains in clinical samples. Primers targeting RE region or other more repetitive genes seemed to be more sensitive. However, further comparative studies are recommended using a large number of suspected clinical samples to confirm variable sensitivities. Cloned *Toxoplasma* templates may facilitate easier and cleaner preparation of *Toxoplasma* reference samples especially when animal houses are defective in research or diagnostic laboratories.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1581-1587.
https://doi.org/10.3889/oamjms.2018.398
eISSN: 1857-9655
Basic Science

Clinical and Morphological Aspects in Assessing the Safety of OSPL-502 with Repeated Dose Administration

Sergey A. Zhuchkov[1*], Alexandr S. Kinzirsky[2], Irina V. Koroleva[2], Yuriy B. Vicharev[2]

[1]Department of Histology, Cytology, and Embryology, Orel State University, Orel, Russia; [2]Laboratory of Pharmacology, Institute of Physiologically Active Compounds of the Russian Academy of Sciences (IPAC RAS), Moscow, Russia

Citation: Zhuchkov SA, Kinzirsky AS, Koroleva IV, Vicharev YB. Clinical and Morphological Aspects in Assessing the Safety of OSPL-502 with Repeated Dose Administration. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1581-1587. https://doi.org/10.3889/oamjms.2018.398

Keywords: OSPL-502; N,N'-substituted 3,7-diazabicyclo[3.3.1]nonanes; Subchronic toxicity; Preclinical studies; Target organs

***Correspondence:** Sergey A Zhuchkov. Department of Histology, Cytology, and Embryology, Orel State University, Orel, Russia. E-mail: zhuchkov@ymservices.ru

Received: 11-Jul-2018; **Revised:** 05-Sep-2018; **Accepted:** 07-Sep-2018; **Online first:** 23-Sep-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: OSPL-502 is a new potential medicinal drug which stimulates a cognitive function. It is necessary to reveal clinical manifestations of its general toxic effect and determine organs that are most heavily affected by this pharmacological substance.

AIMS: To describe and estimate clinical and histopathological changes in the organism of experimental animals in response to the repeated administration of pharmacological substance OSPL-502.

MATERIAL AND METHODS: The study was conducted by the OECD Guidelines (Test No. 407) on Sprague-Dawley rats. The drug was administered at the dose of 20, 60 and 180 mg/kg.

RESULTS: The repeated doses of OSPL-502 have not caused any toxic effects on the growth of body weight, food and water consumption of the tested animals, or affected the musculoskeletal system and exploratory behaviour of the rats in the doses of 20 and 60 mg/kg. The dose of 180 mg/kg (1800 times larger than the therapeutic dose) has shown clinical signs of toxicity in females but has not resulted in the death of the animals. Due to morphological methods, we have found histostructural changes in the liver, kidneys and adrenal glands of the rats that were treated with the test substance in the maximum dose. These changes are reversible and reduce within 14 days after the admission of the studied substances is cancelled.

CONCLUSION: OSPL-502 at the dose of 180 mg/kg has a weakly pronounced toxic effect, the dose of 60 mg/kg is the threshold, and that of 20 mg/kg is no-observable-adverse-effect-level (NOAEL); the liver, kidneys and adrenal glands can be considered target-organs for the tested substance.

Introduction

According to epidemiological data, at least 50% of people over 65 years old have cognitive disorders of different intensities, and the risk of such disorders increase with age. Upward of 100 different neurological disorders are accompanied by cognitive dysfunctions which, in some cases, develop into dementia. The latest population studies demonstrate that there are about 46 million people living with dementia, with 50-70% of them diagnosed with Alzheimer's disease [1]. In 2006, there were about 15-20 million patients [2]. According to the estimates provided by the Alzheimer's Association (2016), 5.4 million Americans have Alzheimer's disease [3].

These facts make it vital to search for, synthesise and study pharmacological properties of substances which can compensate neurological deficits and possess neuroprotective properties.

It is known that glutamate receptors (AMPA and NMDA) play an important role in the formation of memory, which is one of the most important cognitive functions. The stimulation of AMPA-receptors improved the memory process [4]. Some substances that are modulators of AMPA receptors (including derivatives of N, N'-substituted 3,7-diazabicyclo[3.3.1]nonanes), which has neuroprotective and cognitive-stimulating effects [5] [6], have been discovered and studied relatively recently [7] [8].

OSPL-502 is a new derivative of N,N'-substituted 3,7-diazabicyclo[3.3.1]nonanes and is a highly active positive modulator of AMPA-receptors. It is a new potential medicinal drug from the pharmacotherapeutic group of nootropics. Since OSPL-502 should be used as a medicinal drug, it is necessary to reveal clinical manifestations of its general toxic action in case of repeated administration and determine organs and organismal systems that are most heavily affected by this pharmacological substance to estimate the safety of this potential medicinal drug.

Considering the data mentioned above, the current study aims to characterise and estimate clinical and pathological changes in the organism of experimental animals in response to the repeated administration of pharmacological substance OSPL-502.

In this regard, we planned to study clinical and morphofunctional changes of internal organs and the degree of reversibility of discovered changes after repeated oral administration of OSPL-502 to Sprague-Dawley rats of both sexes.

Material and Methods

The study was conducted by the OECD Guidelines (Test No. 407: Repeated Dose 28-day Oral Toxicity Study in Rodents). The tested, and control substances were administered to animals on a daily basis seven days a week within 28 days, which is in strict compliance with the research protocol. The substance administration into the stomach was provided by a probe at the same time before noon. The volume of the administered drug did not exceed 10 ml/kg of the animal's weight.

The volume and concentration of the administered pharmacological substance solution were daily corrected in response to mass body gain to maintain a constant dose of the substance in necessary proportions to the body mass of a growing animal. The drug was administered at the dose of 20, 60 and 180 mg/kg, and the control animals were administered with a dispersion medium in the same volume.

We used 10 animals (five female and five male) at each dose level and control. The satellite group of ten animals (five per sex) is used in control and the top dose group for observation of reversibility, persistence, or delayed the occurrence of toxic effects, for at least 14 days post-treatment (Table 1). The necropsy was performed in 24 hours after the end of treatment for the main groups (40 animals) and 14 days later for satellite groups (20 animals).

Table 1: Description of the study group

Group / Dose	Male			Female		
	The numerical indexes of animals	The volume for administration (ml/kg)	The concentration of a substance (mg/ml)	The numerical indexes of animals	The volume for administration (ml/kg)	The concentration of a substance (mg/ml)
1 / control	11-20	1.6	-	11-20	2.8	-
2 / 20 mg/kg	21-25	1.6	12.4	21-25	2.8	7.7
3 / 60 mg/kg	31-35	1.6	37.9	31-35	2.8	22.3
4 / 180 mg/kg	41-50	1.6	109.4	41-50	2.8	64.4

To conduct this research, we used *animal species Rattus spp.* (Sprague-Dawley rats: males and females which have never given birth or been pregnant) at the age of five weeks, (101 ± 2 gramme of body weight in female, 139 ± 2 gramme of body weight in male) received from a licensed source which has a valid AAALAC accreditation (Research and Production Enterprise "Laboratory animals farm", Branch of the Institute of Bio-Organic Chemistry, the Russian Academy of Science, Moscow Region, Pushchino). Before the experiment, the animals were quarantined in cages for five days to adapt to group housing conditions. The care and keeping of the rats and all procedures with them were done in a vivarium of IPAC RAS (Institute of Physiologically Active Compounds of the Russian Academy of Science) strictly following the current ethical norms and regulations according to the research protocol and regulatory documents [9] [10] [11].

The animals were housed in groups of three in ventilated microisolator units One Cage 2100 (Lab. Products Inc., the USA) on the flooring LIGNOCEL Rinofix MK 2000 (JRS, Germany). In the animal housing rooms, we used the "day-night" lighting regime (12-hour cycle) and controlled the environment (temperature 18-25°C; relative humidity 30-70%).

We fed the animals with standard sterile granulated compound feeding stuff for SPF animals entitled "Chara" (OOO "MultiTorg", Russia) which was balanced in nutrients and vitamins, and autoclaved at 121°C. The control over the autoclave functioning in feeding stuff sterilisation was performed using dipsticks in every cycle of autoclaving. The rats were given autoclaved water for drinking which had undergone additional purification against excessive salt and ferrous ions. The food and water were given to the rats without any restrictions.

Throughout the research, we were registering clinical signs of intoxication, body mass and food consumption. At the same time, we were recording effects of the tested substance on locomotor activity, muscle strength, and orienting behaviour through special physiological tests. We also carried out the ophthalmological examination.

OSPL-502 is a derivative of N,N'-substituted 3,7-diazabicyclo[3.3.1] nonanes (3,7-bis (1,3-benzodioxol-5- carbonyl)-1,5-dimethyl-3,7-diazabicyclo [3.3.1]nonane-9-OH) and is a highly active positive modulator of AMPA-receptors which synthesis in IPAC RAS and has neuroprotective and cognitive-stimulating effects [5] [6] [12] (Figure1). The

pharmacological substance is crystals of white colour which are insoluble in water but well soluble in organic solvents. The substance purity is > 95%.

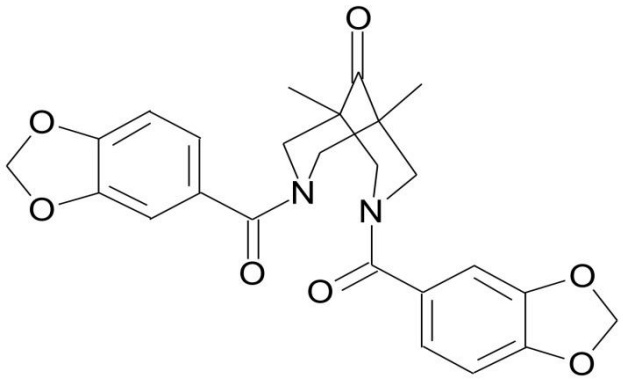

Figure 1: The structural formula of the OSPL-502

Doses were prepared by a provisor on a daily basis just before the administration. The administration solutions were the tested substance suspended in a dispersion medium of 1% starch gel with distilled water. The dose calculation, as well as the regime and duration of the drug administration, were done according to the current regulating documents [13] [14] [15] [16] [17] and results of the previous research [18].

In 24 hours after the final administration of the substance (main groups of animals) and 14 days of the recovery period (satellite groups of animals), the animals underwent CO_2-euthanasia with the following exsanguinating using blood draw from the inferior vena cava. Then we provided the complete examination of external conditions of a body and internal organs. During the necropsy, all changes in organs and tissues were registered. Also, brains, thyroid glands, hearts, lungs, thymus, spleens, livers, kidneys, bladders, stomachs, duodenum, colons, adrenal glands and gonads were taken for histological tests from each animal.

The biomaterial for the pathomorphological study was kept in 10% neutral formalin (pH 7.4). Processing, embedding, microtomy, and staining of specimens was done according to standard techniques [19] [20] with some alterations. The organ slices were put into standard marked cassettes for further procedures.

The processing of samples with an embedding medium was carried out using an automatic tissue processor Leica ASP 200S (Leica, Germany). After the preparation tissue samples were embedded into paraffin wax using tissue embedding system Leica EG1160 and then each block was cut by motorised rotary microtome Leica RM 2265 with microtome blades A35 (Feather, Japan). The produced standard cross sections five microns thick were placed on slides of common thickness (Menzel-Glazer, Germany) and stained by haematoxylin and eosin. Staining of all the samples was simultaneously provided by multicoloured stainer Leica ST5020 Multistainer in automatic mode. The stained samples were covered by standard coverslips.

The further morphological analysis of the slides was provided using light microscopes Leica CME (Leica, Germany) and Micros MC100 (XP 5MP) (Micros, Austria). Concurrently, we used computer image analysis program Microvisible (Image Analyzing Software v. 1.11.10) together with Freeware Image Tool (the USA, University of Texas) calibrated by a stage micrometre for transmitted light OMP with spacing between tick marks equal to 0.005mm.

The study was carried out by the Directive 2010/63/EU of the European Parliament and Council (2010) on the protection of animals used for scientific purposes [21] and with The Guide for the Care and Use of Laboratory Animals [10] [11]. The research protocol was approved by the Ethics Committee of IPAC RAS on August 15, 2012 (Protocol No. 8).

Group arithmetic means (M) and standard deviation (SD) were calculated for quantitative data. The statistical analysis was performed with program Statistic Base for Windows on Russian version 6.4. Male and female rates were tested independently. Differences were detected when a significant point was $p < 0.05$, tendencies were found when a significant point was $p < 0.1$ using the Student's t-test for independent samples and the Mann-Whitney nonparametric test.

Results

Throughout the research, the survival rate of the animals was 100% which proves the accuracy of the chosen dose regimes. The general state of health of the animals receiving the tested substance was also satisfactory. Intoxication manifestations differed depending on the animal’s sex.

The retardation in mass body gain could be seen in female rats when the maximum dose of the tested substance was administered starting from the seventh day of the experiment. This retardation (averagely by 7%) remained within the whole period of the pharmacological substance administration.

In 14 days after the substance administration, the mass body gain of all experimental groups receiving the maximum dose did not differ from that of the control group (Figures 2 and 3). The tested substance given in three different doses did not cause any significant effect on the animals' feed and water consumption.

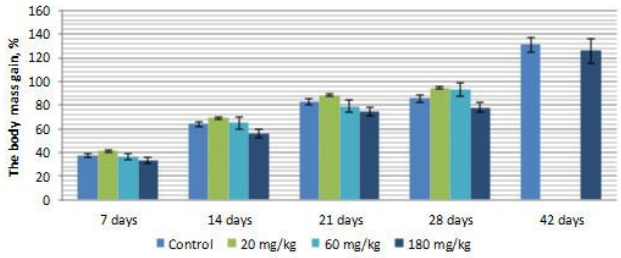

Figure 2: The mass body gain in the female rats during the experiment (n = 5)

We have found no locomotive system disturbances. The female rats administered the tested substance at the dose of 60 mg/kg were able to hang on the cage with increased strength. After the 28-day period of the drug administration, the orienting behaviour of both male and female rats in experimental groups was not different from the orienting behaviour of rats in the control group.

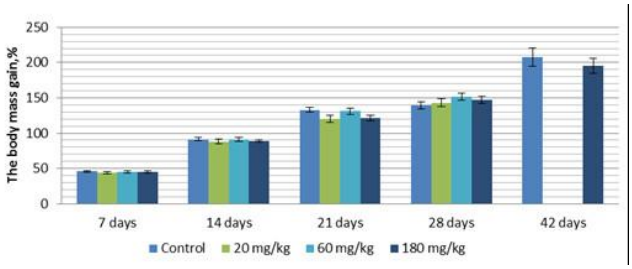

Figure 3: The mass body gain in the male rats during the experiment (n = 5)

The ophthalmological examination showed no exophthalmos, sclera reddening, corneal and lens opacity or any other changes in the retina. The pupillary light reflex retained.

In two weeks after the withdrawal of the pharmacological substance OSPL-502, no clinical signs of intoxication were found that can indicate the absence of cumulative properties in the tested pharmacological substance.

The animals' necropsy showed no haemorrhages, visible pathological changes of internal organs, etc. The microstructure of the brain, heart, thymus, spleen, lungs, thyroid glands and bladder also corresponded to the norms. We have revealed no pathological changes in these organs related to the toxic influence of the substance.

There were no macroscopic signs of mucous degeneration, ulcerations and necrosis in the stomach (where the substance was administrated to) of the animals receiving pharmacological substance OSPL-502 at the doses of 20, 60 and 180 mg/kg. The histologic structure of a stomach wall of the animals in the control group and the animals receiving pharmacological substance OSPL-502 at the doses 20, 60 and 180 mg/kg intragastrically corresponds to the norm, i.e. all four envelopes of the alimentary duct (mucous, submucous, muscular and serous) have a typical structure with no signs of degeneration or necrosis. Also, no ulcerogenic effect was detected as a result of the drug administration. The data mentioned above indicates the absence of the toxic influence on stomach walls during the 28-day administration of the tested substance. The structure of the duodenum wall showed no pathological changes caused by the drug influence. This organ had a typical structure with four envelopes, including the mucous, submucous, muscular and serous. We have also found no signs of epithelium necrosis.

Duodenal glands had a typical structure. The insignificant plethora of microcirculatory vessels was detected. The colons of both male and female rats receiving the intragastrical administration of the tested substance at the doses of 20, 60 and 180 mg/kg had a typical structure and were not different from the structure of the colon of the rats in the control group. No signs of degeneration or necrosis were found. Inflammatory changes were absent. Therefore, OSPL-502 at the tested doses produces no effect on walls of the digestive tract of the tested rats.

The list structure of adrenal glands samples of the intact animals and the animals receiving OSPL-502 was unharmed. In the studied samples, the cortex was distinguished from the medulla, the border between them was clear, but the latter was not always visible in all the cross-sections. There were cells with hydropic degeneration (from a small number of cells to moderate) in the upper third of the zona facilitate of the suprarenal cortex of all the animals. Nearly all cross-sections demonstrated cell clusters with small oval nuclei and oxyphilic cytoplasm. We have noted the moderate defatting of suprarenal cortex cells cytoplasm. The cells deprived of lipids were clustered in the form of triangular cords which started from the region where the zona glomerulosa bordered the zona fasciculata and then went down to the reticular zone. The zona reticularis and medulla were largely vascularized. The noted changes were reversible and reduced in 14 days after the substance was withdrawn. The histostructure of the adrenal glands of the animals receiving OSPL-502 at the doses of 20 and 60 mg/kg was not much different from that of the animals in the control group. The histostructure of adrenal glands of the female rats receiving OSPL-502 at the dose of 180 mg/kg had more acute changes in comparison to the rats in the control group. The cells with signs of hydropic degeneration were located diffusely and mainly in the upper third of the zona fasciculate (Figure 4).

The morphological examination of the liver and kidneys has shown some morphofunctional changes.

The histologic structure of the liver in the rats receiving OSPL-502 at the doses of 20 and 60 mg/kg was not different from that of the intact rats, i.e. the structure of the lobules with radially arranged plates of

hepatocytes was visible with no signs of fibrosis, infiltrates, etc. Hepatocytes were of a regular size and shape. However, there were single cells with signs of degeneration of different degrees (to the extent of hydropic). The hepatocytes nuclei were normochromic without pathological changes.

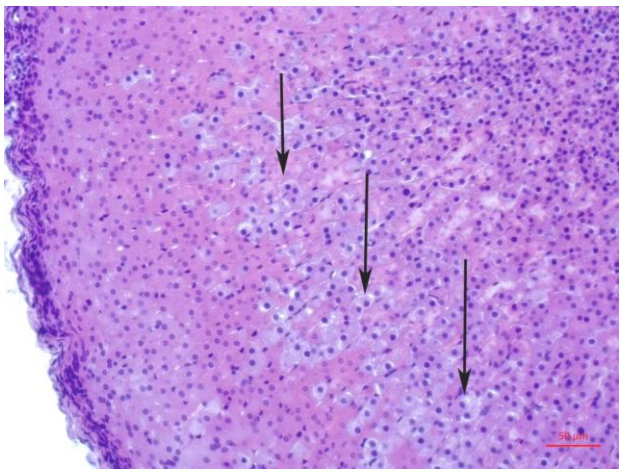

Figure 4: A fragment of the rat's adrenal glands administered with 180mg/kg body weight of OSPL-502. Staining with hematoxylin and eosin. Magnification x200 showing the cells deprived of lipids and the cells with hydropic degeneration (with points)

The intralobular sinusoids were without changes; other vascular system components were also in the same conditions. The female rats receiving OSPL-502 at the dose of 180 mg/kg intragastrically had an increased number of degenerated hepatocytes in comparison to the control group. There were signs of regeneration processes activation which manifested itself by an increased number of immature hepatocytes radially moving down along the lobules in periportal areas and the region of terminal plates. We should note that these changes were reversible and were not found in the animals in after the 14-day substance administration withdrawal, or if the dose of the administered substance was 20 and 60 mg/kg (Figure 5).

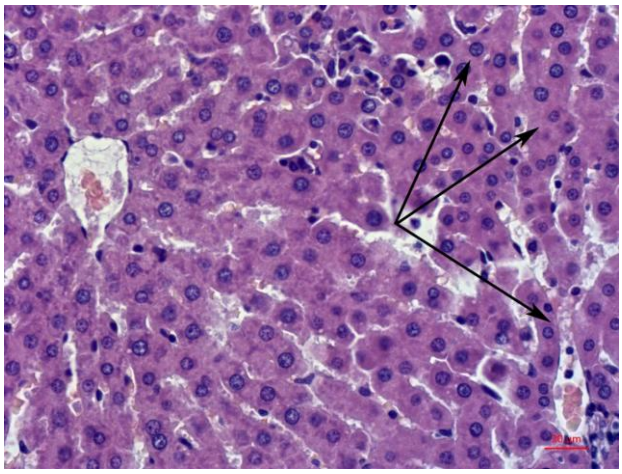

Figure 5: A fragment of the rat's liver administered with 180mg/kg body weight of OSPL-502. Staining with hematoxylin and eosin. Magnification x400. Showing the immature hepatocytes (with points)

The rats from intact groups and the rats receiving the substance at the doses of 20 and 60 mg/kg had no differences in the histostructure of their kidneys. The kidney parenchyma had a typical structure; renal corpuscles were slightly increased, though, their histological structure was without any changes. Glomerular capillaries were filled with blood, the capsule of glomerulus was without changes. There were single shrunken and obsolete glomerulus (one or two in a cross-section).

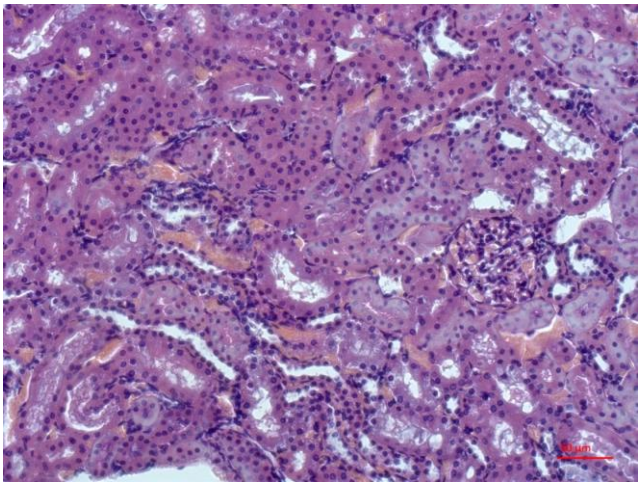

Figure 6: A fragment of the rat's kidney administered with 180mg/kg body weight of OSPL-502. Staining with hematoxylin and eosin. Magnification x200. Showing the tubules with albuminous and hydropic degeneration

Nephron tubules remained unchanged, though, there were single tubules with signs of hydropic degeneration, especially in the subcapsular area. The medullary substance of kidneys was without changes. The female rats receiving OSPL-502 at the dose of 180mg/kg intragastrically had most of their renal corpuscles unchanged, their glomerular capillaries were filled with blood, and the glomerular capsule was without changes (Figure 6 and 7).

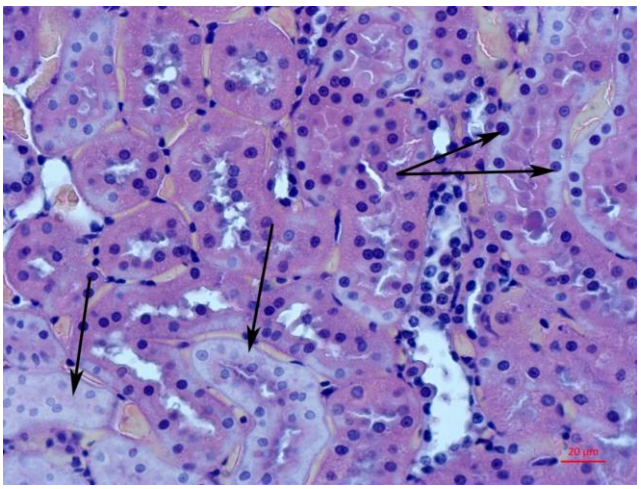

Figure 7: A fragment of the rat's kidney administered with 180 mg/kg body weight of OSPL-502. Staining with hematoxylin and eosin. Magnification x400. Showing the tubules with albuminous degeneration (with points)

In half of the cases, there were tubules with albuminous and hydropic degeneration in cortical and medullary substances with some desquamation of tubules epithelium. There was no swelling of the interstitium. Within 14 days after the withdrawal of the substance administration the kidney structure of the animals in experimental groups was the same as that in the control group.

Discussion

The results of the undertaken study indicate mild morphofunctional changes in the liver and kidneys of the female rats receiving OSPL-502 at the dose of 180 mg/kg, which exceeds the therapeutic dose by 1800 times. The liver had signs of regeneration processes activation which manifested itself in the increase of immature hepatocytes radially moving down along the lobules in periportal areas and the region of terminal plates. Their kidneys had single tubules with albuminous and hydropic degeneration in cortical and medullary substances. It is necessary to denote that these changes were reversible and could not be found in the animals after the 14-day substance administration withdrawal of the dose of 20 and 60 mg/kg. Therefore, the noted changes are reversible which corresponds to the known data [22]. Histostructural changes in adrenal glands of the rats receiving a maximum dose (the moderate defatting of cytoplasm of suprarenal cortex cells, mild degenerative processes) can either indicate that the pharmacological substance has a very weak toxic effect, or be a response to the stress caused by the long-time intragastric administration of the tested substance. We should note that morphofunctional changes are expressed mildly; they are not widespread and appear in both control and experimental groups. The changes mentioned above seem to be a result of tension as a response to the long-time external influence accompanied by stress which can lead to a decrease in cortisol level and signs of suprarenal cortex insufficiency [23] [24]. The noted changes are reversible and reduce within 14 days after the substance administration is withdrawn. The research results are comparable with another study [18] and literature data about the toxicity of N, N'- substituted 3,7-azabicyclo[3.3.1]nonanes [5] [6] [7].

We can conclude that the repeated doses of OSPL-502 have not shown any toxic effects on the growth of body weight, food and water consumption of the tested animals or affected the musculoskeletal system and exploratory behaviour of the rats in doses of 20 and 60 mg/kg. The dose of 180 mg/kg (1800 times larger than the therapeutic dose) resulted in only clinical signs of toxicity in the female rats but did not cause the animals' death. We have only found histostructural changes using morphological methods in the liver, kidneys and adrenal glands of the rats treated to the test substance in the maximum dose. These changes are reversible and reduce within 14 days after the cancellation of the studied substances. Therefore, our studies have revealed that the target organs for the test substances are the liver, kidneys and adrenal glands.

The identified changes are reversible after the withdrawal of the substance; OSPL-502 at the dose of 180 mg/kg has a weak toxic effect, the dose of 60 mg/kg is the threshold, and 20 mg/kg is no-observable-adverse-effect-level (NOAEL).

In conclusion, the female rats are more sensitive to the tested substance than the male ones when it comes to the repeated oral administration of the substance, but this gender difference is evident only in case of the maximum tested dose of 180 mg/kg. Considering the obtained data, the liver, kidneys and adrenal glands can be regarded as target-organs for the tested substance. The dose of 20 mg/kg exceeding the therapeutic dose by 200 times is non-toxic (NOAEL-No-Observable-Adverse-Effect-Level). Morphofunctional changes in the liver, kidneys and adrenal glands are reversible and reduce within 14 days after the substance withdrawal.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1588-1593.
https://doi.org/10.3889/oamjms.2018.332
eISSN: 1857-9655
Clinical Science

Respiratory Viruses and Atypical Bacteria Co-Infection in Children with Acute Respiratory Infection

Nevine R. El Baroudy[1], Amira S. El Refay[2*], Tamer A. Abdel Hamid[1], Dina M. Hassan[3], May S. Soliman[3], Lobna Sherif[2]

[1]Department of Paediatrics, Faculty of Medicine, Cairo University, Cairo, Egypt; [2]Departments of Child Health, National Research Centre, Cairo, Egypt; [3]Department of Clinical and Chemical Pathology, Faculty of Medicine, Cairo University, Cairo, Egypt

Citation: El Baroudy NR, El Refay AS, Abdel Hamid TA, Hassan DM, Soliman MS, Sherif L. Respiratory Viruses and Atypical Bacteria Co-Infection in Children with Acute Respiratory Infection. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1588-1593. https://doi.org/10.3889/oamjms.2018.332

Keywords: Acute Respiratory tract infection; atypical bacteria; viral; coinfection

***Correspondence:** Amira S. El Refay, PhD, Department of Child Health, National Research Centre, El-Bohouth Street, Dokki, Cairo, Egypt P.O:12622, Affiliation ID: 60014618. Tel: +20 012 298 3076; fax: +37601877; E-mail: amirasayed.ak@gmail.com

Received: 29-May-2018; **Revised:** 28-Jul-2018; **Accepted:** 29-Jul-2018; **Online first:** 23-Aug-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Acute respiratory infections (ARI) are one of the prevalent pediatric diseases. Coinfections of respiratory viruses and atypical bacterial respiratory pathogens are common.

AIM: This study aimed to determine the prevalence of co-infection between respiratory pathogens including viruses, bacteria and atypical bacteria in a sample of Egyptian children presenting with symptoms of acute respiratory tract infection.

METHODS: This one-year prospective cohort study conducted in Abo El Rish Pediatric Hospital, Cairo University over one year included children presenting with symptoms of acute respiratory infection. Enrolled children were subjected to nasopharyngeal swabs or throat swabs and then processed to detect viral, bacterial and atypical bacterial causative agents by culture), retrotranscription polymerase, Monoplex polymerase chain reaction (PCR) and Multiplex PCR.

RESULTS: Viral etiological agents were detected in 20 cases (20.8%), while 76 patients (79.2%) had no definite viral aetiology. The most abundant virus detected was Rhinovirus in 36 (27.3%), followed by 21 (15.9%) were positive for RSV, 12 (9.1%) were positive for HMPV, 6 (4.5%) were positive for adenovirus and 3 (2.3%) were positive for influenza B. For Atypical bacterial causes Mycoplasma were positive for 9 (6.8%) cases and one case was positive for Bordetella parapertussis. Viral and atypical bacteria Co infection were detected in 14 (10.6%) of cases.

CONCLUSION: These results suggest that coinfection with bacteria or atypical bacteria in children with acute respiratory tract infection is common and this co-infection can induce serious illness. The multiplex reverse-transcriptase polymerase chain reaction should become an essential tool for epidemiological studies and can fill the gap between clinical presentation and definitive diagnosis.

Introduction

Acute respiratory infections (ARI) are one of the prevalent pediatric diseases all over the world. In children below five years [1] [2] [3], acute respiratory infections are responsible for 18–33% of all deaths. Unfortunately, more than half of this mortality is recorded among developing countries as low social level and malnutrition double the burden [2] [3] [4].

Acute respiratory infections can be caused by pathogens like bacteria, viruses, fungal and atypical bacteria include Streptococcus pneumoniae, Mycoplasma pneumoniae, Haemophilus influenzae, and respiratory viruses (influenza A and B, adenovirus, respiratory syncytial virus and parainfluenza) [5] [6].

Although viruses are usually the main responsible for most of both upper and lower acute respiratory infections (ARIs) [7], but studies documented that patients may be infected with both bacterial and viral pathogens, making it difficult to identify the clinical characteristics that allow the physician to differentiate viral disease from bacterial disease in the early course of the disease [8].

Recently, many new emerging respiratory viruses have been identified including human coronavirus (HCoV), NL63 [9] and human bocavirus

(HBoV) [10]. Moreover, various emerging respiratory viruses led to epidemics and pandemic (H1N1) virus infection and H5N1 [11].

Atypical pathogens Mycoplasma pneumoniae, Chlamydophila pneumoniae, and Legionella pneumophila are considered important agents are causing mild, moderate, or even severe acute respiratory tract infections (ARTIs) in children worldwide [12].

This study aimed to determine the prevalence of co-infection distribution between respiratory pathogens including viruses and atypical bacteria among a well cohort of febrile children presenting by acute respiratory tract infections symptoms.

Material and Methods

This one-year prospective cohort study conducted in Abo El Rish Pediatric Hospital, Cairo University over one year from the first of June 2016 to 30th of June 2017 on children presenting with symptoms of acute respiratory infection.

Ethical statement

The study followed the regulations of the medical ethical committee of the Cairo University and the medical ethical committee of the National Research Centre and. Signed informed consents were collected from legal child guardian before participation after explaining the purpose of the study.

Any child presented with Influenza-like illness (ILI) symptoms the chest clinic Abo El Rish Pediatric Hospital, Cairo University was considered eligible. ILI was defined according to CDC criteria as having measured fever > 38˚C and cough and/or a sore throat [13].

Exclusion criteria

Any child receiving immunosuppressive therapy started antibiotic therapy, children with chronic lung diseases and patients who were unwilling to participate in this study were excluded from the study.

Data collection

Data were collected by a self-designed questionnaire include demographic data, risk factors (smoking, passive smoking and contact with birds). Throat washes and nasal swabs were collected from each child as appropriate.

Laboratory analysis

For Routine Bacterial and fungal culture

A throat wash was taken from each child when appropriate, in the case of a young infant throat swab was taken instead.

Specimens then were cultured on blood agar, chocolate agar, MacConkey agar and Sabarauds dextrose agar (Oxoid Co. England). Isolates were identified by conventional methods such as culture characteristics and biochemical reactions.

For Viral PCR

Nasopharyngeal swabs were taken from each participant by measuring the distance between the ear lobule and the ala nasi by the NP swab and divided by 2, and the swab marked at this distance to ensure the insertion of the swab in the proper site. This flexible, sterile tip flocked with nylon fibre swab applicator was inserted into the nostril and back to the nasopharynx and left in place for a few seconds. It was then slowly withdrawn with a rotating motion. The swab was placed in a 15 mL centrifuge tube labelled with the unique patient ID and containing 2 mL viral transport media (VTM: consisting of a sterile solution of bovine albumin fraction V, HEPES buffer, penicillin and streptomycin in HANK's balanced salt solution). The applicator stick was then cut off.

The received swabs inside the 15 ml tube were agitated vigorously for 10 seconds using a vortex mixer to free cells from the swab tip, and then swab was removed from the tube and discarded using a forceps. The VTM was immediately placed in a freezer (-70°C) until tested with PCR for the presence of respiratory viruses. If samples are positive for Influenza A, then further testing was performed by PCR to determine if the virus is a seasonal influenza variant or swine H1N1 2009 virus.

PCR testing for Respiratory viruses

Viral Nucleic Acid Extraction: Viral Nucleic acid extraction was done after centrifugation of the sample for 10-20 min and 200ul from the sediment taking as starting material using the QIAamp® Viral RNA Mini cat number 52904 according to the manufacturer's instructions. Elution was done with 60ul buffer AVE, and reelution step was added to increase the nucleic acid concentration.

CDNe was done using 15ul from the extracted RNA, for only the testing of the following RNA viruses (Rhinovirus, Influenza, RSV and HMPV). For Adenovirus conventional PCR testing was done directly from the extracted nucleic acid (DNA). The kit used for complementary DNA synthesis was Sensifast cDNA (cat# BIO-65054).

PCR: Conventional PCR was done by 3 different reactions for each sample as follows for the following viruses:

- Monoplex PCR for Rhinovirus testing using primers sequence, concentrations and conditions as [14];
- Monoplex PCR for Adenovirus testing using primers sequence, concentrations and conditions as [14];
- Multiplex PCR for InfA, InfB, HMPV and RSV testing from the following work [15] (Table 1).

Table 1: Primer list for detection of Respiratory viruses by different PCR types

Virus-tested	Type of PCR	Primers			Final Primers Conc	Master Mix used	Annealing Temp	Ref
Rhino	Monoplex	EVP4	5'-CTACTTTGGTGTCCGTGTT-3'		0.2µM	One Taq Hot Start Quick-Load 2X Master Mix (M0488S)	55°C	[2]
		OL68-1	5'-GGTAAYTTCCACCACCANCC-3'					
Adenovirus	Monoplex	AdnU-S'2	5'-TTCCCCATGGCNCACAAYAC-3'		0.2µM	One Taq Hot Start Quick-Load 2X Master Mix (M0488S)	59°C	[2]
		AdnU	5'-TGCCKRCTCATRGGCTGRAAGTT-3'					
InFA and B, RSV HMPV	Multiplex	RSV	vrs P1	GGA ACA AGT TGT TGAGGT TTA TGA ATA TGC	0.3µM	Qiagen multiplex MM	55°C	[1]
			vrs P2	TTC TGC TGT CAA GTC TAG TAC ACT GTA GT				
		InFA	mia 1	CAG AGA CTT GAA GAT GTC TTT GCT GG	0.3µM			
			mia 2	GCT CTG TCC ATG TTA TTT G				
		InfB	mib 1	AAA ATT ACA CTG TTG GTT CGG TG	0.3µM			
			mib 2	AGC GTT CCT AGT TTT ACT TG				
		hMPV	hmpv 1	CCC TTT GTT TCA GGC CAA	0.3µM			
			hmpv 2	GCA GCT TCA ACA GTA GCT G				

Statistical method

Data were coded and entered using the statistical package SPSS (Statistical Package for the Social Sciences) version 23. Data were summarised using mean, standard deviation, median, minimum and maximum in quantitative data and using frequency (count) and relative frequency (percentage)

Results

The study was conducted from first June 2016 to June 2017. 132 children presented by ILI were enrolled in the study selected from Pediatric Hospital Cairo University. 57 (43.2%) were males, and 75 (56.8%) were females.

The age of patients ranged from 1month-10 years (120 months) with a mean age of 2.95 ± 2.46 years (Table 2), this study was designed to estimate the prevalence of respiratory pathogens in 132 children presented with ARI. Overall, samples from 114 individuals (86.3%) were found to be positive for at least one pathogen, and 18 of them were positive for two or more pathogens. Rhinovirus was the most commonly detected agent, followed by *Streptococcus pneumoniae*.

Table 2: Demographic data of the studied children

Age (months)		
Mean ±SD	29.34 ± 28.29	
Range	1-120	
	Frequency	Percentage
Gender		
Female	57	43.2%
Male	75	56.8%
Exposure to smoking		
Yes	63	62.8%
No	49	37.1%
Exposure to birds		
Yes	61	46.2%
No	71	53.8%

Viral etiological agents were detected in 78 cases (59.09%), while 54 patients (40.9%) had no definite viral aetiology. The most abundant virus detected was Rhinovirus in 36 (27.3%), followed by 21 (15.9%) were positive for RSV, 12 (9.1%) were positive for HMPV, 6 (4.5%) were positive for adenovirus and 3 (2.3%) were positive for influenza B (Table 3).

Table 3: Etiological agents detected in the studied children

Etiological agents	Frequency	Percentage
Viral agents		
Rhinovirus	36	27.3
RSV	21	15.9
HMPV	12	9.1
Inf A	0	0
Inf B	3	2.3
Adenovirus	6	4.5
Bacterial agents		
Klebsiella	17	12.9
Enterobacter	11	8.3
Pseudomonas spp.	11	8.3
Acinetobacter	9	6.8
Klebsiella and enterobacter	1	0.8
Atypical bacteria agents		
Chlamydophila pneumoniae	0	0
Bordetella parapertussis	1	0.8
Bordetella pertussis	0	0
Legionella pneumophila	0	0
Mycoplasma pneumoniae	9	6.8

Bacterial agents were detected in 49 cases (37.1%), Klebsiella was detected in 17 (12.9%) child followed by Enterobacter in 11 (8.3%), Pseudomonas spp. 11 (8.3%), Acinetobacter 9 (6.8%) and Klebsiella and Enterobacter co-infection in one child (0.8%).

For Atypical bacterial causes, Mycoplasma was positive for 9 (6.8%) cases, and one case was positive for Bordetella parapertussis (Table 3).

Table 4: Co-infection distribution among studied cases

Co-infection	No
Streptococcus pneumoniae, mycoplasm, Rhinovirus and HMPV	3
Streptococcus pneumoniae, and Rhinovirus	1
Streptococcus pneumoniae, Rhinovirus and HMPV	2
Streptococcus pneumoniae and Rhinovirus	6
Rhinovirus and HMPV	3
Mycoplasm and RSV	3

Viral and atypical bacteria Co infection were detected in 14 cases illustrated in Table 4.

Discussion

Acute respiratory disease present about 75% of all acute morbidities in developed countries, and most of the cases (about 80%) are viral [16]. On average, five to eight respiratory viruses are detected in pediatric patients every year [17] [18]. These viruses are well-known causes of acute respiratory tract 6+03infections (ARTIs), which are a major source of morbidity and mortality in infants and young children [19].

The PCR technology development allowed a wide range of etiological viral agents to be detected with more sensitivity and specificity [20].

Several recent studies have analysed the epidemiologic pattern of respiratory viral infections; Rhinovirus is usually the most common single pathogen found in ARI surveillances samples with prevalence range 24. -50% followed by RSV 22-25% and influenza viruses 7.2-8% either by detection in nasal washes or nasopharyngeal swabs [21] [22] [23], which comes in accordance with an Egyptian study by Amin et al., 2012 RSV virus was detected with a high predominance (51.9%) in Egyptian asthmatic children with acute respiratory tract infection [24].

In the current study, the most abundant virus detected was Rhinovirus in 36 positive samples (27.3%), followed by RSV in 21 (15.9%) positive samples. Although it is non-statistically significant, all the positive cases are distributed all over different age group.

Human metapneumovirus (HMPV) is a recently identified Paramyxovirus first isolated from hospitalised children with acute respiratory tract infections (ARTI) in 2001 [25] Since then the hMPV reported prevalence ranged between 1.5 to 17.5% [26].

In the current study positive samples for hMPV was 12 (9.1%), This ratio may be considered lower than similar studies [26] [27] [28] and that can be explained by that child included in our study were enrolled from the patient presenting by ILI symptoms and the hMPV is more common in hospitalized children with pneumonia .

The dual viral infection or the multiple viral isolates occurrence was reported by some studies [29] [30] on the contrary; other studies failed to detect more than one viral agent [24]. In our study, we reported 3 cases of rhino and hMPV dual viral infection.

Atypical bacteria as Mycoplasma pneumoniae, Bordetella parapertussis and Chlamydia pneumoniae are common respiratory pathogens in children 5 years of age and older [31]. Infection may be preceded by an upper respiratory infection. Of the 132-child presented by ILI in our study 9 (6.8%) children were positive for Mycoplasma pneumoniae, and one case (0.8%) was positive to Bordetella parapertussis.

Coinfections with respiratory viruses and bacterial respiratory pathogens are common, often via synergistic interactions between the viruses like influenza virus, the bacteria, and the human host, However, the interaction between viruses and atypical bacteria still unexplained [32].

This study aimed to demonstrate the rate of co-infection between viral and bacterial agent. Although all the children enrolled in this study had the similar initial clinical presentation but the causative agent varied from single or multiple and bacterial or viral .

Viral and bacterial Co-infection was detected in 18 (22.7%) of children in the current study, of them 6 children were positive to Rhinovirus and another agent either bacterial (Streptococcus pneumoniae), atypical bacteria (Mycoplasma), viral (HMPV) or multiple causative agents.

The dual infection or multiple causative agent infections had been studied evidently in recent researches as multiplex PCR enabled laboratorians to detect a panel of viruses simultaneously while reducing hands-on time and with greater analytical sensitivity. There are several multiplex assays evaluated for detection of respiratory viruses [33].

In a study on respiratory pathogens in hospitalized children, of 1281 children, 449 (35%) had an acute respiratory tract infection caused by at least one of the organisms studied; there were 29 cases of dual infection [34]. In another study Serology with paired samples and PCR on nasopharyngeal aspirates and throat cultures were used to identify bacteria and viruses and mixed viral/bacterial pathogens in 26 patients (20.5%) The main etiological agents were adenovirus, respiratory syncytial virus (RSV), Mycoplasma pneumoniae, Streptococcus pyogenes and Chlamydia pneumoniae [35].

These results suggest that coinfection with bacteria or atypical bacteria in children with acute respiratory tract infection is common and this co infection can induce serious illness.

Unfortunately, most of respiratory tract infection are still treated empirically [36] with antibiotic in spite that most of the causative agents are viral on the other hand Most of pneumonia caused by atypical bacteria usually occurs after infection of the upper respiratory tract [32].

Certainly, the early management of respiratory tract infection is essential to ensure good prognosis and avoid complications. The diagnosis based on clinical finding alone is no more suitable the need of non-traditional diagnostic test now is becoming a must as molecular methods such as multiplex Real-Time PCR (RT-PCR) facilitate identifying many causative agents which in turn

reduce the excessive use antibiotics has caused rising bacterial resistance.

The multiplex reverse-transcriptase polymerase chain reaction should become an important tool for epidemiological studies and can fill the gap between clinical presentation and definitive diagnosis.

In conclusion, co-infection with multiple pathogens with the predominance of viruses is emerging. The Large-scale multicentric studies are recommended to address the epidemiological gap, and hence a new management strategy can be developed.

Limitation of the study: Small sample size and a low number of co-infection cases preclude any robust statistical analysis.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1594-1598.
https://doi.org/10.3889/oamjms.2018.337
eISSN: 1857-9655
Clinical Science

Protective Role of Vitamin C Intake on Muscle Damage in Male Adolescents Performing Strenuous Physical Activity

Shreef G. N. Gabrial, Marie-Christine R. Shakib*, Gamal N. Gabrial

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

Citation: Gabrial SGN, Shakib M-CR, Gabrial GN. Protective Role of Vitamin C Intake on Muscle Damage in Male Adolescents Performing Strenuous Physical Activity. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1594-1598. https://doi.org/10.3889/oamjms.2018.337

Keywords: Vitamin C; Oxidative stress; Strenuous activity; Muscle damage; Creatine kinase; Malondialdehyde; C-reactive protein

***Correspondence:** Marie-Christine R Shakib. Nutrition and Food Science Department, National Research Centre, Dokki, Giza, Egypt. E-mail: chris_shakib@yahoo.com

Received: 02-Aug-2018; **Revised:** 23-Aug-2018; **Accepted:** 24-Aug-2018; **Online first:** 11-Sep-2018

Funding: This study is supported by a grant from the National Research Centre, Egypt

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

Abstract

BACKGROUND: Strenuous non-regular exercise increases reactive oxygen species ROS level leading to an impaired balance between the endogenous antioxidant defence system and the free radicals production. Antioxidants intake can detoxify the peroxides produced during exercise, attenuating the inflammatory responses and therefore may prevent exercise-induced muscle damage.

AIM: The purpose of this study was to determine the role of vitamin C intake in attenuating markers of muscle damage, oxidative stress and inflammatory responses in male adolescents performing the non-regular strenuous exercise.

MATERIAL AND METHODS: Twenty recreationally active male adolescents were assigned to participate in the study. Eligible subjects performed strenuous recreational exercise (2-3 times per week) were randomly divided into two groups: The vitamin C (VC) group that consumed 500 mg of capsulated vitamin C after breakfast for a period of 90 days and the placebo (PL) group that consumed identical capsules in form and aspect that contained 500 mg of maltodextrin for the same period. Aspartate aminotransferase (AST), creatine kinase (CK), lactate dehydrogenase (LDH) were assessed for muscle damage. Malondialdehyde (MDA) was evaluated as a marker of lipid peroxidation. Plasma creatinine, uric acid and urea were determined to monitor kidney function. C-reactive protein, a marker of systemic inflammation was also measured.

RESULTS: In comparison between PL and VC groups, the plasma concentrations of muscle damage markers, oxidative stress markers, kidney function and inflammatory markers showed no significant difference in their baseline values ($P > 0.05$). The plasma concentrations of CK, LDH, MDA, urea, uric acid and CRP were significantly decreased in the VC group ($P < 0.05$) as compared to their values before the intake of vitamin C.

CONCLUSION: The present results support the intake of vitamin C as an antioxidant for attenuating exercise-induced muscle damage, oxidative stress and inflammatory markers in male adolescents performing the strenuous physical activity.

Introduction

A growing amount of evidence indicates that regular and chronic exercises produce physiological adaptations and enhance the endogenous antioxidant system, minimise the oxidative stress and therefore protect the body against adverse effects of oxidative damage that may occur after an acute bout of exercise [1]. However, strenuous non-regular exercise increases reactive oxygen species ROS level leading to an impaired balance between the endogenous antioxidant defence system and the free radicals production [2]. This disturbance in cellular homeostasis induces oxidation of cellular macromolecules such as proteins, lipids and DNA in the contracting muscles, and then lipid peroxidation occurs leading to muscle damage [3]. It has been postulated that the generation of reactive oxygen species occurs mainly by contracting skeletal muscles, inflammatory processes and increased the release of catecholamine [4]. The antioxidant vitamin supplementation effect on detoxifying peroxides produced during exercise has been given a special focus in recent years. It has been suggested that the endogenous antioxidant systems within tissue cells in subjects under strenuous activities are not able to maintain optimal tissue levels of antioxidant vitamins, even if they consume the recommended daily allowances in their diet [5]. Antioxidants supplementation can detoxify the peroxides produced

during exercise as they are capable of scavenging peroxyl radicals and attenuating the inflammatory responses to exercise and may, therefore, prevent exercise-induced muscle damage [6].

The purpose of the present study is to determine the effectiveness of the vitamin C intake in attenuating muscle damage, oxidative stress and inflammatory markers, in male adolescents performing the non-regular strenuous exercise. Our hypothesis is that muscle damage, and oxidative stress is likely to be worse following strenuous exercise in the placebo group. The extent of these changes could be reduced for the group receiving vitamin C.

Materials and Methods

Twenty recreationally active male adolescents, at a mean age of 19 ± 0.5 years, 68.9 ± 7.3kg weight, 178.9 ± 4.6 cm height and body mass index (BMI) of 24.3 ± 0.6, were assigned to participate in the study. Eligible subjects performed the strenuous recreational exercise (2-3 times per week) and considered healthy without a history of the disease or medication use. They reported that they had not engaged in any intense training, including eccentric contractions in the 3 months before the experiment. They completed a medical history, diet and supplementation history, and a physical activity questionnaire to determine eligibility. All subjects were non-vegetarian, non-smokers, nor did they use anti-inflammatory drugs, or antioxidant supplements three months before the study period. Subjects were informed about all procedures and possible risks involved, and they wrote consent to participate in the study at the National Research Centre. The study was carried out according to the Medical Research Ethics Committee, National Research Centre, Cairo, Egypt. All participants were instructed to maintain their normal diet during the study period.

The 20 subjects were randomly divided into two groups: Vitamin C (VC) group (n = 10, age: 19.0 ± 0.2 years, height: 179.6 ± 6.2 cm, weight: 66.7 ± 7.1 kg), and placebo (PL) group (n = 10, age: 19.3 ± 0.5 years, height: 178.6 ± 3.9 cm, weight: 67.3 ± 7.9 kg). The VC group consumed 500 mg of vitamin C packed in the hard gelatinous capsule (C-Retard, slow-release ascorbic acid Hekma Pharma, Egypt) once a day after breakfast for 90 days. The PL group consumed identical capsules in the form and aspect that contained 500mg of maltodextrin for the same period.

Venous blood samples were collected from all subjects at morning in heparinised tubes, at baseline and after 90 days of vitamin C intake after performing strenuous exercise. Blood was centrifuged for 15 minutes at 3000rpm, and plasma was stored at -70°C until assays were conducted. Aspartate aminotransferase (AST) was determined according to the method of Reitman and Frankel [7], Creatine kinase (CK) was determined using the colourimetric method [8], and Lactate dehydrogenase (LDH) was measured using the colourimetric enzymatic assay [9]. Malondialdehyde (MDA) was determined as an indicator of lipid peroxidation by the thiobarbituric acid (TBA) method [10]. Plasma creatinine [11], uric acid [12] and urea [13] were determined to monitor kidney function. C-reactive protein, a marker of systemic inflammation was determined by the method of Saxtad et al., [14].

The data was analysed statistically using Statistical Package for the Social Sciences SPSS software for Windows (SPSS Inc., Chicago, IL, version 17.0) was used for the statistical analysis. Results are expressed as mean ± SEM. The changes in VC and PL groups before and after the intake were tested by using the Student's t-test. *P*-Value < 0.05 indicated a statistically significant difference for all tests.

Results

The changes in plasma markers of muscle damage and oxidative stress of male adolescents in response to vitamin C intake are shown in Table 1. In comparison between PL and VC groups, the plasma concentrations of muscle damage markers, oxidative stress markers, kidney function and inflammatory markers showed no significant difference in their baseline values ($P > 0.05$). No significant change was shown in the plasma concentrations of AST, CK, LDH and plasma MDA in the PL group ($P > 0.05$). In VC group, the plasma concentrations of CK and LDH were significantly decreased by 34.6 % and 16.25% respectively ($P < 0.05$) as compared to their values before the intake of vitamin C. Plasma concentration of AST showed no significant change ($P > 0.05$). A significant decrease in plasma MDA was observed in the VC group by 50.9% as compared to its value before the intake of vitamin C ($P < 0.05$).

Table 1: Indicators of muscle damage and oxidative stress

	Groups							
	PL group				VC group			
	Before-intake	After-intake	% change	P value	Before -intake	After – intake	% change	P value
AST (U/L)	24.2±0.6	23.9±1.3	-1.2	0.858	26.3±0.7	22.5±1.3	-14.4	0.026
LDH (U/L)	204.9±5.2	217.4±12.1	6.1	0.382	202.4±5.6	169.5±9.4*	-16.3	0.013
CK (U/L)	227±5.8	234.8±13.1	3.4	0.611	212.3±11.8	138.8±7.7**	-34.6	0.001
MDA (µ M/L)	5.4±0.1	5.8±0.3	7.4	0.224	5.7±0.1	2.8±0.2**	-50.9	0.001

Values are expressed as Mean + S.E.M, *significantly different within VC group (p< 0.05), ** high significant difference within VC group (p< 0.01). AST = Aspartate transaminase, LDH= Lactate dehydrogenase, CK= Creatine kinase, MDA= Malondialdehyde.

As shown in Table 2, the plasma concentrations of creatinine, urea and uric acid showed no significant difference in their baseline values ($P > 0.05$) in the comparison between PL and

VC groups. In the PL group, no significant difference was shown in the plasma concentrations of creatinine, urea and uric acid ($P > 0.05$). In VC group, the plasma concentrations of urea and uric acid were significantly decreased by 18.5% and 8.8% ($P < 0.05$) respectively as compared to their values before the intake of vitamin C, while plasma concentration of plasma creatinine showed no significant change ($P > 0.05$).

Table 2: Effect of vitamin C intake on kidney function

Groups	Creatinine	Urea	Uric acid
PL group			
Before-intake	1.1 ± 0.0	30.5 ± 0.8	5.3 ± 0.1
After- intake	1.0 ± 0.1	31.6 ± 1.8	5.6 ± 0.1
% change	-9.1	3.6	5.7
P value	0.361	0.573	0.059
VC group			
Before-intake	1.1 ±.0.0	27.5 ± 2.1	5.7 ± 0.2
After- intake	0.9 ± 0.1	22.4 ± 0.9*	5.2 ± 0.1*
% change	-18.2	-18.5	8.8
P value	0.087	0.016	0.013

Values are expressed as Mean + S.E.M, ***** significantly different within the VC group ($p < 0.05$).

As shown in Figure 1, the plasma concentration of CRP showed no significant difference in their baseline values ($P > 0.05$) in the comparison between the PL and VC groups. In the VC group, there was a significant decrease in CRP plasma concentration by 62.6% ($P < 0.05$) as compared to its value before the intake of vitamin C.

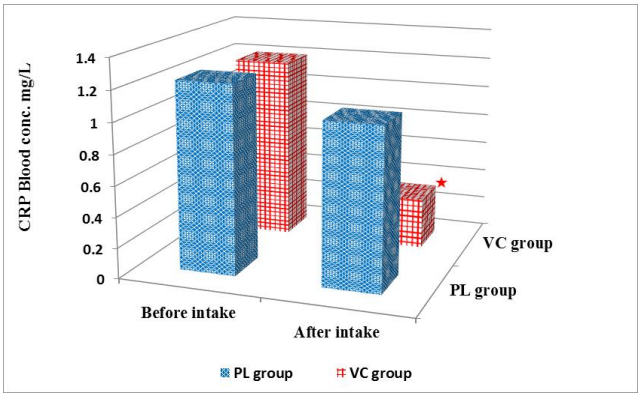

*Figure 1: C-reactive protein concentration in PL group and VC group following strenuous exercise in male adolescents. *Significantly different within VC group ($p < 0.05$)*

Discussion

The purpose of this study was to investigate the effect of vitamin C intake on exercise-induced muscle damage, oxidative stress and inflammatory markers in male adolescents after performing the strenuous physical activity. Initially, a bout of vigorous non-regular exercise results in muscle damage through an inflammatory response mediated by phagocytosis and a respiratory burst of neutrophils leading to the release of ROS [15]. Other studies have shown that ROS are incriminated in the aetiology of muscle damage by causing oxidation of the ion transporting systems [16]. Furthermore, Lipid peroxidation in which free radicals "steal" electrons from the lipids in cell membranes may lead to the release of muscle components such as CK and LDH [17]. Myofibrillar proteins such as CK and LDH are often used as indicators of muscle damage [18] as plasma CK levels alone may not provide a fully accurate reflection of structural damage to muscle cells [19]. Many studies have used plasma malonaldehyde as a measure of oxidative stress caused by exercise. In a previous study, serum MDA has been reported to be increased following a bout of intense exercise [20]. There is a confirming role of exercise in the production of free radicals and subsequent oxidative stress by the significant increase in serum MDA levels about both acute and regular exercise [21].

In the present study, plasma MDA was measured as a marker of lipid peroxidation, as well as the plasma AST, CK and LDH levels were measured as muscle damage markers. Strenuous exercise induced an increase in post-exercise plasma CK, LDH and MDA at baseline values in PL and VC groups, which indicate some degree of myofibre damage [22]. Such results also showed that taking vitamin C reduced the oxidative stress and muscle damage markers through a significant reduction in post-exercise plasma, CK, LDH and MDA in the VC group as it inhibits lipid peroxidation. The potential reason for this phenomenon may be due to the decrease of membrane permeability and reducing the escape of constituents such as CK and LDH through inhibition of lipid peroxidation. This could be due to the antioxidant supplementation [23]. Vitamin C is a potent reducing agent, due to its facility in donating electrons, and has an important antioxidant property [24]. Vitamin C intake significantly blunted the post-exercise high plasma MDA level as it reduces the production of ROS and oxidative stress inflicted by exercise-induced muscle damage. Vitamin C inactivates a variety of reactive species and minimises the damage of body tissues [25].

CRP was measured in blood as an inflammatory marker. In the placebo group, no significant change was shown in post-exercise CRP level, and it was within the normal range. Some studies have shown a slight increase in CRP level after strenuous exercise [26], while in other studies, a decrease in CRP level was observed after prolonged training [27]. Our results are inconsistent with the findings of these previous studies. Differences in serum CRP level after training may be due to the involvement of different mechanisms in the regulation of acute phase responses that may differ from one condition to another [28]. The present study showed that vitamin C intake demonstrated to be efficient in attenuating CRP level. However, in another study of six weeks, vitamin C intake prevented endurance

exercise-induced lipid peroxidation but did not affect inflammatory markers [29].

Furthermore, our study showed that vitamin C intake showed a significant reduction in plasma uric acid levels. A previous study has described mechanisms by which vitamin C reduces serum uric acid SUA and suggested that vitamin C has uricosuric properties, therefore lowering SUA level by inhibiting uric acid synthesis [30]. Vitamin C may act specifically at uric acid reabsorption sites in the apical brush border of the proximal tubule. It is also possible that vitamin C increases the dilatation of afferent arterioles, reduces glomerular microvascular ischemia, leading to an increase in the glomerular filtration rate [31]. Vitamin C as a strong antioxidant decreases free radical-induced damage to the body cells [32] leading to a reduction in the SUA production [33].

In conclusion, the present study revealed that vitamin C intake prevented exercise-induced lipid peroxidation which if uncontrolled, it increases the membrane permeability and may lead to the escape of muscle constituents as CK. Vitamin C intake showed an effect on the systemic inflammatory response by reducing the level of inflammatory marker CRP, and a remarkable reduction in plasma uric acid level. Our findings could, therefore, support the use of antioxidants like vitamin C on attenuating exercise-induced muscle damage markers, oxidative stress and inflammatory markers in male adolescents performing the strenuous physical activity.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1599-1605.
https://doi.org/10.3889/oamjms.2018.237
eISSN: 1857-9655
Clinical Science

Soft Tissue Pathology Detected By Ultrasound Seem To Be Risk Factors for Painful Flare in Osteoarthritic Knee

Douaa M. Mosalem[1], Shothour M. Alghunaim[1], Diaa K. Shehab[2], Ayyoub B. Baqer[3], Aziz K. Alfeeli[4], Mohieldin M. Ahmed[4*]

[1]Physical Medicine and Rehabilitation Departments, Al-Razi Hospital, Kuwait; [2]Mubarak Al-Kabeer Hospital, Jabriya, Kuwait; [3]Farwaniya Hospital, Al Farwaniyah Governorate, Kuwait; [4]Amiri Hospital, Kuwait

Citation: Mosalem DM, Alghunaim SM, Shehab DK, Baqer AB, Alfeeli AK, Ahmed MN. Soft Tissue Pathology Detected By Ultrasound Seem To Be Risk Factors for Painful Flare in Osteoarthritic Knee. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1599-1605. https://doi.org/10.3889/oamjms.2018.237

Keywords: Osteoarthritic knee (OA Knee); Visual Analogue Scale (VAS); Brief Pain Inventory (BPI); Ultrasonography (US)

***Correspondence:** Mohieldin M Ahmed. Amiri Hospital, Kuwait, Kuwait. E-mail: drmohyahmed@yahoo.com

Received: 28-Apr-2018; **Revised:** 23-Jul-2018; **Accepted:** 24-Jul-2018; **Online first:** 18-Sep-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: To our knowledge, the importance of US findings, pain (brief pain inventory (BPI)) and disability in osteoarthritic knee (OA) pain patients remain uncertain.

AIM: The objectives are to evaluate the correlation of US findings, pain (brief pain inventory (BPI)) and disability in OA pain patients.

MATERIALS AND METHODS: Eighty - three patients with OA knee were divided into two groups. The first group was OA as symptomatic knee group and the second group was an asymptomatic control group. The maximum sagittal height of synovial fluid in 12 scans at 0, 30, 60 and 90 degrees flexion knee in 3 major recesses were measured.

RESULTS: There were a significant positive correlation between BPI Pain severity index, or BPI function interference index and a maximum height of effusion at 30-degree flexion angle in a supra-patellar recess in painful symptomatic knees. But, there was a significant negative correlation between BPI Pain severity index, and BPI function interference index and cartilage thickness in painful symptomatic knees.

CONCLUSION: The increase of maximum height of synovial effusion at different angles of knee and decrease of cartilage thickness associated with pain and disability in OA pain patients and are being predictors for pain severity and disability in OA pain patients.

Introduction

To our knowledge, few studies have addressed the relationship between US findings, pain especially brief pain inventory (BPI) and disability in OA pain patients. Thus, the objective is to evaluate the correlation of US findings and pain and disability in OA pain patients.

Osteoarthritis (OA) is one of the most common medical conditions in older adults and 9.6% of men and 18.0% of women aged 60 years have symptomatic OA of the knees [1]. OA is also the most common reason for restricted daily activity with a significant impact on the quality of life among affected people [2].

The importance of soft tissue pathology in pain in knee osteoarthritis remains uncertain. US of the OA knee may be able to visualise inflammation to its full extent and be extremely sensitive in the detection of soft tissue changes in knee OA, including synovial fluid and synovial proliferation. Such abnormalities are correlated with symptomatic flares and have associated prognostic implications [3].

To our knowledge, few studies have addressed the correction of US findings, brief pain inventory (BPI) and disability in OA pain patients. Thus, the objectives were to evaluate the correlation

of US findings, pain and disability in OA pain patients.

Materials and Methods

Eighty-three patients with primary knee osteoarthritis (OA) were divided into two groups according to symptomatic pain knee. The first group (n = 43, 36 females, 5 males and age of 57.37 ± 7.65) was OA with eighty-six symptomatic pain knees (at least 3 months duration) as symptomatic knee group (S) and the second group (n = 40, 33 females, 7 males and age of 53.77 ± 5.22) was OA with eighty asymptomatic Knees group (A) as control group. The brief pain inventory (BPI) and the US of suprapatellar effusion area were done as outcome measures of pain and effusion.

Those patients were chosen from the outpatient clinics, rehabilitation department and diagnosed according to the American College of Rheumatology (ACR) criteria [4]. The European League against Rheumatism (EULAR) recommends that the clinical diagnosis of knee OA should be based on three symptoms (persistent knee pain, limited morning stiffness and reduced function) and three signs (crepitus, restricted movement and bone enlargement). The presence of all these signs and symptoms increases the probability of radiographic knee OA to 99% [5].

Inclusion criteria of our patients included the patients with ages 40-68-year-old and chronic knee pain. The exclusion criteria included patients who had knee surgery, mechanical knee derangement, serious knee pathology (e.g., fractures, tumours, rheumatologic disorders or infective diseases), severe cardiopulmonary disease, pregnant, and a pacemaker or metal implants.

All patients gave their informed verbal voluntary consent to use the recorded data in their follow up sheets according to the protocol approved by the local ethics committee and by the ethical standards of the Helsinki Declaration. This randomised controlled clinical trial began on January 2016 to April 2017.

Outcome measures of knee pain and effusion included visual analogue scale (VAS) and brief pain inventory (BPI) as measured pain and functional disability. Visual analogue scale (VAS) is a measurement instrument that tries to measure pain intensity on a scale from zero (no pain) to 100 (most severe pain). For example, the amount of pain that a patient feels ranges across a continuum from none to an extreme amount of pain [6].

The brief pain inventory (BPI) was originally developed to evaluate cancer pain, but it has been shown to be a valid and reliable instrument for chronic non-cancer pain. The BPI consisted of 11 items, which was designed to evaluate the pain intensity (four items) and pain interference with function (seven items) scores [7]. The BPI items consisted of pain severity (four items) and pain interference with function (seven items) scores. BPI pain severity index consists of four items to measure pain intensity and range of pain severity index from 0-40 (0 = no pain, 10 = pain as bad you can imagine) of 4 items with total 40 point scale. BPI Function interference index consists of the sum of seven items to measure the level of function interference caused by pain (general activity, mood, walking ability, normal work, relations with other persons, sleep, and enjoyment of life) using 0 (no interference) to 10 (complete interference) rating scales and total range of function interference index of 7 items about 70 point scale [8].

Sonographers used the following equipment: 12-MHz, portable ultrasound machine GR LOGIQ, General Electric. The company, USA and Ultrasound scans of the knees were obtained by a linear transducer. All ultrasonographic evaluation of effusion areas were evaluated by an examiner who has good experience in musculoskeletal ultrasonographic evaluation. Grey-scale ultrasound (GSUS) and Color Doppler ultrasound (CDUS) examinations of each knee were performed at the following 3 major suprapatellar pouch recesses: midline suprapatellar, medial parapatellar, and lateral parapatellar recesses. Examination of the midline suprapatellar recess was carried out in the sagittal plane at midline, while that of the medial parapatellar and lateral parapatellar recess was carried out in the midpatellar transverse plane 90° medial and lateral from midline, respectively.

The ultrasound scans of the 3 recesses were performed in the 4 knee positions at 0°, 30°, 60°, and 90° of flexion of the knee joint, yielding a total number of 12 scans for each knee joint. The degree of flexion was established with the use of a standard goniometer. The knee was unsupported by external support (e.g., foam block) during the procedure. For each examination generous amounts of gel were applied to the knee and each sonographer took care in applying only minimal pressure to the transducer during the examination in order not to displace the fluid collection [9]. Ultrasonographers performed a complete ultrasonographic examination of each knee by the EULAR guidelines [10]. The fluid collection was defined as an anechoic or hypoechoic area that is displaceable and does not exhibit Doppler signal according to the Outcome Measures in Rheumatology definition of synovial fluid [11].

An ultrasound examination at the anterior part of the knee was done for each recess in every examined angle of flexion. The greatest effusion height (mm) was calculated automatically by using a sagittal scan as a quantitative measurement of in the supra-patellar, lateral parapatellar, and medial parapatellar recesses by ultrasonography [12]. Moreover, an ultrasound examination of the knee was done to look for hyperemia and thickening of the synovial membrane; and the medial and lateral joint lines to look for osteophytes and meniscal protrusion

of knees. Also, an ultrasound examination of cartilage thickness was done in complete flexion position of knees [12] [13].

Study data were analysed using the SPSS (Statistical Package from the Social Science Program) (version15.0) (SPSS Inc., Chicago, IL, USA). The normality of the population was done during statistical analysis. The Student's t-test indicates the magnitudes of the differences of means ± SD and therefore the magnitude of the observation and to assess the difference between patients and control subjects and considered P value of < 0.05 statistically as significant. Quantitative data were presented as mean (± SD). Correlation between variables was done, and the Pearson correlation coefficient was calculated. All tests were 2-tailed and considered statistically significant at P < 0.05.

Results

Demographic and clinical, findings in symptomatic and asymptomatic knees in patients with primary OA are shown in Table 1. A significant difference of mean (± SD) of VAS, BPI Pain severity index, and BPI function interference index in symptomatic as compared to results in asymptomatic knees in patients with primary OA knee.

Table 1: Demographic, clinical findings in symptomatic and asymptomatic knees in patients with primary OA

Mean ± SD	Symptomatic OA knees (n = 43)	Asymptomatic OA knees (n = 40)	p-value
Age, years	57.37 ± 7.65	53.77 ± 5.22	N.S.
Sex (female./male)	30/13	28/12	N.S.
Body Mass Index (BMI) (kg/m^2)	21.93 ± 2.19	22.39 ± 2.48	N.S.
Disease duration, years	11.3 ± 04	10.55 ± 1.83	N.S.
VAS of pain intensity - point scale.	8.20 ± 1.58	-	-
[1]BPI Pain severity index	-	-	-
– Pain at its worst	8.02 ± 1.45	-	-
– Pain at its least	4.32 ± 1.78	-	-
– Pain on average	5.72 ± 1.51	-	-
– Pain right now	3.95 ± 1.70	-	-
[1]BPI Pain severity index – total point scale.	21.65 ± 3.95	-	-
[2]BPI Function interference index	-	-	-
– General Activity interference	5.76 ± 1.28	-	-
– Mood interference	2.56 ± 1.63	-	-
– Sleep interference	2.97 ± 0.53	-	-
– Enjoyment of Life interference	2.93 ± 0.86	-	-
– Ability to walk	4.95 ± 1.23	-	-
– Ability to work	5.11 ± 0.98	-	-
– People interference	4.18 ± 1.33	-	-
[2]BPI Function interference index- total point scale.	-	-	-
Knee pain involved	28.11 ± 6.78	-	-
– Right knee (n, %)	-	-	
– Left knee (n, %)	41 (47.7%)	-	-
– bilateral knee pain	35 (52.2%)	-	-
– Anti-inflammatory medication(n, %)	10 (52.2%)	-	-
– intra-articular injections of hyaluronic acid (n, %)	43 (100%)	-	-
– Aspiration (n, %)	20 (46.5%)	-	-
	16 (37.2%)	-	-

N.B: OA, osteoarthritis; VAS, Visual Analog Scale; BPI, Brief Pain Inventory; US, Ultrasound. N.S.: not significant, P > 0.05; 1, BPI Pain severity index with range = 0-40 (0 = no pain, 10 = pain as bad you can image) of 4 items and total 40 point scale; 2, BPI Function interference index with range = 0-70 (0 = does not interfere, 10 = interferes completely) of 7 items and total 70 point scale.

Moreover, ultrasonographic findings of maximum effusion height of the three recesses in the four knee positions at 0°, 30°, 60°, and 90° of flexion in symptomatic and asymptomatic OA knee patients were shown in Table 2.

Table 2: Ultrasonographic findings of maximum effusion height of the three recesses in the four knee positions at 0°, 30°, 60°, and 90° of flexion in symptomatic and asymptomatic OA knee patients

Mean ± SD	Symptomatic OA knees (n = 43)	Asymptomatic OA knees (n = 40)	P-value
US maximum effusion height at 0 degree extension , (mean ±SD), mm	-	-	-
– at the supra-patellar recess,	-	-	-
– at the medial para-patellar recess	3.00 ± 0.44	0.68 ± 0.13	p<0.001
– at the lateral para-patellar recess	2.87 ± 0.31	0.56 ± 0.06	p<0.001
US maximum effusion height at 30 degree flexion, (mean ± SD), mm	2.35 ± 0.60	0.30 ± 0.07	p<0.001
– at the supra-patellar recess,	-	-	-
– at the medial para-patellar recess	-	-	-
– at the lateral para-patellar recess	5.02 ± 0.90	0.70 ± 0.01	p<0.001
US maximum effusion height at 60 degree flexion , (mean ± SD), mm	2.97 ± 0.38	0.50 ± 0.02	p<0.001
– at the supra-patellar recess,	3.10 ± 0.59	0.46 ± 0.03	p<0.001
– at the medial para-patellar recess	-	-	-
– at the lateral para-patellar recess	-	-	-
US maximum effusion height at 90 degree flexion , (mean ± SD), mm	-	-	-
– at the supra-patellar recess,	-	-	-
– at the medial para-patellar recess	3.17 ± 0.51	0.68 ± 0.01	p<0.001
– at the lateral para-patellar recess	0.94 ± 0.65	0.63 ± 0.03	p>0.05.
	2.77 ± 0.42	0.35 ± 0.01	p<0.001
	-	-	-
	-	-	-
	2.03 ± 0.33	0.36 ± 0.001	p<0.001
	1.30 ± 0.89	0.21 ± 0.002	p<0.001
	0.40 ± 0.52	0.20 ± 0.001	p>0.05.

N.B: OA, osteoarthritis; US, Ultrasound; **P < 0.001 = highly significant; *P < 0.05 = significant; N.S.= not significant, P > 0.05.

Also, ultrasonographic findings of bone and soft tissue pathology in symptomatic and asymptomatic OA knee patients were shown in Table 3 and Figures (1-7).

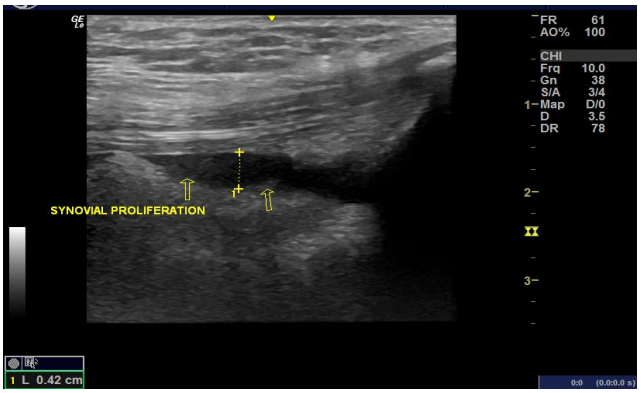

Figure 1: Representative images of synovial hypertrophy and a maximum height of suprapatellar effusion of 42 mm^2

We found increase of mean (± SD) of maximum height of synovial effusion at different angles of flexion in 3 recesses of knee and decrease of mean (± SD) of cartilage thickness in complete flexion position in symptomatic as compared to results in asymptomatic knees in patients with primary OA were shown in Tables 2 and 3.

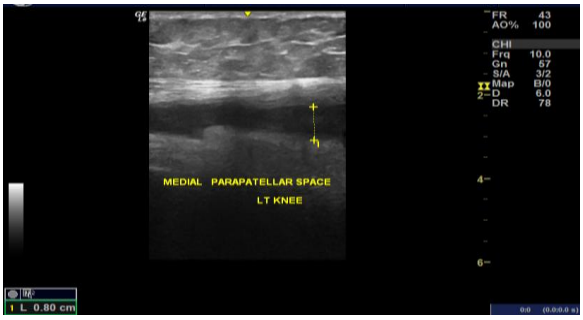

Figure 2: Representative images of the maximum height of medial parapatellar effusion of 80 mm²

Moreover, in Table 4, there was a significant positive correlation between VAS, BPI Pain severity index, or BPI function interference index and maximum height of effusion at 30 degrees flexion angle in supra-patellar recess in symptomatic OK knees (r=0.822, p<0.001, r=0.733, p<0.05 and r=0.820, p<0.05 and) sequentially.

Table 3: Ultrasonographic findings of bone and soft tissue pathology in symptomatic and asymptomatic OA knee patients

Mean +SD	Symptomatic OA knees (n= 43)	Asymptomatic OA knees (n= 40)	p-value
US maximum effusion height at 30 degree flexion, (mean ±SD), mm	- -	- -	- -
-at the supra-patellar recess,	3.68±0.71	0.80±0.13↓	p <0.001
-at the medial para-patellar recess	3.00±0.38 2.87±0.37	0.75±0.16↓ 0.38±0.02	p <0.001 p <0.001
-at the lateral para-patellar recess			
US cartilage thickness, (mean±SD), mm	- -	- -	- -
-at medial epicondyle	1.51±0.24	2.93±0.09	p<0.001
-at intercondylar notch	1.42±0.30	2.76±0.12	p>0.05
-at lateral epicondyle	1.55±0.21	1.31±0.09	p>0.05
-US suprapatellar effusion (n, %)	28(65.1%) 13(30.2%)	5(12.5%) 7(17.5%)	p<0.001 p<0.001
-US synovial hypertrophy(>2 m),(n, %)	- 9(20.9.9%)	- 3(7.5%)	- p>0.05.
-Backer cyst (n, %)			
-US medial meniscal protrusion (>3 mm), n (%)	26(60.5%) -	11(27.5%) -	p<0.001 -
-US lateral meniscal protrusion (>3 mm), n (%)	7(16.3. %) -	7(17.5%) -	p>0.05 -
-US osteophyte (>3 mm), n (%)	8(18.6%)	3(7.5%)	p>0.05.

N.B: OA, osteoarthritis; US, Ultrasound. **p<0.001=highly significant; *p<0.05=significant; N.S.=not significant, p>0.05.

But, there was a significant negative correlation between VAS, BPI Pain severity index, or BPI function interference index and cartilage thickness at medial epicondyle in complete flexion position in symptomatic OA knees (r = 0.691, P < 0.05, r = 0.809, P < 0.05 and r = 0.715, P < 0.05) sequentially.

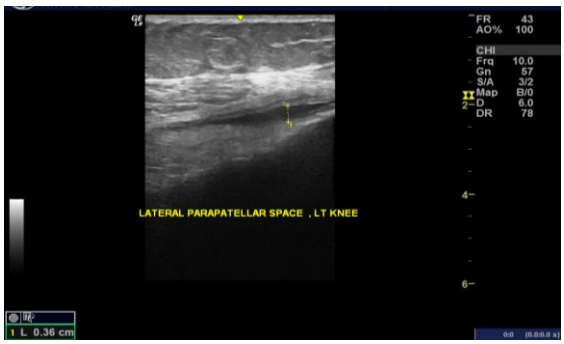

Figure 3: Representative images of the maximum height of lateral parapatellar effusion of 36 mm²

Figure 4: Longitudinal view of irregular cartilage thickness of the complete flexed knee joint

Discussion

Correlation of US findings and pain especially brief pain inventory (BPI) and disability in OA pain patients remains uncertain. Ultrasonographic (US) has become the first-line imaging technique chosen by rheumatologists to obtain real-time imaging information in patients with painful joints [14]. Also, few studies have addressed the relationship between the US of suprapatellar effusion area and knee pain. But, to our knowledge, the current study is the first to report on the association between brief pain inventory (BPI) and US findings in the osteoarthritic knee.

Table 4: Linear regression correlations (r-) between Soft tissue pathology detected by ultrasound and VAS as well as BPI pain severity index and IBP function interfere index in painful symptomatic OA knees

Variables, (mean ± SD)	VAS	BPI pain severity index	IBP function interfere index
Maximum Effusion height detected by ultrasound			
Maximum Effusion height at the supra-patellar recess at 30 degree of flexion , mm	r=0.822** p<0.001	r=0.737** p<0.001	r=0. .820** p<0.001
Effusion height at the medial para-patellar Recess at 30 degree of flexion , mm	r =0.270 p>0.05	r=0. .325 p>0.05	r=0.281 p>0.05
Effusion height at the lateral para-patellar recess at 30 degree of flexion, mm	r=0.323 p>0.05	r=0. .271 p>0.05	r=0.210 p>0.05
Cartilage thickness incomplete knee flexion detected by ultrasound			
Cartilage thickness at Medial epicondyle, mm	r=-0.691** p<0.001	r=-0.806** p<0.001	r=-0.715** p<0.001
Cartilage thickness at the intercondylar notch, mm	r=-0.555 p>0.05	r=-0.707 p>0.05	r=-0.561 p>0.05
Cartilage thickness at lateral epicondyle, mm	r=-0.729 p>0.05	r=-0.817 p>0.05	r=- 0775 p>0.05

N.B: US, Ultrasound; OA, osteoarthritis; VAS, visual analog scale; BPI, brief pain inventory; **P<0.001=highly significant; *p<0.05=significant; N.S.=not significant p>0.05.

Our results showed a significant difference of mean (± SD) of VAS of pain intensity, BPI Pain severity index, and BPI function interference index in symptomatic as compared to results in asymptomatic knees in patients with primary OA. Moreover, there was a significant positive correlation between VAS of

pain intensity, BPI Pain severity index, or BPI function interference index with a maximum height of effusion at 30-degree flexion angle in a supra-patellar recess in painful symptomatic OK knees. But, there was a significant negative correlation between VAS of pain intensity, BPI Pain severity index, or BPI function interference index with cartilage thickness at medial epicondyle of the femur in complete flexion position in painful symptomatic OA.

Figure 5: Longitudinal view of a medial aspect of the right knee joint. Meniscal protrusion present on the medial femoral condyle and tibia of right knee

Our findings are supported other publication; some authors reported that approximately 50% of patients with Inflammation in OA of the knee during flares of knee pain show US evidence of synovitis and/ or effusion as a possible explanation for their pain exacerbation [15].

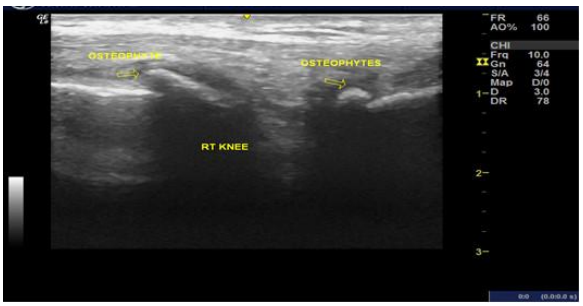

Figure 6: Longitudinal view of a medial aspect of the knee joint. Small osteophytes present on the medial femoral condyle and tibia

Moreover, de Miguel- Mendieta et al. showed that patients with OA of knee and recent onset pain had a higher prevalence of Baker's cyst and joint effusion, as compared with painless OA knees [16].

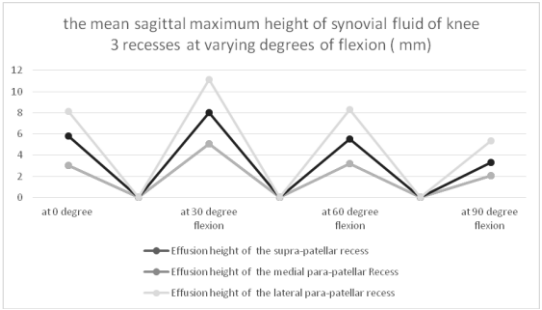

Figure 7: Plot diagram shows the Sonographic evaluation of the mean sagittal maximum height of synovial fluid of knee 3 recesses at varying degrees of flexion, using the extended position (0°) as a reference. Dotted lines represent confidence intervals for each of the corresponding recesses. For each recess, corresponding graphs are marked with matching colours

Some studies reported that ultrasound with knee effusion has a positive correlation with pain score upon walking and stair climbing and this suggested that biomechanical derangement is an important aspect in OA knee pain.

Moreover, the presence of suprapatellar synovitis had higher pain score on sitting, and this suggested that Synovitis was considered to be an important predictor of pain [17]. This could be explained by a previous study conducted by the author that there exist two types of pain among OA knee patients: a mechanical pain and an inflammatory pain. The former, being biomechanical, is associated with joint movements such as walking and stair claiming whereas the latter is caused by flares of joint inflammation [17].

In the OA group, only suprapatellar effusion and medial compartment synovitis were significantly associated with knee pain. Visual analogue pain scale (VAS) scores on motion were positively linearly associated with suprapatellar effusion and medial compartment synovitis [18]. Some studies using MRI showed only moderate correlation/association between effusion/synovitis and pain [19]. Esen et al. correlated the inflammatory episodes in knee OA with suprapatellar effusion [20].

Similar findings are found in others studies. Knee effusion among OA knee has been shown to affect knee mechanics and muscle activity during gait in knee osteoarthritis and therefore can be a cause of the mechanical pain by itself. This suggests that the knee effusion among relates more to mechanical rather than inflammatory pain [21]. Moreover, Knee synovitis is accompanied by knee pain and cartilage destruction, and it induces synovial hypertrophy and the development of effusion in the joint cavity [22]. Several previous studies that used MRI and ultrasonography have reported that knee effusion worsens symptoms and pain [23].

In contrast to these findings, some authors have been demonstrated that imaging findings do not always correlate pain in OA patients and this suggests that The OA pain is multifactorial and the mechanism of its appearance is not completely understood [24] [25]. Other authors found no correlations between pain (VAS and WOMAC score) and joint effusion [26]. Some studies using MRI showed no correlation/association between effusion/synovitis and pain [27]. This finding is also consistent with the EULAR study, which showed no correlation between US inflammatory signs and pain intensity during physical activity [28]. This suggests that the psychological factors can interfere with pain which probably explains the differences with other studies. In contrast, neither US synovitis features nor other US features were associated with knee pain in knees

without OA. The results reflect the importance of synovitis in OA knee pain and the multifactorial origins of pain [29].

The mechanism of pain in OA knee is not well understood. Previous research has shown knee pain in OA to be multifactorial causes [30]. Inflammatory, mechanical, structural, bone-related factors, neurological and psychological factors play a role in the process that results in painful knee OA [31].

Some limitations of our study should be mentioned. One of the major limitations of our study is a small number of participants. The number of participants with more severe OA was excluded, and we studied both knees of one patient as an independent sample. Secondly, an important limitation is the lack of comparison of the US findings with MRI findings as we did not take into consideration the presence of bone marrow lesions as an important source of pain in OA knee.

In conclusion, the increase of maximum height of synovial effusion detected by ultrasound at different angles of flexion in 3 recesses of knee and decrease cartilage thickness incomplete knee flexion detected by ultrasound associated with pain and disability in OA pain patients and is being predictors for pain severity and disability in OA knee.

Acknowledgements

The authors are grateful to our department for supporting the US and for providing statistical support for the study.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1606-1610.
https://doi.org/10.3889/oamjms.2018.334
eISSN: 1857-9655
Clinical Science

Preoperative Oral Pregabalin Reduces Acute Pain after Thoracotomy

Hossein Sattari[1], Morteza Hashemian[1*], Mohammad Reza Lashkarizadeh[2], Hamid Jalalifard[3]

[1]Department of Anesthesiology and Pain Medicine, Kerman University of Medical Sciences, Iran; [2]Department of Thoracic surgery, Afzalipoor Hospital, Kerman University of Medical Sciences, Iran; [3]Department of Anesthesia, Afzalipoor Hospital, Kerman University of Medical Sciences, Iran

Citation: Sattari H, Hashemian M, Lashkarizadeh MR. Preoperative Oral Pregabalin Reduces Acute Pain after Thoracotomy. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1606-1610. https://doi.org/10.3889/oamjms.2018.334

Keywords: Pregabalin; Clinical trial; Thoracotomy; VAS; Post-operative pain

***Correspondence:** Morteza Hashemian. Department of Anesthesiology and Pain Medicine, Kerman University of Medical Sciences, Iran. Mobile: +989121342757. E-mail: moetezahashemian@kmu.ac.ir

Received: 13-Aug-2018; **Revised:** 29-Aug-2018; **Accepted:** 30-Aug-2018; **Online first:** 18-Sep-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Nowadays pain control is one of the most important challenges for physicians, surgeons and anesthesiologists. New drugs and procedures to control pain have always been a major topic for researches.

AIM: In this study, we evaluated the effects of preoperative pregabalin administration on relieving postoperative pain after thoracotomy surgery.

MATERIALS AND METHODS: This study is a randomised, double-blind clinical trial, performed on 60 patients who underwent thoracotomy at Afzalipour hospital in Kerman, Iran. They were divided into case and control groups. Two hours before surgery an oral capsule of 300 mg pregabalin or placebo was given to patients. All patients similarly underwent general anaesthesia. Pain, nausea and vomiting were evaluated based on the visual analogue scale (VAS) and frequency. This study was verified and obtained the ethics committee code of K/92/489 from Kerman University of Medical Sciences.

RESULTS: The average age of the pregabalin group was 39.7 ± 5.8 years and the control group 41.3 ± 6.1 years. The average pain score after regaining consciousness was 6.1 ± 0.2 in the case group and 7.9 ± 0.1 in the control group, and there was a significant difference between the 2 groups (p-value = 0.002). In the control group, 2 patients and the intervention group 3 patients, experienced nausea and vomiting. There was a significant difference between the overall average pethidine consumption and the average visual analogue scale in both groups.

CONCLUSION: Pregabalin administration before thoracotomy is effective to reduce postoperative pain in patients. More research is needed to determine the optimal dose of pregabalin for preoperative administration.

Introduction

Postoperative pain is a problem that can cause various complications if it is not controlled efficiently. Proper postoperative pain control is of great importance in preventing complications such as; tachycardia and hypertension, myocardial ischemia, decreased alveolar ventilation and poor wound healing [1].

Thoracotomy procedure provides access to intra-thoracic organs (heart, lungs, oesophagus, thoracic aortic), is associated with severe postoperative pain which is considered to be excruciating. Besides pain, it has adverse physiological and pathological effects on surgery and disease outcome and prognosis. Postoperative pain causes patients to become less mobile and try to relieve their pain by taking shallow breaths and having fewer chest movements. As a result, this reduction can cause hypoxia and pulmonary dysfunction that can lengthen wound healing. On the other hand, the patient's immobility can cause atelectasis and predispose that can lead to infection and respiratory failure [2]. This pain can cause an increase in neuroendocrine and sympathetic tone systems activity, immunosuppression, hyper-coagulation; increased catabolism, restricted movement, breathing problems, and delay in getting out of bed. Consequently opioids are usually recommended to

suppress this pain. However, opioids have side-effects like respiratory suppression, sedation, gastrointestinal symptoms, and urinary retention. Nowadays attempts have been made to use alternative substances for postoperative pain reduction [3] [4]. It seems that in many cases pain is not controlled efficiently, considering the necessity of pain control especially when it is too severe [5]. Therefore, the need to assess various methods which can reduce postoperative pain is evident. By searching in the academic literature, pregabalin seems to have postoperative pain controlling and preventive properties, and this could reduce the need to administer opioids after surgery [6].

Pregabalin is a drug which binds to the alpha-2-delta subunit of presynaptic calcium channels and prevents calcium from entering. This blocks the release of excitatory neurotransmitters like glutamate, noradrenaline, serotonin, dopamine, and substance p. The central sensitisation syndrome in dorsal horn neurons and centres with high-density synaptic connections such as the amygdala, neocortex and hippocampus is responsible for the increase in postoperative pain. Increase production of alpha-2-delta subunits probably plays a role in central sensitisation. It seems that pregabalin reduces pain by reducing this syndrome [7] [8].

The studies mentioned above show that other researchers have conducted similar studies on the effects of pregabalin as a postoperative pain reliever such as limbs and abdominal surgeries. Since thoracotomy is one of the most common surgeries in medical fields and has one of the most excruciating postoperative pains, the necessity of pain control and reducing opioids consumption to reduce side-effects after this surgery is quite evident. However, no study was conducted on this type of surgery, hence; we decided to study the effects of preoperative pregabalin administration on relieving postoperative pain after thoracotomy operation.

Materials and Methods

This research is an interventional clinical trial study. After getting approval from the ethics committee and obtaining a written informed consent, 60 male and female patients' between the age of 20 to 30 years old with ASA class I and II who were candidates for thoracotomy surgery at Afzalipour Hospital affiliated to Kerman University of Medical Sciences were selected. It is worth stating that the same surgical procedure (Lobectomy) through thoracotomy was performed for all patients.

The sample size was calculated based on results from similar studies and using the same sample size formula with type 1 error (α) of 0.05 for 30 patients in each group with the total of 60 patients.

Criteria for exclusion was; pregnancy, history of allergy to pregabalin, history of treatment with pregabalin or gabapentin, history of chronic pain syndromes and consuming painkillers, addiction to alcohol or drugs, allergy to pethidine, psychiatric drugs consumption, surgery lasting more than 4 hours, uncontrolled blood pressure, history of convulsion and patients who had preoperative pain based on the visual analogue scale (VAS).

Patients were randomly divided into two groups of intervention and control (the first one receiving pregabalin and the second one placebo). Neither the patients nor the drug administers and the person in charge of recording pain scores based on the visual analogue scale (VAS), knew which type of drug was being administered. 300 mg of oral pregabalin was administered 2 hours before surgery as a premedication for pain relief. The control group received placebo (a similar looking capsule without any active pharmaceutical ingredients). VAS was explained to everyone before anaesthesia.

Method of anaesthesia was similar for all patients. First, 0.05 mg/kg midazolam and 3 μg/kg fentanyl as premedication 3 min before induction and then 5mg/kg thiopental and 0.5 mg/kg atracurium and 5 mg/kg sodium thiopental were injected intravenously and then patients were intubated with double lumen tubes. Anaesthesia was maintained with 100% O_2 and 1/2% (one MAC) isoflurane. During surgery, 0.2 mg/kg atracurium injections were repeated once every half an hour and 50 μg fentanyl injections were repeated once an hour to keep blood pressure and heart rate between 20% standard limits. After surgery when they were transferred to recovery, the patients' experienced pain scores were recorded using VAS every 15 minutes during the first hour after regaining consciousness and every four hours in the first 24 hours after being transferred to the ward.

Twenty-five mg of opioids (pethidine) were administered intravenously in case of having a higher pain score of 3 or more.

All information including the patients' demographic information, pain scores at different intervals and the amount of administered opioids were recorded. The obtained data were analysed with SPSS software version 21 using descriptive statistics indices. To determine the relationship between age, height, weight, amount of pethidine consumption, and experienced postoperative pain levels (VAS) in the two groups, a t-test was used.

In this study, in addition to collecting a written informed consent from the participants, two phone numbers were given to them to call the project executive and the department of research and technology of Karman's University of Medical Sciences to ask any questions or to report any complaint. All ethical principles for medical research in

the 2009 declaration of Helsinki were observed. At the same time if any patient was unwilling to participate, they were assured that this would not affect their treatment process.

Results

This study was conducted on 60 patients who were admitted to Afzalipour academic medical centre for thoracotomy surgery. Each group consisted of 30 patients (Figure 1).

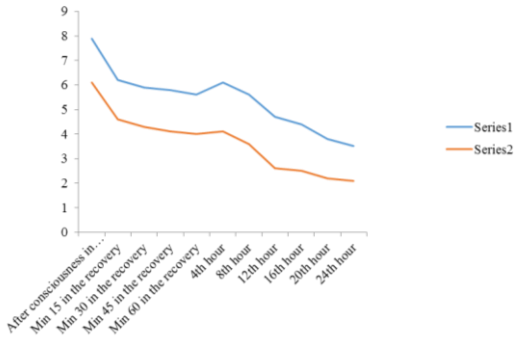

Figure 1: Pain level changes based on VAS

Table 1 shows each group's demographic characteristics. The patients' age, height and weight variables were evaluated, and based on these demographic characteristics; there were no statistically significant differences between the two groups (P > 0.05).

Table 1: Demographic characteristics of the groups

	Pregabalin	Control	P-value
Age (year)	39.7 ± 5.8	41.3 ± 6.1	0.08
Weight (kg)	72.4 ± 10.2	74.6 ± 14	0.2
Gender	17male/13female	14male/16female	0.26
Height (cm)	165.1 ± 9.4	170.4 ± 12.9	0.09

Evaluating pain VAS scores in the recovery room (after regaining consciousness), average pain scores were 7.9 ± 0.1 for the control group and 6.1 ± 0.2 for the pregabalin group, which showed a statistically significant relationship between the two groups (P value = 0.002). Table 2 presents the data. Based on this chart, pain score was significantly different between the two groups during the first 15 minutes in the recovery room, at which time some patients received pethidine (patients with a pain score of 3 or higher). During the next 24 hours, pain scores were generally on a diminishing course for both groups. In comparison, pain scores were lower in the pregabalin group during each evaluation times, and a significant difference was observed between the two groups.

Table 2: Comparing pain VAS scores at all evaluation times

	Control	Pregabalin	P-value
After consciousness in the recovery	0.1 ± 7.9	6.1 ± 0.2	0.002
Min 15 in the recovery	6.2 ± 0.3	4.6 ± 0.4	0.01
Min 30 in the recovery	5.9 ± 0.2	4.3 ± 0.3	0.01
Min 45 in the recovery	5.8 ± 0.2	4.1 ± 0.2	0.01
Min 60 in the recovery	5.6 ± 0.3	4.0 ± 0.2	0.01
4^{th} hour	6.1 ± 0.1	4.1 ± 0.4	0.002
8^{th} hour	5.6 ± 0.1	3.6 ± 0.3	0.001
12^{th} hour	4.7 ± 0.2	0.1 ± 2.6	0.001
16^{th} hour	4.4 ± 0.1	2.5 ± 0.1	0.001
20^{th} hour	3.8 ± 0.4	2.2 ± 0.2	0.001
24^{th} hour	3.5 ± 0.3	2.1 ± 0.1	0.003

Average pethidine consumption changes during the first 24 hours of evaluation are presented in Table 3. All in all, there was a significant difference, considering pethidine consumption between the two groups during evaluation times.

Table 3: Average pethidine consumption (mg) changes in the 2 groups during evaluation hours

	control	pregabalin	p-value
First 15 minutes in the recovery	43.3±2.1	37.5±2.3	0.02
4^{th} hour	33.3±2.2	26.6±1.2	0.001
8^{th} hour	23.3±1.1	15.00±2.2	0.0001
12^{th} hour	21.6±1.6	5.8±1.9	0.0001
16^{th} hour	17.5±2.1	4.1±1.7	0.001
20^{th} hour	15.0±2.2	3.3±1.5	0.001
24^{th} hour	12.5±1.7	2.5±1.3	0.001

These changes were in a way that average pethidine consumption levels were higher in the control group while on average the pregabalin group received less pethidine. Also, Figure 2 shows that pethidine consumption was decreasing more dramatically in the intervention group.

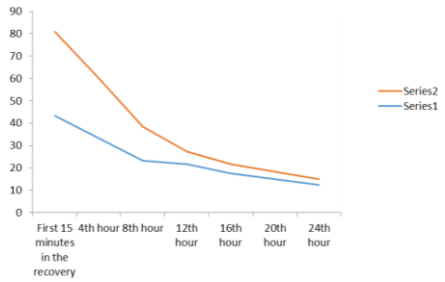

Figure 2: Average pethidine consumption (mg) changes in the 2 groups

Considering the side-effects of pregabalin which was expected based on the drug's pharmacodynamics, our evaluations showed that only three cases of nausea and vomiting were reported in the pregabalin group, whereas only two cases were reported in the control group. Hence, this was not statistically significant. This showed that these symptoms were probably caused by anaesthetics or had other causes. In our other evaluations in the first 24 hours, no other side-effects than the two were observed and all of the patients responded well to supportive treatment and made a full recovery. Of course, pregabalin's side-effects may occur after 24 hours or in the long term, which was not evaluated in this study.

Discussion

In this study, observation showed that pregabalin significantly lowers postoperative pain VAS scores. It also lowers postoperative pethidine consumption levels. The Akhavan Akbari et al., the study showed that administering 150 mg of pregabalin before orthopaedic surgery can cause better pain control and by decreasing pethidine consumption, it can reduce the drug's side-effects [9].

The Entezary et al., a study on preemptive effects of pregabalin vs placebo on acute pain after abdominal hysterectomy showed that a 300 mg administration of pregabalin before surgery could reduce postoperative pain and malaise and also reduces the need for opioids. However, side-effects like dizziness increased in the pregabalin group [10].

The Imani et al., study on the effects of a single dose of pregabalin before surgery on postoperative pain after minor surgery revealed that even a 150 mg administration of oral pregabalin before minor surgeries could reduce pain score and opioids consumption in the first 24 hours after surgery. It is well worth mentioning that this amount of pregabalin had no side-effects [11].

The Imani et al., study on the effects of adding oral pregabalin to patient-controlled intravenous analgesia (PCIA) on pain levels after orthopaedic surgery showed that oral consumption of 300 mg pregabalin during the day after surgery could reduce pain score and opioids consumption in the next 48 hours after operation [12].

In their study titled "Evaluation of a single preoperative dose of pregabalin for attenuation of postoperative pain after laparoscopic cholecystectomy", Agarwal et al., concluded that a single 150 mg preoperative dose of pregabalin significantly reduced pain levels and opioids consumption after laparoscopic cholecystectomy. Also, the side-effects were similar to the control group [13].

Experimental models of neuropathic and inflammatory pains have shown that aminobutyric acid analogues such as gabapentin and pregabalin have analgesic and anti-nociceptive components [14]. It is believed that hypersensitivity of central nerves may lead to a more severe postoperative pain. Preoperative administration of gabapentin, before inflammatory trauma or surgical stimulation, might reduce central nerves hypersensitivity [15].

Compared to gabapentin, pregabalin is a lipid-soluble alternative and can cross the blood-brain barrier, and has better pharmacokinetics and less drug interaction due to a lack of hepatic metabolism.

In the present study, the occurrence of postoperative nausea and vomiting were evaluated. Results showed that there were similar minor side-effects in both groups. In separate studies, side-effects amongst patients were different. In a study on patients who had undergone dental surgery [16] and gynaecological surgery [17], symptoms like vomiting, nausea and stomachaches were observed. However, in studies on patients who had undergone hysterectomy [18], laparoscopic cholecystectomy [19], and orthopedic surgeries [20], no side-effects were observed [21] [22]. In this study, pain levels were evaluated based on the visual analogue scale (VAS) and the observation was that this drug can significantly lower the average pain score. Most studies [19] [20] [21] [22] have achieved similar results. For example, in other studies pregabalin, consumption reduced postoperative pain, while in a minor gynecological surgeries [17]; 150 mg preoperative administration of pregabalin had no pain-relieving effects, which might have been due to its low dosage.

One property of this drug that was observed in many studies is the reduction of postoperative opioids and analgesics consumption levels in the groups receiving pregabalin. These include studies which showed that pregabalin significantly lowers postoperative opioids consumption levels and also reduces opioids side-effects like vomiting. Those studies also showed that pregabalin reduces opioids consumption in patients with acute neuropathic pain. Another study [23] [24] showed that pregabalin reduces opioids consumption after hip arthroplasty.

Considering the effects of pregabalin, we suggest further studies to be conducted on patients in various age groups and bigger sample size. We also suggest that types of surgery, patients' BMI and the pregabalin dosage to be considered as variables. Finally, the effects of this drug in major surgeries should be studied based to see if there are any interfering factors. Finally, more research is needed to determine the optimal dose of pregabalin for preoperative administration.

In conclusion, preoperative administration of pregabalin can yield better pain control, and since this drug reduces the need for analgesics consumption, it can reduce hospital costs and adverse drug reactions.

Ethics Committee Approval

Ethics committee approval was received for this study from the ethics committee of Kerman University of Medical Sciences, Iran. Written informed consent was obtained from patients who participated in this study.

Author Contributions

HS, MH, MRL, and HJ conceived and designed the experiments, performed the experiments, analysed the data, and wrote the paper: All authors have read and approved the final manuscript

Acknowledgement

The authors would like to acknowledge the vice-chancellor of research at Kerman University of Medical Sciences for funding this project.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1611-1616.
https://doi.org/10.3889/oamjms.2018.321
eISSN: 1857-9655
Clinical Science

The Effect of Psychological Stress on Salivary Testosterone in Puberty Children

Dimas Bagus Prastyo*, Melda Deliana, Yazid Dimyati, Karina Sugih Arto

Department of Child Health, Medical School, Universitas Sumatera Utara, Medan, Indonesia

Citation: Prastyo DB, Deliana M, Dimyati Y, Arto KS. The Effect of Psychological Stress on Salivary Testosterone in Puberty Children. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1611-1616. https://doi.org/10.3889/oamjms.2018.321

Keywords: Stress; Salivary testosterone; Puberty

***Correspondence:** Dimas Bagus Prastyo. Department of Child Health, Medical School, Universitas Sumatera Utara, Medan, Indonesia. E-mail: beckscie@gmail.com

Received: 21-May-2018; **Revised:** 24-Jul-2018; **Accepted:** 27-Jul-2018; **Online first:** 18-Sep-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Psychological stress is a condition that is experienced by many adolescents which affect the Hypothalamic-Pituitary-Gonadal axis. Testosterone is known as a sex steroid hormone that is susceptible to acute stress and can be measured through saliva. Disruption of the reproductive system can affect the sexual maturation process.

AIM: To understand the difference in salivary testosterone levels in puberty children before and after given a stressor.

METHODS: A quasi-experimental intervention study was conducted at Antonius Bangun Mulia junior high school, Medan, North Sumatera, in July-October 2017. Subjects were students aged 12-14 years with sexual maturity G2 for boys and M2 for girls. Psychological stress intervention was generated by the Wechsler intelligence scale for children fourth edition (WISC IV). Saliva was collected before and after the intervention. The analysis was done with Wilcoxon test and a P value < 0.05 was considered significant.

RESULTS: Forty-two subjects of 24 male students and 18 female students with sexual maturation Tanner II (54.8%) and Tanner III (45.2%). This study obtained that there was a statistically significant difference in salivary testosterone levels before and after the subject was given a stressor (P = 0.015, CI 95%). This difference also was seen within sexual maturation Tanner II (P = 0.045, CI 95%). No difference was observed in testosterone levels based on gender, male students (P = 0.065, CI 95%) and female students (P = 0.112, CI 95%).

CONCLUSION: Stress can affect salivary testosterone levels. There was a statistically significant difference in salivary testosterone levels before and after psychological stress in puberty children.

Introduction

Puberty is an important stage in the child's developmental process that describes a complex biological process of sexual development and the reactivation of the Hypothalamus-Pituitary-Gonadal (HPG) axis [1]. Studies have shown that variation in age and development of puberty are influenced by genetic factors and environmental factors, such as psychological stress [2]. Although the current study has not determined the incidence of stress in adolescents, US survey in 2013 found that teenagers experienced higher stress than adults during school years [3], and academic exams were considered to be the stressor that evoked anxiety [4].

The widely accepted approach to understand about stress is the process of interaction from resolution request from the environment (transactional model) [5]. Psychological stress itself is defined as a state of perceived threat to homeostasis [6]. Stress is almost considered as a symptom that occurs in adult individuals, but some studies in Brazil show a prevalence of stress in children of 30% to 60% with similar characteristics in adult individuals [7].

The response to stress involves the activity of the autonomic nervous system and the Hypothalamus-Pituitary-Androgen (HPA) axis. The HPG axis has some component structures and neural circuits against the HPA axis, and both of these axes work by maintaining a regulatory balance, in which the relationship is mutual [8] [9]. Acute or chronic stress,

either physically or psychologically, is known to cause HPA axis activation and HPG suppression [10] [11]. Several studies have found different results; it is thought that the mechanism involved is the role of glucocorticoid rather than suppressing but maintain luteinizing hormone secretion [11] [12].

Testosterone is a sex steroid hormone that can be measured in both men and women. From research, it is known that testosterone levels are influenced by obesity, drugs, ageing, physical stress, psychological stress, and actual stress (such as surgery or fasting) [4] [13] [14]. Testosterone levels during puberty depend on age and maturity stage [1] [9]. A study in Germany has found that testosterone levels are susceptible to acute stress and increased in both male and female during acute stress [12]. Measurement of testosterone levels in saliva reflects free testosterone serum levels, although at a condition in which there is a change in the amount of saliva in the mouth [1] [12]. The advantages of this method in pediatric patients are it is noninvasive, and it allows repeated examination with stable results. Examination of morning testosterone levels from 07.00 to 09.00 am recommended for stable results [15]. This study aims to determine whether there is any difference in salivary testosterone levels in puberty children after given stressor.

Methods

A quasi-experimental intervention study was conducted to see the difference in salivary testosterone levels among adolescents before and after given stressor at Antonius Bangun Mulia junior high school, Medan, North Sumatera, in July-October 2017. Samples were students aged 12-14 years with sexual maturation G2 in boys and M2 in girls. The exclusion criteria were students with the long-term use of steroids or glucocorticoids, history of chemotherapy or radiotherapy, orchitis, inflammation of oral cavity, and malnutrition. Samples were obtained by simple random sampling method. This study was approved by the Health Research Ethical Committee, Medical School, University of Sumatera Utara.

All students who fulfilled the inclusion criteria were enrolled in this study after given consent. We interviewed both parent and student to obtain demographic data. Student's stature was measured using microtoise. Sexual maturation was evaluated by trained personnel according to Tanner sexual stage. The testicular volume of male students determined by using orchidometer, and breast budding on female students through inspection and palpation. Saliva was collected at 08.00 am, previously students had been instructed not to eat, drink, or brush their teeth at least one hour. Students were asked to rinse their mouth with water, and then passive drooling method was used to collect approximately 1.5 ml of saliva.

Furthermore, students were asked to complete the questions from WISC IV which consist of mathematical and verbal questions within 15 minutes. The use of mathematical and verbal tests from WISC IV in children can lead to mild to moderate stress [10] [12]. After finished, saliva was collected in the same way as before. All saliva samples were stored in an ice box with temperature 2-8^{o}C until processed in the laboratory. Salivary testosterone examination was performed using enzyme-linked immunoassay kit DRG® Salivary testosterone (SLV-3013).

Data analysis was done with statistical software. Wilcoxon test was used to determine the difference in salivary testosterone levels before and after given stressor. Statistical calculation was done at 95% confidence interval and P-value <0.05 was considered significant.

Results

Of all 63 students, 50 students fulfilled the inclusion criteria. Five students were unwilling to take part in the study, one student was absent, and two students could not be sampled due to the oral lesion. Data collection and sampling were conducted on 42 students who were willing to participate and got approval from parents. Of 42 students aged 12-14 years consist of 24 male (57.1%) and 18 female (42.9%), we found that students median age were 12.8 (min-max 12-13.9) years. Table 1 shows the distribution and baseline characteristics of students, including weight, height, and stage of puberty.

Table 1: Baseline characteristics of students

Characteristics	Samples n=42
Gender	
Male, n(%)	24 (57.1%)
Female, n(%)	18 (42.9%)
Age (years), median (min-max)	12.8 (12-13.9)
Weight (kg),median (min-max)	39.4 (27.6-52.3)
Height (cm),median (min-max)	147.9 (130-156)
Stage of puberty	
Tanner II, n(%)	23(54.8%)
Tanner III, n(%)	19 (45.2%)

Figure 1 can be seen the boxplot of pre- and post-stress testosterone levels distribution. Median values for pre-stress testosterone levels were 5.79 pg/mL, whereas post-stress 11.40 pg/mL. Boxplots for both testosterone levels show that the data of testosterone value was not symmetrical. The value of the first quartile (Q1) for pre-stress testosterone was 2.90 pg/mL, and the third quartile (Q3) was 14.58 pg/mL with minimum value 1.9 pg/mL and maximum value 36.80 pg/mL. Some of the outlier values of pre-stress testosterone levels cause abnormal data distribution.

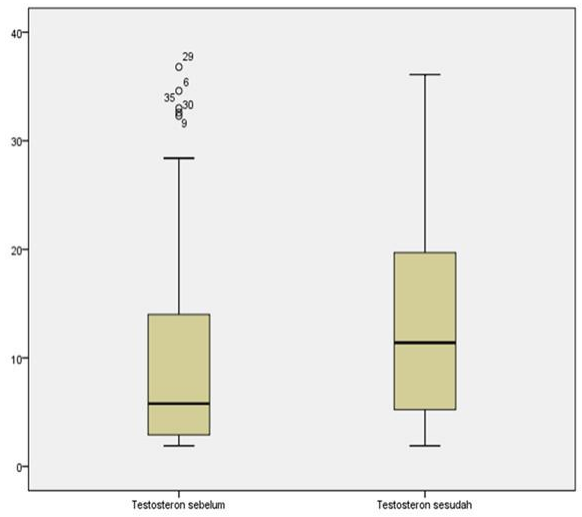

Figure 1: Boxplot of testosterone levels before and after stress

The Q1 value for post-stress testosterone was 5.22 pg/mL, and the Q3 was 19.83 pg/mL, with minimum value of 1.90 pg/mL and maximum value 36.10 pg/mL. The post-stress testosterone levels more evenly distributed, and there was no outlier value.

Table 2: Testosterone levels based on gender

	Testosterone levels (pg/mL) Median (min-max)		P*
	Pre-	Post-	
Gender			
Male	7.69 (1.90–36.80)	17.80 (1.90-36.10)	0.065
Female	5.77 (1.93–33.00)	8.55 (1.98-20.50)	0.112

*Wilcoxon test.

Normality test was done using Shapiro-Wilk test toward pre- and post-stress testosterone levels, and the result showed that data were not normally distributed. Bivariate analysis for pre- and post-stress testosterone was done with Wilcoxon test. From Table 2, testosterone levels were assessed based on gender. Pre-stress levels in male was 7.69 pg/mL (min-max 1.90-36.80), higher when compared with female 5.77 pg/mL (min-max 1.93-33.00). Post-stress levels in male (17.80 pg/mL, min-max 1.90-36.10) were also higher than female (8.55 pg/mL, min-max 1.98-20.50).

The higher the level of puberty, the higher the testosterone levels is. Table 3 shows pre-stress levels Tanner II of 5.40 pg/mL (min-max 1.90-34.60), lower than Tanner III of 6.80 pg/mL (min-max 1.95-36.80). Post-stress levels on Tanner II (10.90 pg/mL, min-max 1.90-36.10) were also lower than Tanner III (13.70 pg/mL, min-max 1.98-35.30).

Table 3: Testosterone levels based on puberty stage

	Testosterone levels (pg/mL) Median (min-max)		P*
	Pre-	Post-	
Stage of puberty			
Tanner II	5.40 (1.90 – 34.60)	10.90 (1.90 – 36.10)	0.045
Tanner III	6.80 (1.95 – 36.80)	13.70 (1.98 – 35.30)	0.198

*Wilcoxon test.

With the Wilcoxon test, we found that there was no statistically significant difference in pre- and post-stress levels in male (P = 0.065), as well as on female (P = 0.112). Maybe this was because the number of samples was too small for each gender and also was not normally distributed. The statistically significant difference in pre- and post-stress testosterone levels were found on subjects with sexual maturation Tanner II (P = 0.045), but not with puberty level Tanner III (P = 0.198).

Table 4: Testosterone levels difference pre- and post stress

n = 42	Median (min-max), pg/mL	P*
Testosterone levels pre-stress	5.78 (1.90-36.80)	0.015
Testosterone levels post-stress	11.40 (1.90-36.10)	

*Wilcoxon test

Discussion

Puberty can be viewed as a transitional period and is a very sensitive phase because of major changes in the neuroendocrine system. Development of puberty is also a time when the responses to the stressor will increase hormonal stimulation and reactivity, thus affecting emotions, behaviour and the process of sexual maturation [2] [3] [16] [17]. Research in America in 2013 found that hormones of the HPG axis are sensitive to stress stimuli in adolescence [16].

The onset of puberty is known to be affected by stress, where early life stress causes early puberty and prepubertal stress will result in late puberty [2] [18] [19]. In general, stress and reproduction have a negative relationship; under stressful conditions will inhibit reproductive function. However, existing studies have shown inconsistent results. Some studies in children and adults have found that in acute stress there will be an increase in testosterone levels that can be measured through saliva [4] [12] [16] [20]. Stress influence during puberty depends on whether the stress is acute or chronic, the degree of stress, and individual cognitive conditions [17].

Testosterone is a lipophilic molecule that can cross the blood barrier and salivary glands [21]. Level of morning testosterone is the highest level [15] [22], so it is often used as a basal testosterone index to get the picture of the maturation process [22]. A sampling of saliva by passive drool method gave better results than using salivettes [23] [24]. Examination of salivary testosterone with enzyme-linked immunosorbent assay (ELISA) method proved to have high sensitivity and specificity with stable results [15], but saliva samples were very sensitive to blood contamination [24]. Testosterone may increase or decrease depending on the subjective factors associated with the assessment of the situation so that the stress response is determined by prior experience and how the experience is interpreted [22].

Testosterone is thought to be a stress-sensitive hormone and is susceptible to acute stress [12] [16] [20] [21]. Testosterone also plays an important role in secondary sexual characteristics and reflects the maturation process [21]. Chronic stress will decrease testosterone levels because glucocorticoids can increase apoptosis of Leydig cells in male individuals thus suppressing steroidogenesis in the testes [20] [25]. But different mechanisms occur in acute stress conditions, where glucocorticoids precisely maintain LH and FSH secretion, resulting in increased testosterone levels [12] [19] [25]. Endogenous release of testosterone can be triggered by stressful challenges and increases in 15 minutes to 60 minutes [10] [12] [21]. The WISC IV test is an IQ test given to children aged 6-16 years. From other research, it is known that series of mathematics and verbal questions from the WISC IV test for 15 minutes has been able to stimulate the occurrence of mild to moderate stress [12].

This study found a significant difference between saliva testosterone levels before and after being given psychological stressor in puberty children; salivary testosterone was an increase in both male and female students. This finding consistent with the theory of the relation of hormonal adaptation response to stress conditions, that is testosterone secreted under LH regulation not only has a key role in sexual differentiation and function but also relates to aggression, competitive behavior, proactive responses, and triggers social status search or motivation of social dominance [20] [25] [26] [27]. Increased testosterone levels in adolescent individuals are associated with stress and competition that occurs strongly in both sex [21]. Research on the adolescent subjects in Iran in 2016 found increased saliva testosterone levels in psychological stress conditions. In the study, it was said that the increase in testosterone was not due to psychological stress alone, but also influenced by sex factors, personality traits, and emotional control [4]. Similar results were also found in Germany study in 2014 against 62 students aged 14-15 years, which showed an increase in salivary testosterone after given stressor, and followed, by increased fine motor skills [12].

In Table 2 it can be seen that there is no statistically significant difference based on gender, but it appears that testosterone levels before and after given stressor is higher in the male. This finding is similar to studies in Germany and Iran. In both studies, although the increase was not statistically significant, there was a higher increase in the male subject [4] [12]. A reasonable explanation is because testosterone is the main hormone in men and the association of testosterone with puberty are found more closely in boys [21].

Sex differences were seen to affect behavioural patterns associated with testosterone reactivity, which were consistent with previous studies of sex factor differences on androgen levels, HPA axis responses, and neurotransmitters [28][29]. Other studies have suggested that testosterone levels in men are associated with sensitivity to social status motivation [30].

Social relationships are thought to have contributed to changes in testosterone levels [25]. A study in Japan in 2015 found adult individuals with increased levels of testosterone in the early stages of stress depending on stable social relationships, but the difference was found mainly in women [20]. Although no significant direct association was found between stress factors and saliva testosterone levels, the study showed that in women with low testosterone levels there is social support from surrounding environment that can make the physiological response to stress decreased. Whereas in women with fewer social support will increase the stress response resulting in increased testosterone levels. Limitation of that study is the least number of male subjects [20].

Age is another factor that affects testosterone, and its relationship is inversely, but there is an exception during puberty. Increased testosterone levels found in higher maturation stage. Puberty and testosterone have a very close relationship, where increased testosterone will accelerate the maturation process [21]. Sexual maturation assessment with Tanner method, performed by a physician, can be used to evaluate pubic hair growth, breast, and genital development [9]. This study also found that testosterone levels were higher in the Tanner III maturation group (Table 3), by the literature that testosterone was higher as the maturation stage increased [17]. However, a statistically significant increase was found in the Tanner II maturation stage, which is estimated because in this study the number of subjects in the Tanner II maturation stage was more than subjects with Tanner III.

Higher levels of chronic stress during adolescence are associated with cognitive impairment and the development of future psychiatric disorders [17] [21] [31]. Increased testosterone levels are found to correlate with the emergence of family problems and negative emotions in male, but not in female children [16].

Some limitations of this study were the small number of subjects. The limited number of samples conducted in one school alone has made the results of this study couldn't be generalised, so more extensive research is needed involving more schools and larger sample quantities. Second, IQ assessment or subject achievement index was not performed, wherein these factors influence the individual stress response that may have an impact on changes in testosterone levels. This was a cross-sectional study design, so it only describes the stress response without assessing the previous stress and disturbance factors.

In conclusion, stress can affect testosterone levels as measured through saliva. This study can consider that in children with puberty disorders, we need to evaluate the stress factor as one of the causal factors.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1617-1621.
https://doi.org/10.3889/oamjms.2018.258
eISSN: 1857-9655
Clinical Science

Comparison of Predictor of Desaturation Disorders and Daytime Sleepiness Based On Epworth Sleepiness Scale and STOP-BANG Questionnaires in Mild to Moderate Obstructive Sleep Apnea Patients

Zohreh Kahramfar, Besharat Rahimi*

Department of Pulmonary and Critical Care Medicine, Advanced Thoracic Research Center, Tehran University of Medical Sciences, Tehran, Iran

Citation: Kahramfar Z, Rahimi B. Comparison of Predictor of Desaturation Disorders and Daytime Sleepiness Based On Epworth Sleepiness Scale and STOP-BANG Questionnaires in Mild to Moderate Obstructive Sleep Apnea Patients. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1617-1621. https://doi.org/10.3889/oamjms.2018.258

Keywords: Obstructive sleep apnea; Stop bang; EPWORTH scale; Desaturation disorders

***Correspondence:** Besharat Rahimi. Department of Pulmonary and Critical Care Medicine, Advanced Thoracic Research Center, Tehran University of Medical Sciences, Tehran, Iran. E-mail: besharatrahimi@yahoo.com

Received: 04-Jun-2018; **Revised:** 26-Jul-2018; **Accepted:** 27-Jul-2018; **Online first:** 18-Aug-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Obstructive sleep apnea (OSA) is characterised by recurrence in upper airway obstruction during sleep.

AIM: This study aimed to compare the predictive values of the Epworth Sleepiness Scale (ESS) and STOP-BANG in the desaturation of patients with mild to moderate obstructive apnea based on the apnea-hypopnea index (AHI) scale.

METHODS: A group of 79 patients (43 male and 36 female) were selected. The suspected patients were introduced to the sleep clinic, and the ESS and STOP-BANG questionnaires were filled up, then subjected to polysomnography test, and the scores of the disease were also determined based on an apnoea-hypopnoea index (AHI). Finally, the desaturation rate (SO_2 < 3% based on the baseline) and desaturation index were determined in patients. Consequently, the finding was compared with the results of the questionnaires.

RESULTS: Patients with STOP-BANG score above 3 had significantly higher weight, oxygen desaturation index (ODI) index and average desatu, while peripheral capillary oxygen saturation (SpO_2) base and average SpO_2 were lower than those with scores below 3 ($P < 0.05$). However, there was no significant difference between the patients with the ESS questionnaire score above 10 and below 10 ($P > 0.05$).

CONCLUSION: The results of these two questionnaires reflect the unsaturated oxygen index in the blood, and can be considered for the evaluation of the severity of the disease.

Introduction

Obstructive sleep apnea syndrome (OSAS) is the second most common disease among respiratory disorders after asthma. This disease is characterised by the recurrence of the complete or partial collapse of the upper airway during sleep, which leads to a disruption of air flow for over 10 seconds (apnea) or hypopnea and followed by transient awakening. The syndrome has many negative effects on the health and behaviour of adults millions around the world [1] [2]. OSAS can affect any age group, but it appears to be more common in middle-aged men [3][4]. Sleepiness is one of the most important symptoms of OSAS, which is characterised by the frequent awakening of sleep to end apnea and hypopnea. Other daily symptoms such as the inability to concentrate, morning headaches, amotivation, depression and decreased libido have been described, which are caused by drowsiness throughout the day. Other complications of this disease include hypertension, abnormal endothelial dysfunction, cancer, diabetes and other disorders, as well as insulin resistance and hyperlipidemia [5] [6] [7] [8] [9]. The severity of the disease is assessed by a series of indicators including excessive daytime sleepiness (EDS), apnoea-hypopnoea index (AHI), oxygen desaturation index range (ODI), polysomnography (PSG), nighttime oximetry, and

apnea testing [9] [10]. Also, diagnosis of this syndrome requires a combination of clinical manifestations of abnormal breathing during sleep and clinical features. Furthermore, imaging studies using X-rays are important for identifying craniofacial anatomical changes. Questionnaires also have a prominent feature [11] [12].

The most common questionnaires are the Berlin questionnaire (BQ), STOP-BANG, and Epworth Sleepiness Scale (ESS) that have been widely used for detecting OSA. These questionnaires had an immense range of sensitivity and characteristics in various strains [13] [14] [15]. In parallel, ESS was originally designed to distinguish the hazard of daytime sleepiness, is proposed as a tool for OSA identification as well as the ESS had the highest characteristic for anticipating mild, moderate, and severe OSA [16].

The STOP-BANG questionnaire revealed a significant relationship between high-risk OSA (STOP-Bang ≥ 3) and elevated hazard of desaturation, critical care admission, and airway problem. This questionnaire contains several questions associated with snoring, tiredness during daytime, observed apnea, body mass index, age, neck circumference, high blood pressure, and in surgical patients, it indicates a moderately great level of sensitivity (65.6%) and specificity (60%) in evaluating OSA (AHI > 5 events/h). The sensitivity and specificity of this questionnaire for moderate-to-severe OSA (AHI > 15 events/h) are 74 and 53%, respectively. For severe OSA (AHI > 30 event/h), the sensitivity is 80%, and the specificity is 49%. Additionally, the sensitivities of this questionnaire with an AHI more than 5, 15, and 30 as cutoffs are 83.6, 92.9, and 100%, respectively [17] [18].

Therefore, we conducted this study to compare the predictive values of the ESS and STOP-BANG in the desaturation of patients with mild to moderate obstructive apnea based on the AHI scale and then the relationship between desaturation and drowsiness level was examined by using these two questionnaires.

Material and Methods

The study was approved by the ethics committee of the Tehran University of Medical Sciences. Participants who contacted us indicated their consent by signing a written consent form. They then completed questionnaires requesting socio-demographic information.

A sample of 79 patients (36 women and 46 men), who admitted to the respiratory centre of Imam Khomeini Hospital with daily sleepiness complaints about sleep testing, completely answered to the ESS and STOP-BANG questionnaires, included in this study.

The STOP-BANG questionnaire consists of four subjective (STOP: snoring, tiredness, observed apnea, and high blood pressure) and four demographics items (Bang: body mass index [BMI], age, neck circumference, gender). Answering yes to three or more items are classified as a great hazard for OSA, and also, the ESS has an eight-item questionnaire to measure daytime sleepiness. ESS has a four-point Likert reply format (0–3), and the score ranges from 0 to 24. ESS score ≥ 11 demonstrates inordinate daytime sleepiness and high hazard for OSA [19] [20] [21].

Patients with answering ‘‘yes’’ to two or more of items of these questionnaires were suspected to having OSA. Then, the polysomnogram (PSG) test was performed for them at the sleep test Center. After interpreting their testing by lung specialists, OSA was determined based on the AHI (mild and moderate) criteria. In standard PSG, electrocardiogram, electroencephalography, electrocoagulation, pulse oximetry, snoring voice recording, electromyogram (submental and bilateral anterior tibialis), thoracoabdominal movements, thermal sensors, and oronasal airflow were recorded.

Subjects with mild to moderate OSAS (5 ≤ AHI < 30) were evaluated in this study. Then, the desaturation rate (sulfur dioxide (SO_2) drop more than 3% based on the baseline) and desaturation index were determined in these patients, and the results were consequently compared with the results of the questionnaires. Exclusion criteria include patients with hypnotic drugs, AHI < 30, as well as patients who did not agree to participate in the study. The variables such as age, sex, height, weight, STOP-BANG scores, ESS, AHI per hour, desaturation rate and desaturation index were measured.

SPSS version 17.0 (SPSS Science, Apache Software Foundation, Chicago, IL, USA) was used. Patients’ characteristics or qualitative variables are presented as means (± standard deviation) or percentages. *A p* value less than 0.05 was defined as statistically significant.

Results

After scrutinising the results of the questionnaires, ESS was determined to be below 10 in 32.9% of cases, while it was above 10 in 67.1% of participants. Moreover, the findings of the STOP-BANG questionnaire showed that the score of the questionnaire was below 3 in 39.2% of the cases and above 3 in 60.8% of the subjects.

Table 1: Demographic data of the participants in the study of the variables studied

Variables		Frequency	Per cent
Sex	Female	43	54.4
	Male	36	45.6
ESS	<10	26	32.9
	>10	53	67.1
STOPBANG	< 3	31	39.2
	> 3	48	60.8
AHI	<15	26	32.9
	>15	53	67.1

The AHI index was also considered to be 15 for 32.9% of the cases, while 67.1% of patients were defined by an AHI index increase of >15. Population information is summarised in Table 1 and Figure 1.

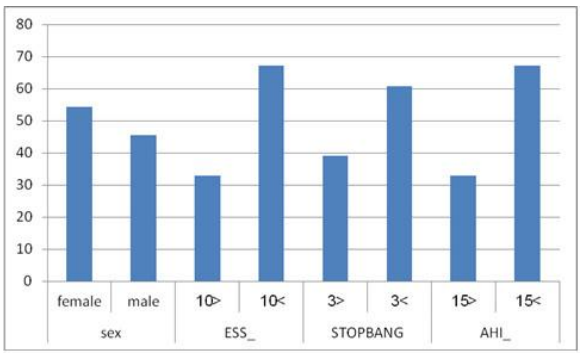

Figure 1: Demographic data of individuals

As shown in Table 2, there were only significant differences in weight (p = 0.002) between patients with higher and lower STOP-BANG scores. This means that weights variables were significantly higher than other among subjects with a mean score of >3.

Table 2: Comparison of demographic data of patients with STOP-BANG score (≥3 points or ≤3 points)

STOP-BANG		Age	Weight	Height	BMI
<3	Mean	45.55	77.90	159.5700	28.717419
	SD	12.871	12.330	30.55032	4.2491928
>3	Mean	51.44	89.31	165.5625	33.074375
	SD	14.862	16.844	9.69735	8.0332249
Total	mean	49.13	84.84	163.2110	31.364684
	SD	14.324	16.151	20.59868	7.1002533
P-value		0.07	0.002	0.2	0.007

However, there were no significant changes in patients with ESS scores above or below 10.

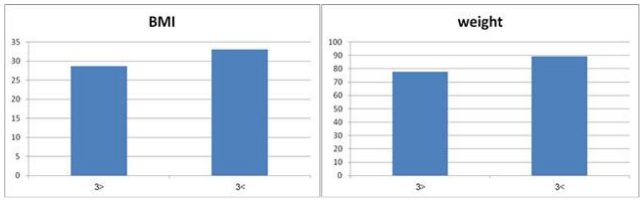

Figure 2: Body mass index and patient weight with STOP-BANG score (≥ 3 points or ≤ 3 points)

Based on the data presented in Table 4, there has been a significant difference in age among patients with AHI ≥ 15, and those with AHI ≤ 15**.**

Table 3: Comparison of demographic data in patients with ESS above or below 10

ESS		Age	Weight	Height	BMI
< 10	Mean	50.65	82.08	166.3077	29.675000
	SD	15.590	13.305	8.59195	5.0371669
> 10	Mean	48.38	86.19	161.6919	32.193585
	SD	13.755	17.335	24.36844	7.8290490
Total	Mean	49.13	84.84	163.2110	31.364684
	SD	14.324	16.151	20.59868	7.1002533
P-VALUE		0.5	0.2	0.3	0.1

This means that people with a mean AHI ≥ 15 were significantly older when comparing others.

Table 4: Comparison of demographic data in patients with AHI above or below 15

AHI		Age	Weight	Height	BMI
<15	Mean	44.15	81.19	166.4615	29.326538
	SD	12.945	13.943	9.34764	4.9433583
>15	Mean	51.57	86.62	161.6164	32.364528
	SD	14.450	16.969	24.21931	7.7958967
Total	Mean	49.13	84.84	163.2110	31.364684
	SD	14.324	16.151	20.59868	7.1002533
P-VALUE		0.03	0.1	0.3	0.07

As summarised in Table 5, there has not been a significant difference between the round neck and the AHI index (≥ 15, and ≤ 15), as well as ESS (≥ 10, and ≤ 10).

Table 5: The relationship between Round neck and STOP-BANG score, ESS and AHI

		Mean	SD	P-Value
STOP-BANG	< 3	38.45	2.850	
	> 3	41.71	3.464	0.001
	Total	40.43	3.594	
ESS	< 10	40.42	3.466	
	> 10	40.43	3.687	0.9
	Total	40.43	3.594	
AHI	< 15	39.54	3.524	0.1

The results of the blood oxygen levels revealed that there had been a significant difference between the subjects with the STOP-BANG score ≥ 3, and those with STOP-BANG score ≤3 regarding SpO_2, mean SpO_2, ODI index and average desatu ($p < 0.05$). However, based on the ESS questionnaire, there was no statistically significant difference between the oxygen indexes in the blood. The average of the ODI index was significantly higher in those with AHI above 15 as compared to others (Table 6).

Table 6: Evaluation of blood oxygen levels among AHI scores and the STOP-Bang and ESS questionnaires

STOP-BANG		SpO_2 base	SpO_2 min	Average SpO_2	Od index	Average desatu
STOPBANG	Mean	93.394	84.74	93.232	11.358	3.829
<3	SD	2.5432	8.037	2.6487	6.4699	0.6876
STOPBANG	Mean	91.531	81.29	90.979	16.735	4.313
>3	SD	2.9594	7.871	3.4616	8.6827	0.8557
P-value		0.005	0.06	0.003	0.004	0.01
ESS <10	Mean	92.627	84.54	92.319	12.573	3.981
	SD	2.5332	5.791	3.0039	7.5299	0.7082
ESS >10	Mean	92.083	81.72	91.640	15.632	4.192
	SD	3.1176	8.876	3.4963	8.5068	0.8735
P-VALUE		0.4	0.1	0.3	0.1	0.2
AHI <15	Mean	92.115	82.50	91.819	8.285	3.881
	SD	3.3980	10.148	3.9598	4.6693	0.7376
AHI >15	Mean	92.334	82.72	91.885	17.736	4.242
	SD	2.7086	6.932	3.0294	7.8942	0.8450
Total	Mean	92.262	82.65	91.863	14.625	4.123
	SD	2.9330	8.065	3.3384	8.2770	0.8243
P-value		0.7	0.9	0.9	0.001	0.06

Discussion

Over 85 % of individuals with OSA have never been diagnosed or treated; therefore applying credible and trustworthy tools are necessary for screening [18]. Among the various methods used to diagnose the disease, use of questionnaires can be useful in screening these patients. In parallel, in recent years, the STOP-BANG questionnaire was utilised broadly for screening OSA [18]. According to studies by Abrishami et al., 2010 and Farney et al., 2011 the STOP-BANG questionnaire has a high sensitivity in predicting moderate to severe OSA [22] [23]. Patients with a score of 0 to 2 can be classified as low risk for developing moderate to severe OSA, while those with a score of 5 to 8 are at high risk of moderate to severe OSA. In this regard, individuals with scores of 3 and 4 require other indicators, such as BMI, for division [24]. In a study by Zhong et al., 2012 reported that people with the ESS of over 10 were more likely to have a higher BMI. There was a significant difference in waking SpO2 of severe OSAS between EDS group No-EDS group [25].

Nevertheless, in the present study, we aimed to use the predictive value of the STOP-BANG, and ESS instead of performing the hypoxic assessment and PSG tests to determine the OSA in individuals with symptoms of sleep disorders. In this regard, it would be hoped that the results of the questionnaire would be used to detect the possibility of hypoxia which can be useful to provide more qualified centres for those who need more detailed examination. Our findings are similar to the studies by Azagra-Calero et al., 2012 and Young et al., 2004, where demonstrated increasing weight gain are associated with a higher incidence of disease, and the severity of the disease could increase with increasing age [22] [26]. The results of this study also revealed that there had been a significant difference between the subjects with the score of the STOP-BANG > 3 and < 3 regarding SpO_2, mean SpO_2, ODI index and average desatu. Meanwhile, there was no significant difference between these indices and the results of the ESS in both groups with score > 10 and < 10. Thus, it can be interpreted that the results of the STOP-BANG questionnaire were a good reflection of the unsaturated oxygen index in the blood. Because in individuals with an AHI index >15, the average ODI value was significantly higher, STOP-BANG findings could also be considered for the evaluation of the severity of the disease. In parallel, Du et al., 2015 assessed the predictive value of STOP-BANG and ESS in the screening of patients with obstructive sleep apnea, who showed the STOP-BANG questionnaire, had the highest sensitivity.

Moreover, a recent meta-analysis based on the STOP-Bang questionnaire in different populations demonstrated that the diagnostic attributes of STOP-Bang were commonly consistent with high sensitivities and low specificities [27] [28]. Although the classifications by two questionnaires revealed differences between the mean values of oxygen levels, only the STOP-BANG questionnaire showed significant differences, indicating a higher potential and predictive value of this questionnaire for determining desaturation disorders. This is consistent with previous studies, such as reported studies from Chung et al., 2016, Tan et al., 2016 and Nagappa et al., 2015, and the accuracy of this questionnaire was confirmed again for predicting OSA [28] [29] [30]. Also, it was found that STOP-BANG is not only important in predicting the incidence of OSA but also can even be effective in assessing the severity of the disease.

The main limitation of our study is that we have failed to optimise our findings to whole sleep clinics in the entire population regardless of symptoms as well as our population was not an exact sample of the general population. The populations of the sleep clinic have abundant diversities, and variety of these patients is different from one clinic to another depending on their communication to various medical sections. Although we tried to overcome this limitation with including a large number of patients, another limitation, it is also possible certain medical assistants and physicians did not feel invested in the quality improvement project and chose not to administer/review the questionnaire. Despite these limitations, the findings of our study still deserve attention.

In conclusion, we found that the patients with mild to moderate obstructive pulmonary obstruction, (which are calculated based on weight and height), and older ages can significantly cause sleep disorders. Also, the range of blood oxygen levels was significantly higher among those classified according to the STOP-BANG questionnaire than the ESS questionnaire. Therefore, the results of this questionnaire not only reflect the unsaturated oxygen content of the blood but also can be used to evaluate options such as the severity of the disease and the prediction of nocturnal desaturation and the occurrence of daytime sleepiness. In summary, it is essential to use reliable questionnaires such as STOP-BANG in one session.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1622-1626.
https://doi.org/10.3889/oamjms.2018.346
eISSN: 1857-9655
Clinical Science

Study of Radial Nerve Injury Caused By Gunshot Wounds and Explosive Injuries among Iraqi Soldiers

Reza Akhavan-Sigari[1,2*], Dorothee Mielke[1], Afshin Farhadi[3], Veit Rohde[1]

[1]Department of Neurosurgery, University Medical Center Göttingen, Göttingen, Germany; [2]Department of Neurosurgery, Bouali Hospital, Tehran Medical Sciences Branch, Islamic Azad University, Tehran, Iran; [3]Department of Orthopedic Surgery, Tehran Medical Sciences Branch, Islamic Azad University, Bouali Hospital, Tehran, Iran

Citation: Akhavan-Sigari R, Mielke D, Farhadi A, Rohde V. Study of Radial Nerve Injury Caused By Gunshot Wounds and Explosive Injuries among Iraqi Soldiers. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1622-1626. https://doi.org/10.3889/oamjms.2018.346

Keywords: Gunshot wounds; Radial nerve; Humerus fracture; Upper limbs; Seddon's classification

***Correspondence:** Reza Akhavan-Sigari. Department of Neurosurgery, University Medical Center Göttingen, Göttingen, Germany; Department of Neurosurgery, Bouali Hospital, Tehran Medical Sciences Branch, Islamic Azad University, Tehran, Iran. E-mail: reza.akhavan-sigari@med.uni-goettingen.de

Received: 13-Aug-2018; **Revised:** 29-Aug-2018; **Accepted:** 31-Aug-2018; **Online first:** 21-Sep-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Gunshot wounds and blast injuries to the upper limbs produce complex wounds requiring management by multiple surgical specialities.

AIM: We sought to determine the pattern of peripheral nerve injuries among Iraqi soldiers in the war.

METHODS: We performed a 3 year retrospective cohort analysis based on medical records of patients with sustaining gunshot wounds and blast injuries to the upper limbs. Ethical approval was obtained from the institutional review board. The patients included were male, serving military personnel of all age groups and ranks presenting with weakness or sensory loss of radial nerve. Three hundred eighteen patients aged 24 years or older with a high-energy, diaphyseal fracture of the humerus and complete motor and sensory radial nerve palsy were reviewed retrospectively. In these patients, the physical examination and electrodiagnostic study were carried out by experienced neurologists. Seddon's classification system was used to assess the severity of the injury. The data related to the types of fracture, the type of damage, the factors causing damage and the failure of treatment were entered into the IBM SPSS 23 software after extraction of files. Based on mid-range indicators and data distribution, traumatic injuries among Iraqi soldiers in the war against ISIL were then investigated.

RESULTS: A group of 318 patients with mean age of 25.41 ± 6 years were enrolled in the study, of which 127 patients were included with an open fracture and 191 patients with closed lesions. All 127 patients with a transected radial nerve had an open humerus fracture and were part of a complex upper-extremity injury. 113 of 127 subjects had primary repair of the radial nerve and recovered well. 14 of 127 subjects were not recovered. 3 of them had iatrogenic radial nerve injury due to the internal fixation device. Furthermore, all 191 patients with closed injuries recovered well. The average time to initial signs of recovery was 8 weeks (range, 1–27 weeks). Axonotmesis and Neurotmesis were found in 283 (89%) subjects. The average time to full recovery was determined to be 6 months (range, 1–22 months). The blast was found to be the main cause of nerve injury in 236 (74.2%) cases, followed by gunshot damage (21.4%, 68 subjects), falling from height and motor vehicle accidents (4.4%, 14 subjects) and multiple injuries (17%, 54 cases).

CONCLUSIONS: Trauma caused by factors such as explosions and gunshot worsens the condition of the injuries and presents the treatment conditions with many challenges. However, the success rate in post-surgical recovery of humerus fracture and injured radial nerve can be remarkably higher in young people as compared to other age groups.

Introduction

The radial nerve is the largest neural network and major peripheral nerve of the upper limb. The nerve arises in the brachial plexus and receives from the lower parts of the cervical segment of the spinal cord and upper parts of the thoracic segments. This nerve affected some forearm muscles in the lower parts of the elbow and involved in providing cutaneous sensory innervation for a large part of the back of the hand. The control of the forearm extensor muscles, wrist and fingers are also provided by this nerve [1] [2]. Therefore, if the radial nerve is damaged in the arm, elbow, or upper part of the forearm, the person will not be able to raise the wrists and fingers from the back to the forehead due to paralysis of the forearm's extensor muscles, where cannot extend at the metacarpophalangeal joints, leading to the wrist drop as the most common symptom of radial nerve

damage [3].

Studies have shown that men are more likely to be injured in the upper extremity, especially the radial nerves due to some issues such as occupation, driving accidents, insignificant safety issues more than women [4]. Among the most important signs of radial neuropathy, pain, paralysis, loss of ability, numbness, bending of the wrists, stretching, lack of coordination, tingling and atrophy can be pointed out [5] [6].

The most common mechanisms of damage to the radial nerve are in the head of the humerus and the dorsolateral humerus [7]. Radial nerve damage commonly occurs at the spiral groove of the humerus which can be linked to compression, and also another reason may be involved in the occurrence of radial nerve damage such as fractures of the humerus or external pressure [8]. Radial nerve damage is one of the most common damages to the peripheral nervous system associated with humerus bone fracture [8]. The bone fracture of the humerus accounts for about 3 to 5% of all fractures, and radial nerve paralysis (RNP) occurs in 2 to 17% of the humerus bone fractures.

RNP can be partial or complete, and humerus fractures account for about 3 to 5% of all fractures, [9] [10] [11]. Radial nerve damage due to trauma can be commonly found in situations such as battle scenes where people are exposed to direct gunshot and explosions. Most types of sensory and motor injuries result from trauma for the patient and cause many problems in the daily activities of individuals [12].

The type and location of traumatic nerve injury are very important regarding the area of the nerve involved and how to prognosis [13]. In general, penetrating wounds, which occurred during the war, are often the result of projectiles to the combatant's body. These injuries can be caused by direct bullet collisions or from fragmentation munitions or fragmentation munitions caused by explosive ordnance such as mines, grenades, mortars and bombs [14]. Such war injuries have a high potential for nerve damage, especially in motor organs. The explosions or gunshot have caused extensive destruction and damage to the tissues due to the high speed and direct transfer of energy to the organs and tissues, as well as their high temperatures. These injuries not only leave a different tissue traumatic pattern but also severely complicate the treatment process [15]. Radial nerve damage treatment is very important due to the extremely complex anatomy of the nerve area [16]. Based on the time of injury, radial nerve damage is divided into two primary and secondary groups. Loss of function occurs at the time of injury, in the primary form, and mainly due to closed fractures. However, in the secondary type, loss of function occurs due to conservative treatment or trapping the nerve in the fracture holes or broken parts of the bone, as well as following surgery [11] [16] [17]. According to published data, secondary damage to the radial nerve occurs in 4% to 32% of patients undergoing surgery for stabilising the fracture [18]. Therefore, the adoption of a therapeutic approach is very important. Treatment for radial nerve injuries caused by penetrating ulcers is a surgical repair of the nerve.

Some researchers in this field believe that when radial nerve injury is caused by pressure or stretching, a preservative and protective treatment can be used for a limited period [19]. Other therapies for the treatment of radial nerve damage can be referred to the transfer of the tendon; this treatment is used when there is no indication of nerve repair due to the atrophy of extensor muscles and replacement of the fibrous tissue [20]. Each of the therapies used to treat radial nerve injuries depends on a variety of factors, such as the type of injury, the site of injury, and the degree of nerve damage.

Regarding the pivotal role of this issue and problems above, the present study was aimed to investigate the types of radial nerve injury caused by the bullet and explosive injuries among Iraqi soldiers in the war against ISIS fighters.

Material and Methods

We performed a 3-year retrospective cohort analysis based on medical records of patients with sustaining gunshot wounds and blast injuries to the upper limbs, which conducted from June 2015 to April 2018 in collaboration with Islamic Azad University and Bouali Hospital. During this period, patients were enrolled by inclusion and exclusion criteria. The sampling process was to initially cover all Iraqi soldiers and military personnel available in all age groups exposed to bullet wounds and explosive injuries and other traumatic injuries in fighting ISIS fighters, including operations military, vehicle accidents, clashes, etc. Among these patients, patients with weak or radial sensory loss in their medical records were selected after being referred to the military emergency hospital. Demographic data, trauma history, trauma characteristics, the severity of the injury, electrophysiological study and physical examination were extracted. Seddon classification system was used to assess the severity of injury in these patients.

Finally, medical records of 318 patients who suffered from humeral fractures and complete radial nerve palsy were investigated. Data were analysed by SPSS 23IBM software, which presented by descriptive statistics (frequency, percentage, mean ± standard deviation, etc.).

Results

This retrospective study was performed on 318 patients with a minimum age of 24 years who were injured by gunshot or blast injury with humerus fractures and complete paralysis of the radial nerve at Tehran's Bouali Hospital between 2015 and 2018. The mean age of these patients was determined to be 25.41 ± 6 years. 127 patients with open fracture and complicated injuries in upper limbs and 191 patients with closed injuries and radial nerve palsy were included (Figure 1).

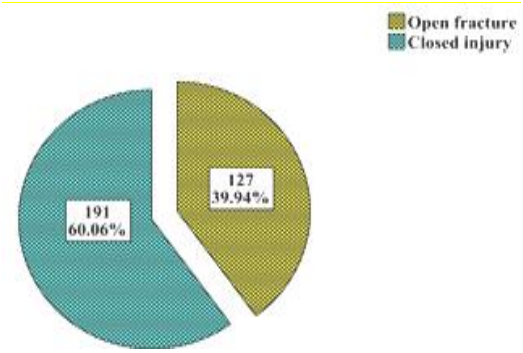

Figure 1: Frequency of open fracture and closed injuries in patients

Out of 127 patients, 113 patients with an open fracture (Figure 3) improved by primary repair of the radial nerve, but 14 patients (11%) did not recover at all. 3 of them with absolutely no improvement had iatrogenic radial nerve injury due to the internal fixation device. Our findings indicated that all 191 patients with intact explored nerves were improved. The average recovery time was determined as 8 weeks (the range was between 1 and 27 weeks), and the total average recovery period was calculated as 6 months (range 1-22 months). In the current study, Axonotmesis and Neurotmesis have seen in 283 (89%) subjects. According to the data, the explosion was found to be the main cause of radial nerve injury in 236 individuals (74.2%), followed by gunshot damage (21.4%, 68 subjects), falling from height and accidents caused by motor vehicles (4.4%, 14 subjects) and multiple injuries (17%, 54 case), (Figure 2).

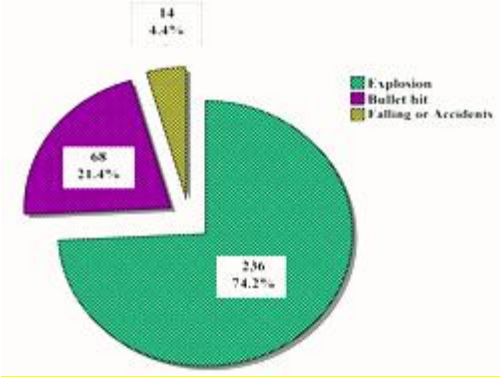

Figure 2: Frequency of major causes of nerve injury

A large number of the lesions were ≤ S2 and M2 levels before the operation. The results were classified into three groups. Good outcome was defined as ≥ M4 and ≥ S4, the fair outcome was represented by M2-M3 / S2-S3, and poor outcome was ≤ M1 and ≤ S1 (Table 1 and 2).

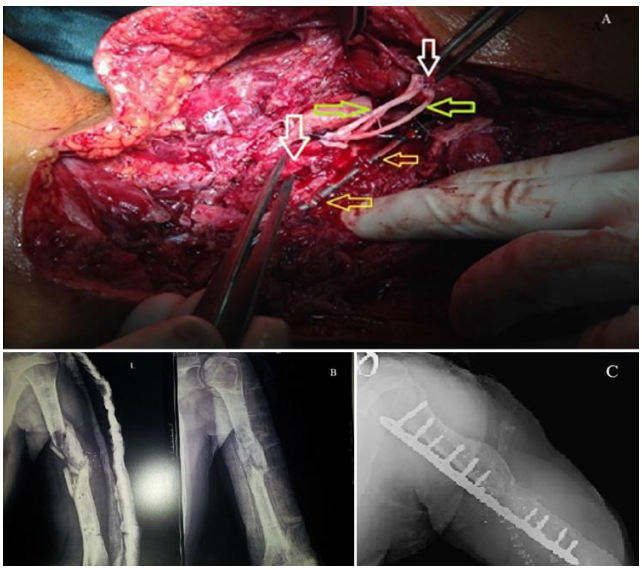

Figure 3: A) Severely injured left upper extremity gunshot wound with segmental radial nerve defect (white arrow). After débridement, segmental radial nerve defect reconstruction with interposition of sural nerve allograft of 6 cm (green arrow) and nerve coaptation wraps in place was performed. The superior and inferior halves of the proximal and distal radial nerve trunk were anastomosed with three segments of the sural nerve stumps. Each nerve ending was anastomosed using a perineural technique under microscopic magnification. Internal fixation of midshaft humerus fracture (orange arrow); B) Preoperative X-Ray of midshaft humerus fracture caused by gunshot; C) Open reduction and internal fixation with shortening of the humerus and iliac crest bone graft resulted in a union.

Discussion

In addition to incidents and everyday events, battle scenes are high-risk situations that can expose the person to the most severe traumatic injuries. War trauma can be either penetrating or non-penetrating. Penetrating injuries are caused by gunshot, knives and other sharp objects clipart, and non-penetrating injuries are due to accident, falling, explosion wave, etc. [21]. According to the investigations carried out in this area, penetrating injuries as a result of war projectiles are one of the most common causes of injury in the battlefields [22]. Motor organs and long bones are important parts of the body that suffer from trauma, tissue damage and fracture. The humerus is a large bone in the arm or forelimb that may be fractured in its various parts, such as head and neck, trunk, and areas near the elbow joint as a result of accidents, and falling. Each of these fractures has its characteristics and has different treatment regimens. On the battlefield, in addition to closed damage to the

humerus and the bone, Penetrating injuries also occur in the area due to projectiles.

Table 1: Modified British Medical Research Council (BMRC) grading of sensorimotor recovery, and motor recovery on the quality of outcome after radial nerve repair [27]

Motor recovery
Poor
M0 No contraction
M1 Return of perceptible contraction in the proximal muscles
Fair
M2 Return of perceptible contraction in both proximal and distal muscles
M3 Return of perceptible contraction in both proximal and distal muscles of such of degree that all-important muscles are sufficiently powerful to act against resistance
Good
M4 Return of function as in stage 3 with the addition that all synergic and independent movements are possible
M5 Complete recovery
Sensory recovery
Poor
S0 No sensation
S1 Deep pain re-established
Fair
S2 Some response to touch and pin, with over-response
S3 Good response to touch and pin, without over-response
Good
S4 Location and some tactile discrimination
S5 Complete recovery

These injuries are often caused by direct bullet encounters or by the explosives collapse such as mines, grenades, mortar bomb and bombs [14]. Projectile-induced injuries due to high speed, direct energy transfer and extreme heat cause massive damage to the tissue and make the traumatic pattern and treatment process more complex [15].

Table 2: Summary of patients data and grading of recovery

	Total number of patients n=318		
	Closed fracture 191 (60%)	Open fracture 127 (40%)	
	Improved: 191	Improved: 113	Not recovered: 14
M2/S3	7 (3,6%)	51 (45,1%)	
M3/S3	49 (25,6%)	15 (13,2%)	
M3/S4	19 (9,9%)	32 (28,3%)	
M4/S3	22 (11,5%)	12 (10,6%)	
M5/S4	56 (29,3%)	3 (2,6%)	
M5/S5	38 (19,8%)		
	M0/S0		6
	M0/S1		5
	M0/S2		3

The most important complication of humerus trauma and humerus fracture in this area is radial nerve neuropathy. Based on available data, up to 5% of the total fractures are related to the humerus fracture, which is associated with a radial nerve palsy [9][11][23], and often this neuronal injury occurs between two-thirds of the upper and one-third of the lower humerus. Understanding damage patterns, especially in military, and familiarity with the complexities of these types of injuries can play a decisive role in the quality of the treatment process and improve the success rate of patient recovery significantly.

Therefore, research projects focusing on the subjects in the present study are necessary and inevitable. Non-surgical treatment, especially in the case of the elongation and mild rupture, and surgical intervention (early exploration or late exploration) are performed by a specialist diagnosis. The surgical process has complications such as infection, iatrogenic damage to the nerve and the lack of improvements in fracture due to disruption of the blood supply to the tissue. According to the data reported by the American Orthopedic Surgery Board, intramedullary nailing (IMN) and Open Reduction and Internal Fixation (ORIF) have been accounted for 1.5% and 3% of infected d cases. The iatrogenic paralysis rate in IM and ORIF methods has been estimated to be 3.1% and 7.8%, respectively. Furthermore; nonunion of bone fractures has been reported in 3.1% and 1.6% of subjects for IMN and ORIF methods [24]. Compared with the statistics above, which is a result of reports published between 2004 and 2013, we conclude that only 1% of subjects showed absolutely no improvement in radial nerve, which is considerably more favourable than global published data.

Moreover, no infections or nonunion was found in our study. The most important factor which may have affected our results in recovery of the radial nerve e is probably the young age of our patients. Although this issue has become a challenge for researchers and clinicians; however, the high rate of treatment success in our study demonstrated that rapid recovery depends mainly on the age of patients. Due to the young age of patients in our study (mean age of patients 25.41 ± 6), the improvement rate was high, and the treatment complications were surprisingly low. Nouraei et al., (2014) also reported that young patients have fewer complications such as nonunion, infection, neurovascular problems and osteonecrosis [25]. Although healing in healthy older adults (≥ 65 years of age) is not impaired per se, age-related changes are obvious in all phases of wound repair [26].

In conclusion, the diagnosis of radial nerve injury after humeral fractures is easy. However, the verify of the damage degree of the radial nerve is very difficult. It is the degree for damage of radial nerve playing a decisive role in the judgment of whether exploration is necessary or not. Complex peripheral nerve injuries of the upper extremity are challenging to manage. They require an understanding of not only the mechanism and type of nerve injury but also the timing of repair. One has to be careful not to downgrade function by intervening too early in a closed injury. Care should be taken not to intervene too late, precluding motor recovery, in an open nerve injury. Closed injuries benefit from serial examinations to determine the extent of injury and recovery, whereas open or sharp injuries benefit from expeditious operative exploration and repair. These characteristics guide surgical decision making. Options for repair are numerous and include primary repair, grafting, nerve and tendon transfer, and free functional muscle transfer. A careful history and physical examination, along with the judicious use of

electrodiagnostic and radiologic studies are just the beginning of the complex management algorithms of peripheral nerve injuries and are tempered with an experienced approach to repair. All the tools in one's armamentarium should be considered, while individualised care in response to the unique attributes of these complex cases is applied.

We recommend early radial nerve exploration (within the first 2 weeks) in patients with open fractures or high-energy closed fractures of humerus with radial nerve injury. To draw a more convincing conclusion on the optimal management strategy, more methodologically improved trials with standardised outcome measures are recommended in future work.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1627-1632.
https://doi.org/10.3889/oamjms.2018.325
eISSN: 1857-9655
Clinical Science

Emotional Processing In Patients with Ischemic Heart Diseases

Shirali Kharamin[1], Mohammad Malekzadeh[2], Arash Aria[3], Hamide Ashraf[2], Hamid Reza Ghafarian Shirazi[4*]

[1]Clinical Psychology, Yasuj University of Medical Sciences, Yasuj, Iran; [2]Health Psychology, Yasuj University of Medical Sciences, Yasuj, Iran; [3]Internal Medicine, Yasuj University of Medical Sciences, Yasuj, Iran; [4]Social Determinants of Health Research Center, Yasuj University of Medical Sciences, Yasuj, Iran

Citation: Kharamin S, Malekzadeh M, Aria A, Ashraf H, Ghafarian Shirazi HR. Emotional Processing In Patients with Ischemic Heart Diseases. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1627-1632. https://doi.org/10.3889/oamjms.2018.325

Keywords: IHD; Emotional processing; CVD; Stress; Psychosomatic

***Correspondence:** Hamid Reza Ghafarian Shirazi. Social Determinants of Health Research Center, Yasuj University of Medical Sciences, Yasuj, Iran. E-mail: gshr3@yahoo.com

Received: 18-Jul-2018; **Revised:** 23-Aug-2018; **Accepted:** 27-Aug-2018; **Online first:** 20-Sep-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Cardiovascular disease is the most prevalent public health problem on a worldwide scale, and ischemic heart disease accounts for approximately one-half of these events in high-income countries. One of the most important risk factors for this disease is mental and psychological especially stressful experiences.

AIM: This research was established to compare emotional processing, as a key factor in stress appraisal, between IHD patients and people with no cardiovascular disease.

METHODS: Using simple sampling, fifty patients were selected from people who diagnosed as IHD in the hospital and referred for treatment after discharging care and treatment. Control group participants were selected as control group peoples, using neighbourhood controls selection. The Emotional Processing Scale was filled by all members of the two groups.

RESULTS: There were significant differences between the two groups on the EPS-25 total scores, as well as on emotional processing dimensions of signs of unprocessed emotion, unregulated emotion; avoidance and impoverished. Also, there was no significant difference between the two groups in the dimension of Suppression. The final step of regression revealed a β of 10.15 and 1.05 for AVO and IEE subscales respectively.

CONCLUSION: The result showed that patients with IHD are using more negative emotional processing styles.

Introduction

Cardiovascular disease (CVD) is the most prevalent public health problem on a worldwide scale [1], and ischemic heart disease (IHD) accounts for approximately one-half of these events in high-income countries [2]. Despite an improvement in treatments and prevention, IHD still caused over 2.1 million deaths (23% of all deaths) in Europe in 2015 and resulted in over 165 million disability-adjusted life-years (DALYs) lost in 2012 (6% of all disability claims) [3] [4].

The relationships between psychosocial risk factors and CVD have been investigated in a variety of laboratory [5] [6] and epidemiologic studies [7] [8] [9] [10]. This connection has been the subject of an ever-growing body of literature over the last 50 years [11] [12]. The majority of these studies have demonstrated the relationship between the chronic and acute stress [13] [14] [15] [16] [17], and its aversive emotional and psychological consequences, such as depression [18] [19] [20], anger [21] [22], PTSD [23] [24], anxiety [25] [26], and CVD. Therefore, the role of negative emotion in CVD has been notable in recent works [27] [28] [29]. To explain associations between psychosocial factors (especially stressor) and CVD, several biological and behavioural mechanisms have been proposed, including inflammatory processes, lack of exercise, and lifestyle-related factors [5] [30].

Negative emotions are a common reaction to stressful experiences, and different approaches to processing these emotions may have distinct consequences for the stress response trajectory [31]. In other words, the types of emotion regulation or processing could change the consequences of stressors as fundamental factors contributing to the pathophysiology of CVD. Therefore, recently, researchers have investigated whether poor emotion regulation and processing capacity could be associated with CVD [31] [32] [33]. Emotional processing can be either helpful or harmful, and the consequences of attending to emotions may depend on the nature of the emotional processing. Emotional

process can be referred to as psychological, psychophysiological and psycho-neurological mechanisms by which distressed emotional reactions in individuals are converted or changed to non-distressed reactions [34]. According to Rachman paper [35], the incomplete abortion or processing emotion could result in direct and indirect signs.

The role of this mechanism in the emergence or maintenance of some psychological disorders such as PTSD [36], panic disorder [37], depression [38] has been investigated in many studies. Also, its contribution to psychosomatic disorders including fibromyalgia [39], chronic fatigue [40] chronic pain [41], inflammatory bowel disease [42] and functional gastrointestinal disorders [43] has been proposed. Literature also showed a relationship between the excessive emotional regulation and some physical illnesses such as cancer [44], cardiovascular diseases [33] and multiple sclerosis [45]. It has also been considered as an important factor in psychotherapy [46] [47].

Despite the strong evidence reporting the role of emotional processing in consequence of stress and linking negative emotion (as the consequence of stress), few studies have been able to examine this relation to the development of CHD, [*29*][48]. For example, *Kubzansky and Thurston* [29] reported that those reporting high levels of emotional vitality (is characterized by a sense of energy and positive well-being in addition to being able to regulate emotions effectively) had multivariate-adjusted relative risks of 0.81 (95% confidence interval, 0.69-0.94) for Coronary Heart Diseases (CHD).

In spite of these studies, (majority investigated emotional regulation), the relationship of emotional processing and CVD has been remained provocative. Therefore, the existence of a comprehensive study with all aspects of its emotional processing is completely felt. This research aimed to investigate this relationship. This study was based on the hypothesis that emotional processes play a key role in IHD; therefore, they should report higher scores in emotional processing scale.

Methods

Using simple sampling, fifty IHD patients were selected. The patients were selected from people who diagnosed as IHD in the hospital and referred to Heart clinic for treatment for after discharging care and treatment. Fifty non-patient people were selected as control group peoples, using neighbourhood controls selection

Emotional processing scale (EPS). The Emotional Processing Scale (EPS) is a 25-item, five-factor self-report questionnaire designed to measure emotional processing styles and deficits [49]. The scale is rated on a ten-point scale (0 for completely disagree to 9 for completely agree). It measures five dimensions namely: suppression (SUP), signs of unprocessed emotion (SUE), unregulated emotion (UE), avoidance (AVO) and impoverished emotional experience (IEE). This scale has reported favourable psychometric properties, including high internal consistency and high temporal reliability.

The coefficient α value for the scale was .92. Internal consistency was high (α ≥ 0.80) for three factors and moderate for two (α ≥ 0.70). The Pearson's test-retest correlation coefficient obtained for the entire scale was 0.74. The psychometric data on final 25-item version also showed internal consistency 0.92, 0.88 and 0.90 for the UK, Italian and Italian & UK data respectively [50] [51].

During one month all questionnaires (EPS) were completed by patients who referred to Heart clinic for treatment after discharge from the hospital. All patients were diagnosed by hospital cardiologist as IHD. After selection, the IHD patient, his/her house address had been determined and among 4 neighbourhoods from left and 4 from right the most similar person to the patient (age, education, gender, economic status, marriage status and ….) was selected as a matched control person. The patient would have been removed from the case group if he/she had reported any psychiatric disorders, additional physical diseases or any cognitive inability.

Data were analysed using SPSS version 22. Frequencies and score means were obtained for demographic variables and were analysed by independent T-test (for age) and chi-square (for gender, education and marital status variables). The average scores of two groups were compared by using independent T-test for SUP and SUE subscales (met criteria for normality) and because of significant level for normality test, Mann-Whitney U test for UE, AVO, IEE subscales and total scores. Also, a logistic regression (backward model) was conducted to determine odds ratios of developing IHD for each variable of interest. The five subscales were included in the analysis as predictor variables and IHD as the dependent variable. A p-value < 0.05 was considered to be statistically significant.

Results

The demographic statistics of the research participants are presented in Table 1. The sample size was 100 (50 IHD and 50 control group). The mean age of the IHD group was 59.84 years (SD = 14.78; ranged 24-88). The mean age of the control group was 58.2 years (SD = 14.60; ranged 22-90).

Table 1: Sample demographic data

Variables	MI group	Controlled group	Values of differences
Age	59.84 (14.63)	58.2 (14.78)	T = 0.56,df 98, Sig 0.59
Sex (%)			
Male	23 (46)	27 (54)	x^2 = 0.16, df 1, Sig 0.84
Female	25 (50)	25 (50)	
Education (%)			
Primary to high school Education	46 (92)	43 (86)	x^2 = 0.9, df 1, Sig 0.26
Academic Education	4 (8)	7 (14)	
Marriage status			
Married	47 (94)	48 (96)	x^2 = 1.04, df 1, Sig 0.31
Single	3 (6)	2 (4)	

The result showed that majority of participants in both groups had under academic education (92% for IHD and 86% for control group) and 95% of them were married (94% for IHD and 96% for control group). Using independent T-test for comparing the age and chi-square for sex, education, and marital status, there was no significant difference between the two groups.

The result indicated that mean of total scores in EPS was 140.26 ± 27.81 for IHD group and 123.56 ± 26.71 for control group. Table 2 presents the group means and standard deviations for the total scores of EPS-25 and the five dimensions of emotional processing. There were significant differences between two groups on the EPS-25 total scores ($Z = 3.048$, $p < 0.002$), as well as on emotional processing dimensions of: signs of unprocessed emotion ($T (98) = 2.39$, $p < 0.001$), unregulated emotion ($Z = 2.33$, $p < 0.02$); avoidance ($Z = 3.48$, $p < 0.001$) and impoverished ($Z = 2.94$, $p < 0.003$). In addition, there was no significant difference between two groups in dimension of Suppression ($T (98) = 0.37$, $p < 0.7$).

Table 2: Means and standard deviations MI and control groups' scores in EPS

		Means(SD)			
Items	**MI group**	**Controlled group**	**Total**	**T**	**P Value**
SUP	25.28(9.38)	24.62 (14.8)	24.95 (8.78)	0.37	0.71
SUE	29.68(6.84)	26.46 (6.66)	28.08 (6.90)	2.39	0.01
		Mean rank		**Mann-Whitney**	
UE	57.26	43.74		912	0.02
AVO	60.59	40.41		745.5	0.0001
IEE	59.03	41.97		823.5	0.003
Total	59.34	41.66		808	0.002

Suppression (SUP); signs of unprocessed emotion (SUE); unregulated emotion (UE); avoidance (AVO) and impoverished emotional experience (IEE).

Table 3 showed the data resulted from logistic regression. The Omnibus Test showed a chi-square of 16.74, df = 2 and $p < 0.0001$. The Hosmer and Lemenshow Test also showed a chi-square of 5.21, df = 8 and $p < 0.73$. Also, the overall predicted percentage for the model was 66, and it explained between 15.6 to 20.8 percentages of variances. In the final step of the backward system of analysis (step 4), the result showed that the only predictor variable with a significant value in this equation was AVO with $\beta = 1.16$ (95% C.I. = 1.03-1.30). In addition, IEE showed a significant value near to significant level with $\beta = 1.05$ (95% C.I. = 0.99-1.10 and significant value = 0.06)

Table 3: Logistic regression for exploring the correlates (emotional processing) of IHD

								95% CI. for EXP(B)	
		B	S.E.	Wald	df	Sig.	Exp (B)	Lower	Upper
Step 1	SUP	-0.005	0.027	0.033	1	0.855	0.995	0.945	1.048
	SUE	-0.009	0.045	0.043	1	0.836	0.991	0.907	1.083
	UE	0.012	0.035	0.125	1	0.723	1.012	0.946	1.083
	AVO	0.140	0.053	7.035	1	0.008	1.150	1.037	1.276
	IEE	0.047	0.040	1.379	1	0.240	1.048	0.969	1.134
	Constant	-4.907	1.662	8.722	1	0.003	0.007		
Step 2	SUE	-0.009	0.045	0.036	1	0.849	0.991	0.908	1.083
	UE	0.013	0.034	0.157	1	0.692	1.014	0.948	1.083
	AVO	0.140	0.053	7.018	1	0.008	1.150	1.037	1.276
	IEE	0.045	0.038	1.404	1	0.236	1.046	0.971	1.126
	Constant	-5.027	1.532	10.762	1	0.001	0.007		
Step 3	UE	0.012	0.033	0.135	1	0.713	1.012	0.948	1.081
	AVO	0.138	0.052	7.115	1	0.008	1.148	1.037	1.271
	IEE	0.041	0.033	1.571	1	0.210	1.042	0.977	1.111
	Constant	-5.098	1.486	11.764	1	0.001	0.006		
Step 4	AVO	0.140	0.051	7.408	1	0.006	1.150	1.040	1.273
	IEE	0.048	0.026	3.396	1	0.065	1.049	0.997	1.105
	Constant	-4.979	1.446	11.853	1	0.001	0.007		

Discussion

The role of psychological factors, especially stress, in heart diseases has been investigated in health psychology literature and possesses from rich evidence-based credit. It seems that the impact of stressors could be changed as a consequence of emotional processing styles. The present study aimed to compare the emotional processing style between IHD patients and normal people.

Demographic data revealed no significant differences between two groups that confirm an acceptable matched samples selection. In another word, the result shows that two groups in the majority of variables that could be confounding are (to some extent) the same. The IHD is the only variable which was different in the two groups.

To our knowledge, this is the first study that examined the relation between IHD and comprehensive aspects (five domains) of emotion processing. We found that patients in four domains (from five domains) of emotional processing had significantly higher scores. That was also the case in total scores of emotional processing. Therefore, the hypothesis that negative emotional processing in IHD is more than none patients group was supported. Similar to previous studies [13] [18], this study showed that patients with IHD reported higher scores in EPS.

In this study, result showed that IHD patients reported significantly higher scores in subscales of SUE, UE, AVO, IEE and total scores than none patients group. The higher scores in this scale, the intended negative emotional processing is used more. In other words, emotional processing with potentially distinct effects on the stress response trajectory is more negative in this group of patients. The binary logistic analysis confirmed the goodness of fit for the model. Although the result of regression showed that only AVO subscale (and to some extent IEE) was a significant predictor variable for IHD, the high correlation between subscale (as a dimension of unit

structure) could be accounted for removing other subscales (SUE, UE) from modelling [52]. This implies that people with more score in AVO are more vulnerable to IHD

Several biological and behavioural mechanisms could be proposed to explain this association.

First, the positive emotional processing and regulation of may lead to health-protective behaviours and lifestyle system [32] [53] [54]. For example, Pressman and Cohen found that greater emotional vitality was significantly associated with less smoking, alcohol consumption, and more physical activity. Second, it may alter disease susceptibility by acting directly on biological systems [54]. For example, recent investigations have demonstrated associations of positive affect with lower heart rate, lower levels of cortisol, and attenuated fibrinogen stress responses as well as with reduced ambulatory systolic blood pressure assessed 3 years later [54] [55].

Third, it may change the stress reaction such as negative emotion. Gross in his theory showed that the individual differences in using different methods of cognitive emotion regulation would carry out different emotional, cognitive, and social consequences. For example, the use of reappraisal styles is related to positive emotional experiences and better intrapersonal practices, and higher well-being [56]. Therefore, better emotion regulation capacity could modify stress reactions associated with certain mental disorders (such as depression, anxiety and anger). For example, the relationship between anger outbursts, depression and anxiety and CHD may (partly) have its basis in emotion regulation [57] [53].

To conclude, the current findings suggest that negative emotional processing style may be associated with producing IHD by potentially distinct effects on the stress response trajectory. In other words, patients with IHD are using more negative emotional processing styles.

This study contains some limitations that are important to acknowledge. The sample consisted of only IHD population. Therefore, it is recommended to use other types of CVD with different types. Secondly, this research studied the emotional processing in this group of patients after the appearance of IHD. Therefore it should be better to investigate this variable in a cohort study in general population and during a long period.

Ethical approval

All procedures performed in studies involving human participants were by the Yasuj University of Medical Sciences Research Ethics Committee and in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1633-1635.
https://doi.org/10.3889/oamjms.2018.375
eISSN: 1857-9655
Clinical Science

Association between Haemoglobin A1c and Uric Acid Levels among Patients with Diabetes Mellitus Type 2 at a Primary Health Care Clinic in North Sumatera, Indonesia

Rusdiana[1*], Sri Suryani Widjaja[1], Muhammad Syahputra[1], Maya Savira[2]

[1]Departement of Biochemistry, Faculty of Medical Universitas Sumatera Utara, Jl. Dr Mansur Kampus USU Medan 20155, Indonesia; [2]Departement of Physiology, Faculty of Medical Universitas Sumatera Utara, Jl. Dr Mansur Kampus USU Medan 20155, Indonesia

Citation: Rusdiana, Widjaja SS, Syahputra M, Savira M. Association between Haemoglobin A1c and Uric Acid Levels among Patients with Diabetes Mellitus Type 2 at a Primary Health Care Clinic in North Sumatera, Indonesia. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1633-1635. https://doi.org/10.3889/oamjms.2018.375

Keywords: Uric acid; Hba1c; Fasting Blood Sugar; Type2 diabetes mellitus

***Correspondence:** Rusdiana. Department of Biochemistry, Faculty of Medical Universitas Sumatera Utara, Jl. Dr Mansur Kampus USU Medan 20155, Indonesia. E-mail: rusdiana7165@yahoo.com

Received: 28-Jun-2018; **Revised:** 22-Aug-2018; **Accepted:** 24-Aug-2018; **Online first:** 22-Sep-2018

Funding: Ministry of Research and Technology and Higher Education Republic of Indonesia. The support is under the research grant BP-PTN USU of Year 2017 Contract Number×97/UN5.2.3.1/PPM/KP-TALENTA USU/2017

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

Abstract

BACKGROUND: Haemoglobin A1c (Hba1c) levels and uric acid levels may be associated.

AIM: This study aimed to determine Hba1c, and uric acid levels are associated among patients with diabetes mellitus type 2 who attend a primary health care clinic in North Sumatera, Indonesia.

METHODS: We conducted a cross-sectional study among patients was conducted on 70 type 2 Diabetes Mellitus patients who attended Primary Health Care in Binjai. Patients with age > 40 years old attend a primary health care clinic in Binjai city, North Sumatera with diabetes mellitus type 2. In each subject demographics, age, sex, body mass index, blood pressure, post health history, fasting blood sugar, Hba1c and uric acid levels were checked and recorded. A student's t-test was used to determine if there was an association between Hba1 and uric acid levels. A total of 70 were included in this study.

RESULTS: The mean age of study subjects was 58.33. The mean Hba1c level was 8.743, and standard deviation (SD) was 1.80. The mean of uric acid was 6.31, and standard deviation (SD) was 1.58. The statistical analysis using T-test found that there was no significant association between Hba1c and uric acid levels among study subjects ($p > 0.05$).

CONCLUSION: We found no significant association between Hba1c and uric acid levels among the study subjects.

Introduction

Diabetes mellitus (DM) type 2 is increasing incidence and prevalence [1]. DM is a leading cause of morbidity and mortality worldwide [2]. About 2-3 % of the world's population is estimated to have DM [3]. People with DM are at higher risk for cardiovascular disease, nephropathy and retinopathy [3] [4].

Haemoglobin A1C (Hba1c) is a measure of glycosylated haemoglobin over the period 3 months due to the usual lifespan of erythrocytes of 120 days and is used to monitor control of blood glucose levels in patients with DM [5].

Serum Uric acid is the final oxidation product of purine metabolism in the circulation. Elevated serum uric acids levels are associated with increased risk for cardiovascular disease and so the metabolic diseases such as metabolic syndrome and diabetes mellitus [6]. Patients with hyperuricemia are significantly more likely to DM [7]. Some study suggests uric acid may be associated with glycometabolic disorders, because of this association between uric acid and glucose metabolic [8]. However, there is not a linear association between uric acid and blood glucose levels. Hyperuricemia in patients with diabetes mellitus type 2 associated with increased risk for diabetic nephropathy [9]. Serum uric acid level among patients with diabetes mellitus type 2 may are with the development of macroalbuminuria

and microvascular disease [10]. Uric acid levels rise with increasing blood glucose concentrations in the normal and prediabetes population [11].

However, among patient with diabetes mellitus type 2, uric acid levels tend to decline with increasing blood glucose concentration [12]. The reason for the inverse relationship is unclear. However, insulin levels are also closely related to uric acid levels [13]. Serum uric acid levels are directly associated with serum insulin levels in diabetic, but the mechanism for this is not clear [14]. We aimed to determine if there is an association between HbA1c and uric acid among patients with diabetes mellitus type 2 who attend a public health care clinic in Binjai city, North Sumatera Indonesia. This place is many Diabetes Mellitus type 2 patients.

Material and Methods

This study is a cross-sectional evaluation of consecutive sampled subjects who attend a primary health care clinic in Binjai city, North Sumatera, Indonesia. Inclusion criteria the subjects of sex aged > 40 years old and a history of confirmed on who were willing to participate in the study. Exclusion criteria were those who were undergoing treatment for cancer therapy or who were taking a diuretic. This study was approved by the Health Research Ethics Committee, Faculty of Medicine, Sumatera Utara Universitas/H. Adam Malik General Hospital number 591/TGL/KEPK FK USU-RSUP HAM/2016.

In each subject, demographic, age, sex, body mass index, abdominal circumstance, blood pressure, and laboratory tests such as fasting blood glucose, HbA1c and uric acid levels were obtained and recorded. The student's T-Test was used to determine an association between HbA1c and uric acid levels. A p-value < 0.05 was considered statistically significant.

Results

A total of 70 subjects were included in the study, 69 % female and 31% male.

Table 1: Age and laboratory result among study (n = 70)

	Min	Max	Mean	SD
Age	40	76	58	9
Uric Acid	3.8	10.9	6.3	1.6
HbA1c	5.7	12.5	8.7	1.8

The mean (range) Uric acid level among study the subjects was 6.3 (3.8-10.9 mg/dl). The mean (range) HbA1c level was 8.7 (5.7-12.5 %). We found no significant association between Hba1c and uric acid levels among the study subjects. We used the statistical analysis with chi-square test and found that there was no correlation at type 2 diabetes mellitus patients in Binjai city of North Sumatera in Indonesia (n = 70, p > 0.05).

Discussion

Over the years, the association between uric acid levels and glucose metabolism has been a hot research topic. A growing number of studies have indicated that there is a bell fit between uric acids and glucose concentrations. Many previous studies have linked uric acid to type 2 diabetes mellitus, but studies linking uric acids to HbA1c are scarce. Some studies have observed an increase in Uric acid levels in type 2 diabetes mellitus and our study found that there was no association between HbA1c and uric acid level in the subjects.

In the research by Yuliang Cui et al. that is an inverse correlation between uric acid and HbA1c, which is dependent on hyperinsulinemia in patients with newly, diagnosed with type 2 diabetes. Some studies have found that serum uric acid levels are inversely correlated with blood glucose concentration in type 2 diabetes mellitus patients. However, until now, it has been unclear as to why this relationship exists and what factors influence this relationship.

High insulin levels may be important factor affecting the correlation between the uric acid and HbA1c, the same with Fengjiang Wei et al., they found that serum uric acid level is inversely associated with HbA1c in Type 2 Diabetes Mellitus patients and according to the research by Walid G Babkir et al show that patients with type 2 diabetes mellitus serum uric acid level has an adverse effect on glycemic control, but the research by V. Pavithra etc. has strongly established an association between uric acid and Hba1c thereby linking uric acid, the end product of purine metabolism to DM.

Acknowledgements

The authours gratefully acknowledge financially supported of the Ministry of Research and Technology and the Higher Education Republic of Indonesia. The study was supported by the research grant TALENTA USU 2018.

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Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1636-1641.
https://doi.org/10.3889/oamjms.2018.348
eISSN: 1857-9655
Clinical Science

Intralesional Neodymium YAG laser to Treat Complications of Polymethylmethacrylate

Alberto Goldman[1], Uwe Wollina[2*]

[1]Clinica Goldman and Hospital Moinhos de Vento, Porto Alegre, Brazil; [2]Städtisches Klinikum Dresden, Academic Teaching Hospital Dresden, Department of Dermatology and Allergology, Dresden, Germany

Citation: Goldman A, Wollina U. Intralesional Neodymium YAG laser to Treat Complications of Polymethylmethacrylate. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1636-1641. https://doi.org/10.3889/oamjms.2018.348

Keywords: Permanent fillers; Polymethylmethacrylate; Adverse reactions; Intralesional Nd: YAG laser; Management of adverse filler events

***Correspondence:** Uwe Wollina. Städtisches Klinikum Dresden, Academic Teaching Hospital Dresden, Department of Dermatology and Allergology, Dresden, Germany. E-mail: uwollina@gmail.com

Received: 18-Jul-2018; **Revised:** 23-Aug-2018; **Accepted:** 08-Sep-2018; **Online first:** 23-Sep-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Dermal fillers are widely used for facial and body contouring. Polymethylmethacrylate (PMMA) is a permanent biphasic filler for soft tissue augmentation. In case of unwanted side effects, drug therapy and surgical excision have been commonly used with mixed results.

AIM: We report on a series of patients with adverse events to PMMA and an innovative minor invasive procedure to reduce disfigurement by nodules and lumps.

METHODS: We employed a subdermal, intralesional 1,064 nm neodymium-doped yttrium aluminium garnet (Nd: YAG) laser in combination with suction using a blunt liposuction cannula of 2.0-2.5 mm of diameter.

RESULTS: For 12 years, a total of 81 consecutive subjects (79 females and 2 males) were treated. The average age of the patients was 43.7 years (range 26 to 76 years). Granulomas and lumps could be removed in a minor invasive procedure with tumescent anaesthesia. In a minority of cases, the procedure had to be repeated. The results were impressive and not adverse events related to intralesional laser therapy were observed, 86.4% of patients were satisfied.

CONCLUSION: The procedure should be used before or in combination with classical surgery to remove PMMA in case of adverse tissue reactions to PMMA.

Introduction

Dermal or soft tissue fillers are versatile tools for correction of wrinkles, loss of volume and facial sagging. A broad range of filler materials is available from biodegradable to permanent products [1] [2]. Polymethylmethacrylate (PMMA) is a hydrophobic permanent biphasic filler for soft tissue augmentation. PMMA microspheres are suspended in either collagen (like in ArteFill® or Artecoll®) or methylcellulose (like in MetaCrill®) [3]. PMMA (Bellafill®) is the only FDA-approved filler for the correction of atrophic acne scars [4] [5]. PMMA injections have become a fashion in Brazil and other Latin American countries during recent years, and various adverse events have been noted.

Filler injections complications have been classified as early (0–14 days), late (14 days to 1 year), or delayed (> 1 year) [6]. PMMA implants have the potential to elicit a cellular immune response in humans, although the mechanism of the late and ongoing inflammation, granuloma formation or nodules is not well understood [7]. One factor contributing to adverse events is the type and localisation of injection. In an animal study, submucosal injections were prone to nodularity in contrast to subcutaneous injections [8]. In humans, granulomatous reactions to PMMA are well-known. Other factors that are being discussed include infections, foreign body formation, and biofilms [7] [9] [10].

Early adverse events after PMMA injections include erythema, swelling and itching. PMMA has

been reported to cause in particular delayed and late adverse effect. In a study from Goiás, Brazil, the authors identified 11 cases of complications of PMMA injections to the midface which started from two to 24 months after the injection. Oedema, erythema, and contour irregularity were seen in 100% of patients, followed by nodules (64%), yellowish xanthomatous pigmentations (36%), and eyelid malposition (18%). Histopathology demonstrated an ongoing inflammation with giant cells. Corticosteroid injection was of minimal effect. Surgical removal was performed in 82% of cases and resulted in an improvement of oedema, erythema, and nodules [11]. Salles et al., [12] observed 32 complications during 15 years of PMMA injections for soft tissue augmentation. They reported acute tissue necrosis (n = 5), delayed granulomas (n = 10), and late chronic inflammatory reactions (n = 10). Lymphedema of the lips was noted in 6 patients, and infection was seen in a single patient [12]. The most severe adverse events observed with PMMA are extended soft tissue necrosis and blindness due to unintended intravascular injection [13] [14].

Many treatments were suggested to improve these symptoms including corticosteroids and other drugs injections, open surgeries, suctions etc. Each adverse event requires a specific treatment or a combination of more than one approach. Acute infections warrant an adequate and specific antibiosis. Culture - if it possible - is mandatory. Granulomatous and inflammatory reactions can be treated with injections of steroids. However, the response is usually very poor. Side effects using steroids injection are very frequent including neovascular formation and subcutaneous adipose tissue atrophy. 5-Fluoracil also represents an alternative injection treatment for granuloma reaction. Oral allopurinol is occasionally employed for the treatment of inflammatory reaction. In our experience, the response was also very poor or limited. Traditional plastic surgery is trying to remove some amount of PMMA what usually represents a challenge. Fibrosis, subcutaneous tissue scarring and the proximity to important vascular and nerves plexus can lead to catastrophic complications. Specific regions such as the lips, nose, glabella, buttocks, legs, hands and penis are probably the most difficult areas to be treated [11] [12] [15].

Patients and Methods

We analysed patients with adverse effects to polymethylmethacrylate (PMMA) injections in the face and body which had been treated between June 2006 and July 2018 at Clinica Goldman de Cirurgia Plastica and Hospital Moinhos de Vento, in Porto Alegre, Brazil. The great majority of our patients already used all the above-cited treatments without result, with very poor improvement or event with exacerbation of the symptoms and deformities.

Often the adverse events had been delayed or late with the first occurrence even after 10, 15 or more years. Adverse late events suddenly appeared with erythema, unevenness, dislocation or migration of the product, pain, inflammatory reactions, deformities (sometimes bizarre deformities), nodules and granulomas, functional limitations and others. In our series, longer lasting redness is a very frequent adverse event. For many years such cases have been treated (initially in the face and after in breast, buttocks, chest, legs) using the subdermal 1,064nm neodymium-doped yttrium aluminium garnet (Nd: YAG) laser in continuous-wave mode. The treatment is based on the same concept as laser-lipolysis and laser-assisted liposuction.

With this procedure PMMA granulomas were treated by Nd: YAG laser in a total of 81 consecutive subjects who underwent the intralesional laser procedure, 79 were female and 2 male. The average age of the patients was 43.7 years (range 26 to 76 years).

All subjects underwent pre-operative assessment including autoimmune evaluation. All subjects provided informed consent. Imaging techniques were also employed in selected patients preoperatively (Figure 1). The procedures were performed in tumescent anaesthesia after subcutaneous infiltration of a solution containing lidocaine with vasoconstrictor and warm saline solution. The solution varies according to the area to be treated. Usually, all procedures in the face were made under local anaesthesia and sedation. Body procedures, including chest, penis, legs or buttocks were performed under general anaesthesia (intravenous anaesthesia). The laser power varied from 6 to 18W.

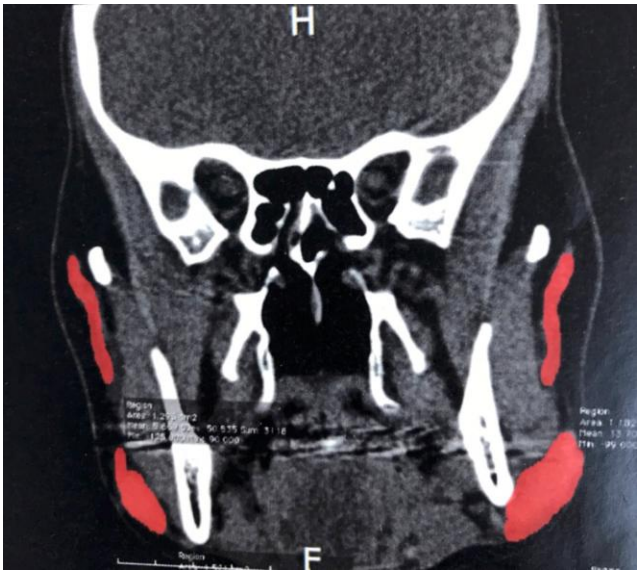

Figure 1: Magnetic resonance imaging demonstrating intense contrast impregnation in the mandibular region and pterygoid muscle (PMMA)

The laser energy is delivered to the affected tissue through a 300-600-micron fibre optic, embedded in a 1 or 1.6 mm diameter stainless steel micro-cannula of variable length. The distal portion of the fibre optic is extended approximately 2 mm beyond the distal end of the cannula allowing the direct contact of the laser energy with the organic tissue and synthetic material/granuloma. The helium-neon (He: Ne) laser source is combined into the laser beam allowing a precise visualisation of the region where the energy is acting, due to cutaneous trans-illumination (Figure 2).

Figure 2: Granuloma in the malar region due to PMMA injection. Treatment by intralesional Nd: YAG laser

The cannula is moved intralesional within the tissue. The external skin temperature is controlled using a digital thermometer. The upper limit of skin temperature is 42^{o} Celsius to avoid a potential skin burn. In some subjects, a thermal camera was used at the same time as the laser action, which provides an accurate and instantaneously skin and tissue temperature control. Cold compresses were applied in the treated laser region to maintain skin integrity and avoid skin damage. Laser treatment is delivered over a variable length of time according to the amount of granuloma, facial or body location, previous treatments, external scar, internal fibrosis and resistance.

Figure 3: a Typical aspect of aspirated PMMA material after laser action

The main concept and aim of the internal laser treatment are to produce tunnels in the affected region and to disrupt the synthetic alloplastic filler facilitating the suction or its surgical excision.

Usually, the endpoint for the laser action is the temperature and mainly the resistance during cannula advancement in the fibrous tissue. The product of the laser action is removed using the negative pressure of around 450 mm Hg in conjunction with suction using a blunt liposuction cannula of 2.0-2.5 mm of diameter. The quantity of material removed from the patient varies according to each situation (Figure 3). The procedure lasts from 30 minutes to 3 hours.

Figure 4: Before (a) and after (b) intralesional Nd: YAG laser therapy of PMMA-induced nodule on the mandibular margin

We have observed patients with a very small quantity of PMMA in the face but severe complications. At the same time, patients with a larger quantity of injected PMMA in the chest/breasts had no inflammatory problems, foreign body granulomas or other clinical manifestations after long years. Probably there is no relationship between the amount of removed material using laser and the clinical improvement as well. Many patients with very small PMMA volume injections in the face had an impressive clinical and aesthetic improvement after laser therapy (Figures 4, 5 and 6).

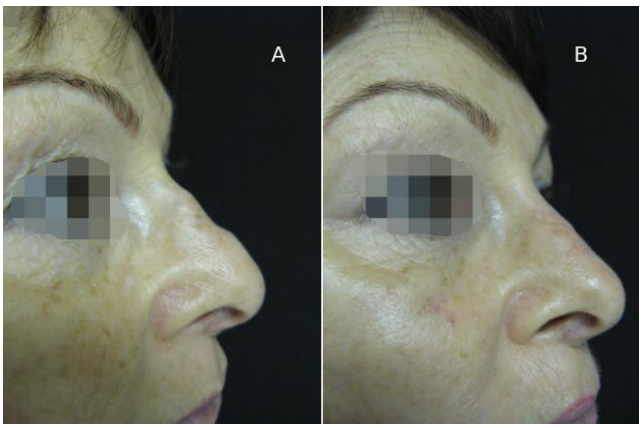

Figure 5: PMMA nodule of the nasal dorsum (a); After treatment with an intralesional laser (b)

Histological studies were performed in all subjects, focusing on the effects of the laser. The laser produces channels along the fatty tissue and fibrous tissue including the granulomas and the alloplastic filler material (Figure 7).

Figure 6: a Typical aspect of a patient with problems related to PMMA injection; (a) Plethoric face, inflammatory aspect, granulomas, deformities and some neovascular formations; (b) Result after intralesional laser treatment on the mandibular border, the malar and zygomatic region

The procedure had to be repeated twice in 8.6%, three times (4.9%), and four times (1.2%; chest and buttocks) for optimum results. Complications included four cases of late seroma. All solved with serial aspiration. No case of infection, burn or necrosis was observed. Transient unilateral temporary paresis of nerve branches was observed in 7 subjects. The paresis disappeared completely during a period of 3 weeks to 3 months.

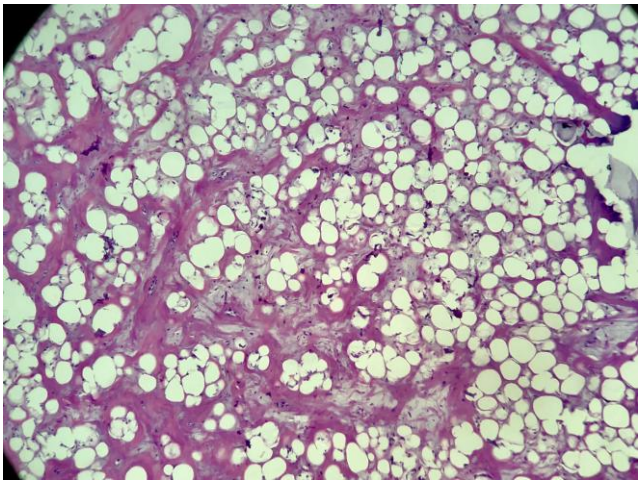

Figure 7: Histology demonstrates spherical particles of exogenous material morphologically compatible with PMMA surrounded by foreign body type granulomas, lymphocytic infiltrates and connective tissue with hyalinization (Hematoxylin-eosin, x4)

Since PMMA induces tissue fibrosis, the main idea was to create tunnels splitting (cleavage isolating the product) the material and fibrosis from the muscle, subcutaneous tissue, fascia, skin or mucosa using the laser as well as trying to disrupt the methacrylate into small particles facilitating the suction using small cannulas. After the laser action, a small cannula was used to aspirate this material. The aspirated material was always sent to the pathologist. In all cases, it was easily possible to identify foreign body material (PMMA) in great quantity and practically no fibrosis or organic tissue around the PMMA. The quantity of PMMA removed without laser was much less than with laser energy.

Seventy of 81 patients (86.4%) were very satisfied by the outcome with an important improvement of the deformities in the mandibular border, malar region, lips and other body rejoins. We noted a substantial improvement of the clinical symptoms like pain, inflammation, nodules and redness.

Some of the patients suffered from other extra-cutaneous complaints such as hypercalcemia and renal dysfunction (creatine elevation). These patients had PMMA in the breast, chest (male patient), and buttocks. All patients were submitted to subdermal laser treatment. In some patients, the procedure was repeated some months after the prior surgery. All patients had important clinical improvement, calcium level control and improvement in renal function. In another words - PMMA removal probably improved not only face and body deformities but systemic clinical complications produced or aggravated by the presence of PMMA.

The follow-up was up to 12 years without any relapse or later complications after Nd: YAG laser therapy.

Discussion

In case of complications caused by filler injections systemic drugs (e.g. corticosteroids, antibiotics etc.) and surgical removal of the material are the most widely used methods [16]. For fillers based on hyaluronic acid, the injection of hyaluronidase is an efficient option [17].

Another possible treatment option would be the removal of the material by intralesional laser treatment. The first group using intralesional laser therapy to handle filler complications was Cassuto et al., [18]. They brought to action the 808 nm diode laser at 6 to 8W with a pulse duration of 500 ms to 1000 ms. Their laser fibre had a diameter of 200 μm. The first twenty patients who were treated, reported a significant improvement of pain, tenderness, stiffness, and size of the nodules or granulomas [18]. The same group published a second paper including 219 patients. Complete disappearance of nodules and lumps was observed in 62%, partial improvement in 30% of patients [19].

Schalke et al., [20] combined an 810 nm diode laser (3-6 W) with a 1,470nm diode laser (0.6-0.8 W) in continuous-wave mode. They used fibre diameters between 200 μm and 600 μm for intralesional treatment of nodules and granulomas in 242 patients. In 92% of cases, they achieved an

improvement, in 9% a complete resolution.

The present study is the first one, using the intralesional Nd-YAG laser in analogy to laser lipolysis with the special focus on PMMA complications [21]. 86.4% of our patients were very satisfied. This is remarkable since we treated only patients wing with complications after PMMA injection in contrast to the other studies mentioned [18] [19] [20]. PMMA is more resistant to treatment than hyaluronic acid-fillers. With 81 patients treated for PMMA complications, this is one of the largest trials.

After puncturing the skin, a bare laser fibre is used to ensure a focused subdermal energy delivery. In this trial, the skin temperature was measured during laser action to prevent skin burns. It is important to avoid carbonisation of PMMA as well [18] [19] [20]. For removal of filler material, a blunt suction cannula with negative pressure was used in contrast to squeeze as suggested by others [18] [19] [20].

The laser energy interacts with adipose or connective tissue or - as in the present study - with PMMA. With the support of a pilot beam, the depth of the bare fibre can be visualised. The interaction of laser energy and polymer ions like PMMA can be described by the following formula: R + A + T = 1. The intensity of laser energy on the irradiated surface is I0, the intensity of the reflected irradiation is IR, and the intensity of the absorbed irradiation is coined IA. The intensity of the transmitted irradiation is named IT. R (IR/I0) is the ratio of reflection, A (IA/I0) the ratio of absorption, and T (IT/I0) the ratio of the transmission. R is higher in crystalline structures than in amorphous structures such as PMMA [22]. The 1,064 nm Nd: YAG laser has a high transmission for PMMA. During laser contact to the polymer surface the temperature increases rapidly, but after cessation of laser irradiation the temperature drops down beneath the melting point of the polymer [23].

The laser energy can cause fragmentation of PMMA by (1) charge-directed fragmentations, (2) charge-remote rearrangements, and (3) charge-remote fragmentations via radical intermediates [24]. The Nd: YAG laser irradiation results in a shallow focal trough with radiating fractures [25] [26]. In a liquid microenvironment, microchannels can be produced [27].

We suppose that microchannels are part of the intralesional laser action in living tissue. Eventual fragmentation of the material allows its evacuation. The liquefaction, we and other observed, is partly due to laser lipolysis.

Intralesional laser therapy is a safe technique in the hands of experienced users. We observed 4 cases of seroma (5%) after the procedure. Burning, post-inflammatory hyperpigmentation, and scarring could be avoided by skin temperature monitoring. We have not observed sterile abscess formation in contrast to other studies [18] [19] [20]. Transient minor impairment of facial nerves disappeared completely without intervention.

Our data argue for significantly improved removal of PMMA in subcutaneous tissue by Nd: YAG intralesional laser application. The procedure - if necessary - can be repeated.

Patients with adverse reactions to PMMA injection usually feel very insecure and depressed [28]. There is not a single definitive, safe and efficient treatment for all cases. Because of this, patients need to be informed regarding the limitation and expectation of each treatment. The necessity or convenience of retreatment using this intralesional laser treatment or even a combined approach must be discussed. It is important to be very clear about the fact that it is usually impossible to remove PMMA completely from the body or face. Clinical and immunologic associated problems such as hypercalcemia, lupus or rheumatic problems must also be treated. An interdisciplinary team, including dermatologist, plastic surgeon, rheumatologist, psychiatrist and other specialists, can lead to an improved and more effective global treatment. Although the intralesional Nd: YAG laser treatment has been shown to be effective and safe in this series, further studies are necessary to improve and clarify the utility of this technique.

Esthetic filler injections become increasingly popular. Those who perform injections of fillers need to know about potential adverse events, their prevention and treatment. The efficacy and favourable safety profile of intralesional laser treatment to treat PMMA-related complications suggest the use of this method before or in combination to classic surgery.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1642-1646.
https://doi.org/10.3889/oamjms.2018.374
eISSN: 1857-9655
Clinical Science

Clinical, Radiological and Bacteriological Profile of Lung Abscess - An Observational Hospital Based Study

Madhusmita Mohanty Mohapatra*, Manju Rajaram, Archana Mallick

Department of Pulmonary Medicine, JIPMER, Pondicherry, India

Citation: Mohapatra MM, Rajaram M, Mallick A. Clinical, Radiological and Bacteriological Profile of Lung Abscess - An Observational Hospital Based Study. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1642-1646. https://doi.org/10.3889/oamjms.2018.374

Keywords: Cavity with fluid level; *Klebsiella pneumoniae*; Lung abscess

***Correspondence:** Madhusmita Mohanty Mohapatra. Department of Pulmonary Medicine, JIPMER, Pondicherry, India. E-mail: drmadhusmita1@gmail.com

Received: 27-Jun-2018; **Revised:** 20-Aug-2018; **Accepted:** 21-Aug-2018; **Online first:** 23-Sep-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: The incidence of lung abscess acquired in the community is unknown, but this is a common clinical problem encountered in developing countries. The incidence of lung abscess was high in the pre-antibiotic era but the advent of susceptible antibiotics it has reduced with an equal fall in mortality to 8.7%. With the emerging antibiotic resistance and change in the trends of bacteriological profile causing lung abscess, it is the need of time to reevaluate lung abscess.

AIM: The study aimed to determine the clinical, radiological and bacteriological profile of lung abscess.

MATERIAL AND METHOD: The study was a non-randomized prospective observational study conducted in the department of pulmonary medicine for 18 months. In the study, patients > 15 years of age with clinical features of lung abscess were recruited and were subjected to chest X-ray, routine blood test. Sputum gram stain and culture, as well as antibiotic sensitivity according to the organism, were evaluated. Reports of all investigations along with patient characteristics and risk factors were analysed statistically using SPSS 20.0.

RESULTS: Forty-six cases of lung abscess were included, and the majority of patients were found to be adults with a mean age of 42.9 years with a male to female ratio of 6.6:1. The most common predisposing factor was an unhygienic oral cavity in 28% of cases with alcohol ingestion being the most important risk factor in 22% of cases. The most common organism found in lung abscess cases was *Klebsiella pneumoniae,* and they were sensitive to ceftazidime.

CONCLUSION: Our study shows that *Klebsiella pneumoniae* should be considered an important pathogen in community-acquired lung abscesses.

Introduction

Lung abscess is defined as an area of necrosis of lung parenchyma leading to the cavity with air-fluid level due to the formation of a bronchopulmonary communication. One cm to two cm sized necrotising abscesses coalesces to become large lung abscess. These lung abscesses can be primary or secondary to underlying lung disease, acute or chronic based on the duration of the disease, community acquired or hospital acquired in nature. The mortality was higher for lung abscess in the pre-antibiotic era, but with the advent of antibiotic therapy, the mortality has reduced to 8.7% [1].

Many risk factors are associated with the formation of lung abscess. Based on aetiology, the type of causative organism for lung abscess also varies. With the advent of antibiotics, the etiological trend has changed, and a large chunk of cases needs to be still evaluated. Anaerobes from oropharyngeal secretion and gram-positive organisms were indicated as the main pathogenic organism in few small studies earlier [2]. But due to lack of skilled physicians for transtracheal and transthoracic lung aspiration, the etiological diagnosis of lung abscess has been rare.

The radiological picture of the cavity with air-fluid level can occur at any site of the lungs. Whether this radiological distribution of lung abscess has any association with the aetiology is still not clear in the literature. With the problem of antibiotic resistance,

clindamycin and penicillin used previously for the treatment of community-acquired lung abscess are no longer effective.

Since little data is available on the clinical correlation/ characterisation of lung abscess and their management, hence there is a need to describe the various risk factors as well as the radiological and bacteriological profile of lung abscess.

The objective of this study is to evaluate the clinical, radiological and bacteriological profile of lung abscess in a tertiary care centre in India

Methodology

This prospective observational study was approved by the Institute scientific and ethical committee. The study was carried out for 18 months, and 46 cases of lung abscess were included over a period of 18 months. Patients were selected from outpatient and inpatient of the department of pulmonary medicine. A detailed history of unconsciousness, alcohol intake, epilepsy and anaesthesia were taken. History of oral and dental infection, dental extraction, diabetes mellitus, pneumonia and sinusitis was also elicited. A detailed clinical examination for evaluating lung abscess was done. A routine investigation like hemogram, sputum for gram stain and acid-fast bacilli, blood culture, sputum culture and antibiotic sensitivity and chest X-ray PA view was done. Apart from the above investigations bronchoscopy, CT scan thorax with CT guided FNAC, serology and immunology tests were done where there was ambiguity. Patients are having chest X-ray PA view showing cavity, and air-fluid level with fever (> 37.8^0 C), purulent sputum, cough were included in the study. Patients less than 15 years of age and with bronchiectasis, malignancy, pulmonary infarction was excluded from the study.

The data was prepared in excel sheet, and statistical analysis was done using SPSS version 20.0. The continuous variables were measured regarding the mean and standard deviation. The categorical variables were measured regarding percentage.

Results

In this study, the majority of the patients were above 40 years (58.7%) with a mean age of 42.9 years and an age range of 15 to 65 years. Out of 46 cases of lung abscess, 40 cases were males (87%) with a male to female ratio of 6.6:1 (Table 1).

Table 1: Demographic profile of lung abscess

Age	No.of cases	Percentage
> 40years	15	32.6%
< 40years	31	67.3%
Mean Age	42.9 years	
Sex	No.of cases	Ratio
Male	40	6.6:1
Female	6	

In our study, about 21 cases (45%) had predisposing factors and the most common predisposing factor was an unhygienic oral cavity in 13 cases (28%) followed by dental sepsis in 6 cases (13%). About 22 cases had associated risk factors and the most common risk factor found was alcohol ingestion in 10 cases (22%) and smoking in 8 cases (17%) (Table 2).

Table 2: Predisposing and risk factor for lung abscess

Predisposing factor	**No. of cases**	**Percentage**
Unhygienic oral cavity	13	28%
Dental sepsis	6	13%
Dental procedure	1	2%
Seizure	1	2%
Risk factor	**No. of cases**	**Percentage**
Alcoholic	10	22%
Smoker	8	17%
Diabetes	4	
COPD	2	

Amongst symptomatology, the most common symptom observed was a cough in 42 cases (91%). Fever was present in 38 cases (83%) (Table 3).

Table 3: Symptomatology of lung abscess

Symptomatology	**No. of cases**	**Percentage**
Fever	38	83%
Cough	42	91%
Expectoration	35	76%
Chest pain	10	21.7%
Hemoptysis	5	10.8%
Breathlessness	5	10.8%
Loss of appetite	8	17%

Radiologically majority of lung abscess was found to be located on the right side with the most common site being right upper lobe in 23 cases (52.17%) followed by right lower lobe in 9 cases (19.56%). Lung abscess was also present on left lung with the most common site being left upper lobe in 6 cases (13.10%). Majority of lung abscess presented radiologically with a cavity and fluid level. 34 cases (74%) represented a cavity radiologically with a fluid level while 8 cases (17%) were empty cavity (Table 4).

Table 4: Radiological site and characteristic of lung abscess

Radiological sites	**No.of cases**	**Percentage**
Right side		
Upper lobe	23	52.17%
Middle lobe	4	8.7%
Lower lobe	9	19.56%
Combined	1	2.17%
Left side		
Upper lobe	6	13.1%
Lower lobe	3	6.52%
Radiological Characteristic		
Cavity with fluid level	34	74%
Empty cavity	8	17%
Cavity with consolidation	4	9%

All lung abscess patients underwent sputum gram stain, and 35 cases (76.09%) were found to have a gram-negative organism. Two cases (4.35%) of lung abscess grew gram-positive organisms (Table 5).

Table 5: Sputum gram stain for lung abscess

Sputum Gram Stain	No.of cases	Percentage
Gram-positive	2	4.35%
Gram-negative	35	76.09%
Mixed	NIL	0%
No organism	9	19.56%

Sputum culture was done for cases which had gram stain results and the most common organism observed in lung abscess was gram negative *Klebsiella pneumoniae* in 23 cases (50%) followed by Escherichia. Coli in 12 cases (26%) cases (Table 6).

Table 6: Sputum culture for lung abscess

Organism	No.of cases	Percentage
Klebsiella	23	50%
E.coli	12	26.08%
Streptococcus. pneumoniae	2	4.35%
Mixed growth	8	17.4%
No growth	9	19.6%

The antibiotic to which the bacteria was sensitive was cetazidime and amikacin (Table 7).

Table 7: Antibiotic sensitivity of sputum in lung abscess

Antibiotic Sensitivity	No. of cases	Percentage
Ceftriaxone	12	26%
Cefoperazone	10	21.7%
Ceftazidime	25	54.34%
Amikacin	16	34.7%
Streptomycin	5	10.86%
Metronidazole	8	17.4%
Clindamycin	14	30.43%
Meropenam	2	4.34%

Discussion

Although the incidence of developing lung abscess has decreased with the advent of antibiotics still its presence is marked by the presence of risk factors. The incidence of lung abscess found in our study was more in adult males with a mean age of 42.9 years. This finding was similar to the findings of JS. Moriera et al. where the majority of lung abscess was in adult males more than 40 years of age with an age range of 15 to 65 years [3].

The most common symptom observed in lung abscess cases was a cough followed by fever. Cough was present as the main symptom in 42 cases (91%), and fever was present in 38 cases (83%). The initial symptom of lung abscess is a cough for a few days followed by expectoration once a bronchopulmonary communication was established. The findings of our study were in accordance to what was observed by all studies [4] [5]. Several predisposing factors are associated with the causation of lung abscess. The unhygienic oral cavity was found to be the most important causative agent for a lung abscess. About 28% of lung abscess cases had an unhygienic oral cavity which was represented in a few studies [1] a [2] [3] [4] [5] [6]. The oral cavity is an abode for many organisms which stays in the gingival crevices and cause lung abscess when aspirated and hence unhygienic oral cavity leads to lung abscess. Dental sepsis was also noted in 6 cases (13%) of lung abscess cases. Alcohol ingestion and smoking were found to be two important risk factors in our study. About 10 cases (22%) gave a history of alcohol ingestion in our study while 8 cases (17%) were smokers. Our study had similar findings as was observed for risk factors in studies done by Takayanagi N and Magalhaes L [6] [7]. Loss of consciousness due to alcohol ingestion, epilepsy leading aspiration of oral secretion was found to be the main cause. Gastroesophageal pathology like oesophagal malignancy, oesophagal stenosis or gastric outlet obstruction due to tumours leading to aspiration of oral secretion has also been found to be an important contributor to lung abscess. In patients on ventilators, lung abscess has been seen due to microaspiration of oral secretions as well as due to hematogenous spread of organism leading to single or multiple lung abscesses.

Radiology plays an important role in identifying lung abscess. Chest X-ray not only helps in identifying lung abscess but also determines the location of the abscess. Plain CT scan thorax is sometimes done where there is confusion in differentiating lung abscess from loculated hydropneumothorax [8]. In our study the majority of the lung, the abscess was found to be located on the right side compared to the left side. Very few lung abscesses were present in bilateral lung fields. The most common location in our study was the right upper lobe followed by right lower lobe. This was because the majority of our patients were alcoholic and might have aspirated the oral secretion when unconscious under the influence of alcohol. Since anatomically the right main bronchus is straighter than left, the majority of our lung abscess was found on the right side. Majority of our lung abscess were represented by a cavity with fluid level, but few cases had an only empty cavity. Our study had similar finding as was observed by studies done by few authors where lung abscess was found most commonly on the right side and in the upper lobe [6] [8] [9].

All lung abscess cases were subjected to investigation by examination of their sputum for acid-fast staining and gram staining and culture sensitivity. In our study, sputum of all lung abscesses was sent for gram stain. Out of 46 cases of lung abscess, only 37 cases had an organism. In the remaining 9 cases, there was no growth of the organism. The most

common organism detected was *Klebsiella pneumoniae* in 23 cases which were followed by *E. coli* in 12 cases. Nine cases did not yield any organism. Two cases had gram-positive *Streptococcus pneumoniae* organism. Our findings did not show any other Streptococcus species except *Streptococcus pneumoniae* though *Streptococcus viridans* was found to be a cause of lung abscess in a study done by Jerng JS et al., [10] while other studies showed *Streptococcus pneumoniae* as a common gram-positive organism for lung abscess [11] [12]. In our study, the most common organism observed was *Klebsiella pneumoniae* and was similar to findings observed by Wang JL et al., [13]. Few studies on the bacteriology of lung abscess have been done due to the low incidence of the disease, and *K. pneumoniae* was reported in small studies during the 1970s. There have been few case reports of *K. pneumoniae* lung abscess occurring as concurrent infection at other sites [14] [15] [16]. In our study, all the *K. pneumonia* lung abscess was not secondary to bacteremia or other foci of infection. Studies have shown that there is pharyngeal colonisation of gram-negative organisms and in alcoholics may contribute gram-negative pneumonia and lung abscess formation due to the aspiration to lower respiratory tract. In children, most studies showed that a common organism causing lung abscess was *Staphylococcus aureus* and *Streptococcus pneumoniae* [17]. There has been a noted difference in the organism causing lung abscess in immunocompetent and immunocompromised patients. It was found that aerobic organisms are mainly responsible for causing lung abscess in immunocompromised patients while lung abscess caused in immunocompetent patients was caused by anaerobic organism [18]. With the upcoming resistance pattern of antibiotics and immunomodulation due to environmental effect, a lot of gram-negative organism as well as gram-positive organisms are involved in the causation of lung abscess instead the conventional anaerobes. Studies done shows that these causes may be important for the pathogenesis of lung abscess due to *K. pneumoniae.*

Culture sensitivity of an organism with sputum gram stain for lung abscess was evaluated, and most of these cases were sensitive to ceftazidime and amikacin suggesting most of the organisms were gram negative. Trails on antibiotics regimen in lung abscess reported that ß-lactamase inhibitors/ß-lactams are the mainstay for the treatment of aspiration pneumonia and lung abscess [19] [20]. Since there may be a mixed pattern of the organism which was either not detected or present as commensal, it is better to treat these cases with a combination of cephalosporins, aminoglycosides and anaerobic coverage with metronidazole or clindamycin.

The limitation of our study is that the majority of lung abscess cases who presented to us had used antibiotics from a local hospital for a fever. Main bacteria like anaerobes could not be isolated effectively due to the prior use of antibiotics.

K. pneumoniae should be considered as an important pathogen in community-acquired lung abscess in addition to anaerobes. Their presentation may be acute but not severe with the presentation of multiple cavities and putrid sputum. With the increased number of *K. pneumoniae* infection and antibiotic resistance, it is safe to give third-generation cephalosporin and metronidazole or clindamycin.

Currently, there is lack of consensus on the best approach to the management of lung abscess, and controversies still surround medical and surgical approaches. Large prospective studies are required to evaluate better factors predicting clinical outcome of pyogenic lung infections. Protected lung specimen by bronchoscopy method or by transthoracic/ transtracheal lung aspiration should be done to evaluate the common etiological agent so that an antibiotic protocol can be framed for treatment of lung abscess.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1647-1651.
https://doi.org/10.3889/oamjms.2018.384
eISSN: 1857-9655
Clinical Science

Pathological Profile, Early Complications, Functional and Oncological Outcome after Radical Cystectomy - Ileal Conduit for Bladder Cancer Patients in Sanglah General Hospital between January 2013 and December 2016

Yudiana Wayan[1], Pratiwi Dinar Ayu[1], Oka A. A. Gde[1], Niryana Wayan[1], I Putu Eka Widyadharma[2*]

[1]Department of Surgery, Medical Faculty, Udayana University-Sanglah General Hospital, Bali, Indonesia; [2]Department of Neurology, Medical Faculty, Udayana University-Sanglah General Hospital, Bali, Indonesia

Citation: Wayan Y, Ayu PD, Gde OAA, Wayan N, Widyadharma IPE. Pathological Profile, Early Complications, Functional and Oncological Outcome after Radical Cystectomy - Ileal Conduit for Bladder Cancer Patients in Sanglah General Hospital between January 2013 and December 2016. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1647-1651. https://doi.org/10.3889/oamjms.2018.384

Keywords: Bladder cancer; Radical cystectomy; Ileal conduit; The oncological outcome

***Correspondence:** I Putu Eka Widyadharma. Department of Neurology, Medical Faculty, Udayana University-Sanglah General Hospital, Bali, Indonesia. E-mail: eka.widyadharma@unud.ac.id

Received: 29-Jul-2018; **Revised:** 08-Sep-2018; **Accepted:** 09-Sep-2018; **Online first:** 23-Sep-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Radical cystectomy is the standard treatment for nonmetastatic bladder cancer (muscle-invasive and selective superficial bladder cancer). There are many types of urinary diversion after this procedure; the ileal conduit is the most and simplest one.

AIM: To asses clinical, pathological profile, early complication, functional and oncological outcome after radical cystectomy and ileal conduit for muscle-invasive bladder cancer patients.

METHOD: Between January 2013 and December 2016, there were 68 patients diagnosed with bladder cancer. From those patients, 24 (35.29%) patients had been performed radical cystectomy with ileal conduit type for urinary diversion (100%). Patients demographic, clinical and pathological profile, early postoperative complication, functional and oncological outcome were collected from the medical record.

RESULT: Among the 24 patients who underwent radical cystectomy, 20 patients were male (83.3%) with the mean age was 57.3 y.o (33–77 y.o). Twelve patients (50%) showed pT4 and pT2 respectively. Based on pathological result 20 patient (83.34%) had the urothelial carcinoma, three patients (12.5%) had squamous cell carcinoma, and one patient (4.1%) had adenocarcinoma. Two patients (8.3%) got neoadjuvant chemotherapy, and nine patient (37.5%) of patients followed adjuvant chemotherapy after surgery. Wound dehiscence, fistula enterocutan, prolong ileus, leakage anastomosis and sepsis were kind of complication after surgery. One year's survival rate is 84%, mortality rate 20.8% and a recurrence rate of 20.8% in 4 years follow up.

CONCLUSION: Radical cystectomy and ileal conduit type of urinary diversion still become the preferable procedure for nonmetastatic bladder cancer with good functional and oncological outcome.

Introduction

Radical cystectomy with bilateral pelvic lymphadenectomy is the standard treatment for muscle-invasive and high-risk non-muscle-invasive urothelial carcinoma of the bladder. There are numerous choices for urinary diversion after radical cystectomy; ileal conduit continues to be the most common form of urinary diversion [1] [2].

Improvements in surgical techniques and modern perioperative care have substantially decreased the rate of perioperative complications and lowered the operative mortality rate. However, this procedure remains complication-prone and is associated with significant perioperative and long-term morbidity ranging from 19% to 64% according to different series [3]. In several large series, the overall recurrence-free survival at five years for patients undergoing cystectomy approaches 70%, ranging from 50 to 60% for stage 3 or 4 tumours and 89 % for stage 2 tumours [3] [4].

Countless retrospective studies unquestionably support radical cystectomy excellent oncologic outcomes and satisfactory postoperative quality of life (QoL) at long-term follow-up. Although much of the clinical evidence coming from these studies are of low quality, major international

guidelines strongly recommend radical cystectomy as the elective treatment for muscle-invasive bladder cancer. For more than 30 years, the ileal conduit (IC) has been considered the ‘‘standard’’ urinary diversion method for most patients submitted to radical cystectomies. It is recognised as the most clinically adequate, reliable, and cost-effective solution.

Some complications are strictly related to IC and have been distinguished between early (before 90 days) and late (after 90 days). Early complication related to bowel complication such as intestinal anastomosis related, ureteral ileal anastomosis leakage, enteric fistula, bowel obstruction, prolonged ileus and conduit necrosis, whereas late complication related to stoma complication such as abdominal wall related, conduit stenosis, ureters enteric anastomosis stricture also Hydronephrosis, kidney failure, and metabolic changes [5].

This study aimed to investigate the clinical, pathological profile and complications related to radical cystectomy and ileal conduit patients with bladder cancer in Sanglah general hospital, Denpasar Bali.

Methods

From January 2013 to December 2016, there were 68 patients had been diagnosed with bladder cancer, 59 patient had the urothelial carcinoma, four patient had squamous cell carcinoma, five patient had adenocarcinoma. 30 patient already underwent chemotherapy, three patient had a partial cystectomy, and 24 patient (35.3%) had been performed radical cystectomies with following ileal conduit type or Bricker’s procedure type of urinary diversion, remain of it 11 patient (16.17%) had refused for treated. We carried out a retrospective study by reviewing the medical records of those 24 patients who treated with open radical cystectomies and ileal conduit type for urinary diversion. Follow up on those patients continued until January 2017. Data were collected through follow-up visits in outpatient clinics, evaluated by the following history included: age and comorbid (diabetes mellitus, hypertension, ischemic heart disease), operative details, histopathological type and grading also the history of neoadjuvant and adjuvant chemotherapy were recorded. We were also collecting postoperative early complications and late complications, at early complications, we divided into diversion related and not diversion related. With a minimum follow up 6-month patients were routinely seen in the outpatient clinic for stoma care, oncologic follow-up and also late complications after the procedure.

Results

Pre characteristics database showed 20 patients (83.4%) was male, with the median age was 57.3 y.o (32-77 y.o). All patients came to the hospital with chief complained initiated gross hematuria or blood clot retention, some of them complained of dysuria too, 33.3% patients had suffered from UTI (Urinary Tract Infection), 20.83% patients had bladder stone and some suffered systemic comorbidities such as cardiovascular diseases (29.2%), type II diabetes Mellitus (8.3%) and prior renal failure (37.5%). Based on clinical, 20.83% patients with staging cT2 tumour preoperatively, 33.3% patients had cT3 and 45.83% with cT4. There is only two patient (8.33%) had neoadjuvant chemotherapy.

Table 1: Preoperative characteristic of bladder cancer patients who underwent radical cystectomies

Variables Preoperative	Result
Sex (%)	
Male	20 (83.4%)
Female	4 (16.6%)
Mean age (range)	± 57.3 y.o (33–77 y.o)
Chief complain	Hematuria (100%)
Local comorbidities (%)	
UTI	8 (33.3%)
Bladder stone	5 (20.83%)
Bladder diverticula	2 (8.34%)
Surgical history of bladder	3 (12.5%)
Systemic comorbidities (%)	
Cardiovascular comorbidity	7 (29.2%)
DM type II	2 (8.3%)
Chronic kidney failure	9 (37.5%)
Clinical staging (%)	
T1	-
T2	5 (20.83%)
T3	8 (33.3%)
T4	11 (45.83%)
Neoadjuvant Chemotherapy	2 (8.33%)

From perioperative data showed that the mean operation time was 351 minutes (310-435 minutes), and mean intraoperative blood loss was 1070cc (500 – 2500cc). 91.66% of patients performed bilateral pelvic lymph dissection. There was, two patients (8.33%) died one month after surgery because of sepsis.

Table 2: Perioperative data from patients with radical cystectomies

Variables Perioperative	Result
Mean Operation time (range)	± 351minutes (310-435 minutes)
Mean intra operative blood loss (range)	±1070 cc (500-2500 cc)
Pelvic Lymph dissection bilateral	
Yes	22 (91.66%)
No	2 (8.34%)

From postoperative and follow up data in the outpatient clinic we found that 50% patient showed pT4, and 50% had pT2, there is no pT3, 83.34% patients showed with transitional Cell ca (TCC) and one patient with a synchronous tumour with TCC and acinar adenocarcinoma prostate, 70.83% was high-grade carcinoma. In all cases, nine patients (37.5%) undergo adjuvant chemotherapy.

Table 3: Postoperative characteristics data from patients with radical cystectomies

Variables Postoperative	Result
Pelvic Lymph dissection (%)	
Yes	22 (91.66)
No	2 (8.34)
Pathological staging (%)	
pT1	-
pT2	12 (50%)
pT3	-
pT4	12 50%)
Lymph Node Pathological	
pN0	13 (54.2%)
pN1	6 (25%)
pN2	3 (12.5%)
pNx	2 (8.3%)
Pathological type (%)	
TCC	20 (83.34%)
SCC	3 (12.5%)
Adenocarcinoma	1 (4.16%)
Pathological grading (%)	
Low grade	4 (16.7%)
Moderate grade	3 (12.5%)
High grade	17(70.83%)
Adjuvant Chemotherapy	9 (37.5)

About the postoperative complication, we divided into an early and late complication, which early was taken 3 months after surgery and late was taken 3 months after surgery. Early complication related to bowel complication such as intestinal anastomosis related, ureteral ileal anastomosis leakage, enteric fistula, bowel obstruction, prolonged ileus and conduit necrosis, whereas late complication related to stoma complication such as abdominal wall related, conduit stenosis, ureters enteric anastomosis stricture also Hydronephrosis, kidney failure, and metabolic changes [5]. From the early complication include one patient had fistula enterocutan and 20.8% suffered prolonged ileus. Fifteen patients still alive in minimum follow up 12 months had a survival rate of 84%. Mortality rate 20.8%, causes of death shown at table 4: one patient (4.16%) died 2 months after surgery because of ileus, 0ne patient (4.16%) died 1 year after surgery because of recurrence and metastasis, and one patient (4.16%) died 21 months after surgery because of metastasis. Recurrency happens in five patients (20.8%), 4 with local recurrence and 1 with distant metastasis. The distant metastasis went through bone metastasis.

Table 4: Complication characteristics from patients with radical cystectomies

Variables	Result
Early complication (%)	
Bowel related	
Prolonged ileus	5 (20.8%)
Fistula enterocutan	1 (4.16%)
Sepsis (<3 month)	2 (8.34%)
Late Complication (%)	
Stoma-related	
Ureter stenosis	1 (4.16%)
Electrolytes imbalance	1 (4.16%)
Wound dehiscence	5 (20.8%)
A hernia incisional	1 (4.16%)
Survive (>12 months)	15 (84%)
Mortality rate	5 (20.8%)
Cause of Mortality rate	
Sepsis	2 (8.34%)
Metastasis	2 (8.34%)
Ileus	1 (4.16%)
Recurrence	5 (20.8%)

Discussion

Radical cystectomy with pelvic lymph node dissection provides the best cancer-specific survival for muscle-invasive urothelial cancer and is the standard treatment, with 10-year recurrence-free survival rates of 50–59% and overall survival rates of around 45% [6] [7] [8] [9]. The primary goals in the selection of a urinary diversion are to provide the patient with the diversion that results in the best local cancer control, the lowest potential for both short and long-term complications and the best quality of life while still allowing the timely completion of chemotherapy and therapeutic goals [10]. Recently, many options for urinary diversion after radical cystectomy, but in our study ileal conduit still considered the "standard" urinary diversion for nonmetastatic bladder cancer. It is universally recognised as being the most clinically adequate, cost and time effective, and reliable solution in long-term. Other option for urinary diversion like neobladder still not yet performed in our institution because of for developing country like Indonesia, many patients with bladder cancer came from intermediate low social state and poor education so that ileal conduit type more acceptable than neobladder. However, a form of incontinent urinary diversion (ICUD) is the method of choice in elderly patients, and in those with high comorbidity (American Society of Anaesthesiologist Score [ASA] > 3), Ileal Conduit remains the preferred method [11].

Radical cystectomy with ileal conduit type of diversion entails simultaneous surgery on the urinary tract, intestines, and lymph nodes; hence, complications frequently occur after this extensive procedure. According to the literature, the incidence of such secondary conditions varies widely (from 19% to 64%). Hollenbeck et al., studied data on 2538 cases obtained from the National Quality Improvement Program, which is a prospective quality management initiative of 123 US Department of Veterans Affairs medical centres, and the results of that evaluation showed that 30.5% of the patients had at least one complication at 30-day follow-up after RC [12] [13] [14]. From postoperative data and follow up outpatient clinic we found that 50% patient had pT4, and 50% had pT2, there is no pT3. Based on pathological result 20 patient (83.34%) had urothelial carcinoma, 3 patient (12.5%) had squamous cell carcinoma, and 1 patient (4.1%) had adenocarcinoma, and one patient had a synchronous tumour with TCC and acinar adenocarcinoma prostate, 70.83% was high-grade carcinoma, while Enein et al. study showed TCC 59.5%, SCC 36%, and Adenocarcinoma 2%, moderate grade 56.5% and T3 46.5% [15].

Fifty percent of our patients came with the high stadium, more than stadium II of cancer, and not few of them came with metastatic bladder cancer. At the time of diagnosis, almost all high-grade bladder

cancer are muscle invasive. It is because of minimum information about cancer especially bladder cancer in our society, and low awareness about it, so there always almost all of the patients came with late bladder cancer. The decision to perform immediate radical surgery depends on several factors, including ASA, age, and life expectancy.

In our study the average patient loses 500ml to 2500 ml of blood during RC, indicating that this type of surgery often leads to considerable loss of blood and, consequently, to transfusions. Blood transfusions are associated with major complications and with high total hospital costs for RC [16] [17].

It is noteworthy that all patients in this study had a minimum follow up, and some of them, not well recorded and loss of follow up also. From complication data, we divided into an early and late complication, early taken before 3 months after surgery and late is made 3 months after surgery. From the early complication, only one patient had ureter stenosis, 20.8% had wound dehiscence, 20.8% patients had prolonged ileus, and 1 (4.1%) had an incisional hernia. The earliest complications in our study were wound dehiscence, it is probably because of bad hygiene, hypoalbuminemia, late immobilisation, and some of our patients had systemic comorbidities like diabetes mellitus type II. Kim et al., have searched about early and late complication, their study showed during the median follow-up of 46.6 months, early, and late morbidities were 29.5% and 19.8%, and complication-related mortalities were 2.2 and 6.6%, respectively [18].

Feared complications immediately after RC include internal anastomotic leak and urinary extravasation caused by anastomotic or reservoir leakage. In a prospectively randomised study [19], perioperative stenting was found to decrease urinary leakage. Even in the absence of available evidence, it seems wise to recommend that drains be left in place until anastomotic integrity is established in the intestinal and urinary tracts. Due to the relatively low extravasation rate associated with RC, it is probably not necessary to perform routine postoperative urography or stentograms in patients with a normal postoperative course [16]. Gastrointestinal events probably represent the most common type of complication during the period after RC. Postoperative intestinal anastomotic leakage has been described in 3% of patients [20], Compared to urinary or intestinal leakage, intestinal obstruction is more common as a complication of RC, and it was found to affect 23% of patients in a recent cystectomy series [20]. Infectious events are the second most common complications of RC, constituting 25% of all early complications within 90 d of the surgery, according to a recent investigation [20]. Adequate perioperative antimicrobial prophylaxis to prevent postoperative infectious complications is standard practice in the care of surgery patients, but information is lacking concerning the optimal schedule in conjunction with RC. Wound-related complications, primarily dehiscence in the early postoperative period, constitute 15% of all early complications of RC [20]. However, when using a midline incision, several factors might affect the incidence of wound separation, including operational, technical issues. In contrast, a recent meta-analysis of 23 randomised studies comparing the interrupted and continuous methods of laparotomy wound closure demonstrated that the interrupted technique was associated with significantly less dehiscence (odds ratio: 0.58), whereas there was no difference concerning hernia risk [21].

In our study, Recurrence has arisen in 5 cases, 4 with local recurrence and 1 with distant metastasis while in a study by Jian et al., there was local recurrence in 9 patients (5.3%), distant metastasis in 23 patients (13.5%) and both in two patients (1.2%) [21].

Five patients (20.8%) had died from various causes; one patient (4.16%) died 2 months after surgery because of ileus, two patient (8.33%) died before one month after surgery because of sepsis, 0ne patient (4.16%) died 1 year after surgery because of recurrence and metastasis, and 0ne patient (4.16%) died 21 months after surgery because of metastasis, while in the study of Jian et al., there were 28 of 171 patients (16.4%) had died from various causes, 20 from metastasis and 8 from causes unrelated to the tumor (two from upper gastrointestinal bleeding, two from myocardial infarction, two from pneumonia, one from stroke and one from a car accident) [21].

Improvements in surgical techniques and modern perioperative care have substantially decreased the rate of perioperative complications and lowered the operative mortality rate. However, this procedure remains complication-prone and is associated with significant perioperative and long-term morbidity ranging from 19% to 64% according to different series. The Ileal conduit remains the most frequent form of urinary diversion, published first in 1952 and became popular and is still the Gold Standard. The technique proved to be quick, safe and straightforward compared to neobladder [11].

In conclusion, the Ileal Conduit can still be considered an appropriate surgical solution after Radical Cystectomy in most patients because of the relative simplicity of the surgical technique, the acceptable complication rate, and the satisfactory postoperative Quality of Life. Our patients still need to be follow up till next 5-10 years after surgery, to maintain the late complications after surgery. After all, yet to be our job to give more education and information about cancer issue, to raise awareness and to reduce morbidity, and gained the better quality of life for cancer patients. Careful patient selection, strict adherence to proper surgical technique and appropriate life-long follow-up are of paramount

importance in the successful management of patients undergoing radical cystectomy for bladder cancer.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1652-1658.
https://doi.org/10.3889/oamjms.2018.364
eISSN: 1857-9655
Clinical Science

Mercury Materno-fetal Burden and Its Nutritional Impact

Enas R. Abdel Hameed[1], Lobna S. Sherif[1], Ola M. Abdel Samie[1], Hanaa H. Ahmed[2], Amira Ahmed[3], Hala Atta[3], Hisham Waheed[1], Reham F. Fahmy[1*]

[1]Child Health Department, Medical Research Division, National Research Center, Cairo, Egypt; [2]Hormones Department, Medical Research Division, National Research Center, Cairo, Egypt; [3]El-Galaa Teaching Hospital, Cairo, Egypt

Citation: Abdel Hameed ER, Sherif LS, Abdel Samie OM, Ahmed HH, Ahmed A, Atta H, Waheed H, Fahmy RF. Mercury Materno-fetal Burden and Its Nutritional Impact. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1652-1658. https://doi.org/10.3889/oamjms.2018.364

Keywords: Mercury; Pregnancy; Cord blood; Nutrition

***Correspondence:** Reham F Fahmy. Child Health Department, Medical Research Division, National Research Center, Cairo, Egypt. E-mail: reham.dodo2@gmail.com

Received: 17-Jun-2018; **Revised:** 09-Aug-2018; **Accepted:** 18-Aug-2018; **Online first:** 24-Sep-2018

Funding: National Research Center 10th research plan "Immunological Profile in Cord Blood and Growth Assessment of the Newborn in Relation to Maternal Exposure to Environmental Contaminant"

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

Abstract

BACKGROUND: Mercury exists worldwide in food, water and air throwing its health hazards on all body systems.

AIM: To show the influence of the presence of mercury in pregnant mothers' blood on its level in the umbilical cord blood; and to display the relationship between the different foodstuff on the mercury levels in pregnant mothers' and umbilical cord blood.

PATIENTS AND METHODS: This cross-sectional study was conducted on randomly chosen 113 pregnant mothers at the time of labour and on their newborns. Full history, sociodemographic data and food frequency questionnaire for dietary assessment were recorded. The Maternal and neonatal anthropometric measurements together with the Apgar scoring were also measured. Serum mercury levels in both mothers' and umbilical cord blood were measured using the Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

RESULTS: A high percentage of mothers (82.3%) were exposed to passive smoking. There was a statistically significant positive correlation between the maternal and fetal umbilical cord blood mercury levels ($p = 0.002$). There was an insignificant negative correlation between the maternal blood and fetal umbilical cord blood mercury levels on one side and each of the different foodstuff on the other side (fish, vegetables, fruits and proteins, for example, meat and legumes). An insignificant positive correlation was found between dairy products and of the maternal blood and umbilical cord blood mercury levels.

CONCLUSION: The fetal umbilical cord blood mercury levels correlate positively with the maternal blood mercury. The different foodstuff can influence the maternal and umbilical cord blood mercury levels whether by increase or decrease. Strict measures should be taken to decrease environmental mercury contamination with attention to pregnant mothers.

Introduction

Mercury is a xenobiotic heavy metal with undisputed toxicity, grading it as number three after lead and arsenic [1] [2].

Although it exists worldwide in soil, food, water and even in the atmosphere, still definitely our developing countries are the much more suffering ones. It can easily gain access to the human food chain and after that goes on bioaccumulating

Unfortunately, mercury adversities start very early where it can cause embryopathies, fetotoxicity, infantile hazards and up to older ages we can meet its detrimental health effects [3] [4].

Mercury has a wide range of catastrophic health insults starting from abortions, stillbirths, craniofacial malformations up to neural tube defects, brain damage and infantile cerebral palsy. As it throws its impact on almost all body systems, hence we encounter anaemia, immunological impairments, autoimmune conditions, renal injuries, endocrinal insults and so many others [5] [6] [7].

Our present study aims to elucidate the impact of the presence of mercury in pregnant mothers' blood on its level in umbilical cord blood and to display the influence of the different foodstuff, eaten by mothers, on the mercury levels measured in both pregnant mothers' blood and umbilical cord blood.

Patients and Methods

This is a cross-sectional study that was conducted on 113 pregnant mothers at the time of labour and their newborns. They were chosen randomly from those attending El-Galaa Teaching Hospital as a research project, funded the by National Research Center 10th research plan, entitled "Immunological Profile in Cord Blood and Growth Assessment of the Newborn About Maternal Exposure To Environmental Contaminant". The study was conducted in the period from September 2016 to June 2017.

The study protocol was approved by the Medical Ethical Committee of the National Research Centre. All mothers gained comprehensive and clear knowledge about the aim of our work, and written consents were signed before enrollment.

The mother's ages ranged between 18 and 40 years. Neonates were of both sexes. Pregnant mothers with a history of chronic diseases or major illnesses during pregnancy were excluded. Neonates with any apparent congenital abnormalities, genetic, metabolic or neurological problems were also excluded.

- Sociodemographic data about mothers included age, social status, economic responsibility, water source availability, sanitary disposal, smoke exposure and education.

- Food frequency questionnaire (FFQ) for dietary assessment of the different foodstuff.

- Gestational age, type of labour, history of delivery problems and chronic diseases.

- Maternal anthropometric measurements of weight in kilograms (kgs) height in centimetres (cm) [8].

- Neonatal Apgar scoring, at one and five minutes, was measured to assess neonatal condition at birth [9].

- Neonatal anthropometric measurements of weight (kgs), height (cm), head circumference and mid-upper arm circumference (MAC) in cm were all taken [10].

- 10 ml of blood were collected from mothers whether in normal or section delivery at the time of labour and put in 3 EDTA tubes.

- Another 10 ml of blood were collected from the cord blood during delivery before placental separation and put in EDTA tubes.

- These blood samples were for measuring the mercury levels in mothers and umbilical cord of fetus using inductively coupled plasma mass spectrometry.

The standards for ICP-MS were prepared from stock solutions of mercury at 10 mg/L concentrations obtained from Sigma- Aldrich, Australia, and labelled as Fluka Trace Cert Ultra. From the stock, we prepared spiked solutions as much as needed. The hydrochloric acid and ultrapure nitric acid (HNO_3) were taken from J.T. Baker Inc. The other solvents and reagents in our study were analytical grade got from Sigma- Aldrich, Australia. The water for washing the laboratory apparatus and glassware together with the standard solutions and sample preparation was deionised (resistance < 18 m, Academic Milli-Q Ultra Pure Water System, Australia) [11].

The ICP-MS was calibrated through the use of standard aqueous solutions (having the same acid concentration used in the samples and additional internal standards) prepared from the stock solutions through dilution in the range of 0.05 to 10 mg/L concerning mercury. First, we prepared a solution containing 10 mg/L of mercury as mentioned before and from it, the final standard solution is made.

Before doing the analysis, there was an optimisation of the operating conditions of the ICP-MS instrument. The ICP-RF power, the nebuliser gas flow rate and the ion lens voltage(s) were particularly adjusted to give the highest possible signal intensities all while keeping low levels of oxides and doubly-charged ion production (where both have to be less than ~ 3%). The appropriate calibration standards were then measured after optimisation of the instrument.

To make a calibration curve with a correlation coefficient of 0.999 or better and as a part of the protocol of quality assurance, we did at least six-point calibrations of different ranges for mercury (0.10 to 10 mg/L and 0.05 to 2000 µg/L).

From the corresponding calibration curve, we determined the concentrations of the sample solution. To make sure that the instrument went on meeting the linearity criteria and the acceptable sensitivity, there was an analysis of the calibration standards at regular intervals during the ICP-MS analytical runs.

The analysis was performed using SPSS version 21 (SSPS Inc., Pennsylvania, USA). Mean ± SD was used for age distribution calculations. The correlation was done using Pearson correlation. $P < 0.05$ value was considered as significant and $p < 0.005$ value as highly significant.

Results

The sociodemographic data of the studied cases are shown in Table 1. It revealed that a high percentage of cases were exposed to passive

smoking (82.3%). Anthropometric and laboratory data of mothers and newborns are shown in Table 2.

Table 1: Sociodemographic data of studied cases

	No	%
Sex		
Male	51	45.1
Female	62	54.9
Delivery mode		
Normal	63	55.8
Caesarean section	50	44.2
Social status		
Married	112	99.1
Divorced	1	0.9
Economic status		
Responsible	11	9.7
Non-responsible	102	90.3
Water		
Available at home	112	99.1
Outside home	1	0.9
Sanitary disposal	113	100
Smoking exposure		
Yes	93	82.3
No	20	17.7
Mother education		
0 = illiterate	10	8.8
1 = primary education	31	27.4
2 = preparatory education	18	15.9
3 = secondary education	41	36.3
4 = high education	13	11.5

Table 3 revealed a statistically significant positive correlation between maternal blood mercury levels and the fetal umbilical cord blood ones (p = 0.002).

Table 2: Anthropometric and laboratory data of studied cases

	Mean	Std. Deviation
Mother:		
Age in years	26.72	5.6
Weight in kilograms (kg)	75.44	14.8
Height in centimeter (cm)	157.73	6.7
Newborn:		
Baby weight in kilograms	2.95	0.6
Baby length in centimeter(cm)	47.44	3.3
Head circumference in centimeter (cm)	34.17	1.9
Mid upper arm circumference in centimeter (cm)	10.22	1.5
APGAR1	5.76	1.6
APGAR 5	8.03	1.6
Gestational age in months	36.90	2.1
Laboratory data:		
Serum maternal mercury (Hg) level ng/ml	28.82	11.8
Serum neonatal mercury(Hg) level ng/mL	15.65	5.9

Table 4 showed the correlation between maternal food intake during pregnancy and serum maternal and neonatal Hg levels. Negative insignificant correlations between the maternal blood mercury levels on one side and the proteins as meat and legumes, vegetables, fruits and fish food items each on the other side were found.

Table 3: Correlation between serum maternal and neonatal mercury (Hg) levels

		Neonatal Hg
	Pearson Correlation	**0.303****
Maternal Hg	Sig. (2-tailed)	**0.002**

**Significant value at p < 0.005.

The same table similarly showed insignificant negative correlations between the fetal umbilical cord blood mercury levels on one side and the proteins as meat and legumes, vegetables, fruits, and fish food items each on the other side. The dairy products food item was the only one which showed an insignificant positive correlation with both the maternal blood and umbilical cord blood mercury levels.

Table 4: Correlation between maternal food intake during pregnancy and serum maternal and neonatal mercury (Hg) levels

Maternal food intake		Maternal Hg	Neonatal Hg
Protein (meat and/or legumes)	Pearson Correlation	-0.094	-0.067
	Sig. (2-tailed)	0.350	0.509
Vegetables	Pearson Correlation	-0.124	0.000
	Sig. (2-tailed)	0.217	0.999
Fruits	Pearson Correlation	-0.145	-0.186
	Sig. (2-tailed)	0.147	0.066
Fish	Pearson Correlation	-0.147	-0.197
	Sig. (2-tailed)	0.371	0.237
Dairy products	Pearson Correlation	0.167	0.149
	Sig. (2-tailed)	0.094	0.140

Discussion

Organic mercury is the most toxic form that can readily cross the placental barrier. It comprises ethyl mercury which is present in medical preparations and can passively diffuse; while methylmercury is present in fish and seafood and can actively cross the barrier via amino acid carriers. On the other hand, inorganic mercury exists in the dental fillings and is also airborne where it is transferred via the different occupations. It is usually caught in the placental barrier [12] [13] [14] [15] [16].

Mercury health hazards are catastrophic because of two reasons. First, its prenatal exposure does not only impose its adversities on the fetus (fetotoxicity), but it can also go on throwing its mal-influences up to the age of fourteen as documented by neurophysiological tests suggesting irreversibility in many occasions [17].

Second, the capabilities of mercury to bioaccumulate seriously impeding the different body system functions. This was proved not only concerning the ingested methylmercury because it is hydrophobic, resistant to metabolism and accordingly, can be found in every level of the food chain, but also concerning mercury vapour. This latter can easily pass to the circulation and once oxidised becomes lipid soluble, hence very liable to bioaccumulate in the brain, liver and renal cortex [3] [17].

The present study showed a significant positive correlation between the maternal blood mercury levels and the umbilical cord blood ones. We resort this to several justifications. First, the capability of mercury to readily cross the placental barrier and moreover to hamper its function. This is due to its prooxidative influences and impeding the antioxidative processes both of which augment the fetal vulnerability. Similarly, other researchers were in agreement with our results [12] [16] [17] [18] [19]. Second, is the escalated binding of mercury to the fetal blood cells not only because of its heightened

affinity to haemoglobin but also due to the higher fetal haemoglobin and hematocrit levels compared to adults.

This explanation was consistent with other work studies which moreover evidenced it by their much higher levels of mercury in umbilical cord blood compared to the maternal blood ones. They also referred this to the higher affinity of methylmercury to fetal hemoglobin [12] [20] [21] [22] [23] [24] [25].

Third; we should also suggest a very important probable justification for that correlation where we found in our study a considerably high percentage of pregnant mothers exposed to smoke (82.3%). Mercury is present in air and smoke. Since it is present in the tobacco plant and cigarette filters and papers, therefore it is released in cigarette smoke. It also gets into air and smoke from the breakage of mercury-containing devices like thermometers, compact fluorescent lamps, thermostats and barometers. School laboratories and the mercurochrome antiseptic are other sources [26].

Inorganic mercury from dental fillings was also reported to be airborne [12]. That heavy load of maternal mercury rationally got to the fetus through the previously mentioned first two justifications.

Matching with our suggestion some studies found out that mercury from air and smoke goes from the respiratory system to the circulating blood, hence to the placental barrier and accordingly gets retained in the embryonic tissues, the most hazardous of which is the brain. They also reported that inhaled mercury gets retained in the body in 80% of the cases [27] [28]. Other researches declared that mercury in smoke could cause vascular endothelial damage [26].

Furthermore, cigarette smoke can cause an elevation of mercury blood levels through its content of cadmium too. Cadmium imposes metallothioneins expression and these in turn bind to zinc and selenium both of which are well known to be mercury detoxificants. Accordingly, we assume that mercury levels are supposed to increase.

Finally, we suggest that during gestation the fetus could act not only as a filter to the maternal mercury but also as a reservoir or container to that mercury; hence its mercury levels rose up to correlate positively with their mothers.

Again, this suggestion was going on with other research workers who confirmed it, reporting that the umbilical cord blood mercury levels even exceeded their mothers' to reach double or even triple those levels [29] [30] [31] [32].

Concerning the negative relation between each of the maternal blood mercury and cord blood mercury levels on one side and fish eating on the other side, this relation though insignificant but it was surprisingly important because fish has always been reported to be a major source of mercury contamination; which could elevate its levels in cord blood (if eaten for example more than two times per week) [19].

However, our finding could resort to the pivotally beneficial fish nutritional contents. These comprise selenium, omega-3 fatty acids, vitamins A, D and calcium. Selenium, which was reported to be abundant in fish, plays a crucial role in protecting against mercury toxicity. Omega-3 fatty acids, vitamins A and D are antioxidants which alleviate methyl mercury hazards. Calcium too is very important because mercury causes cellular oxidative stress and mitochondrial affection ending in intracellular Ca-homeostasis disturbances and increased lipid peroxidation. Hence, we assume in our own words that the presence of these nutrients can lessen mercury toxic effects and levels [21] [33] [34].

Also, it is important to report that not all kinds of fish contain mercury in detrimental levels [23]. Similarly, other researchers found out the same result and gave more or less consistent explanations [35] [36] [37] [38] [39].

Very interesting Chinese work studies reported their results, which were in agreement with ours, to some other factors. These included the kind of fish, the fish organ ate, the water level from which the fish is caught, the accumulation factor of the metal itself and many other environmental risk factors [40] [41].

However, and contradictory to our findings, several researchers proved that frequent fish eating during gestation is associated with higher mercury levels [21].

Hence, fish could be considered a double-edged reciprocal weapon.

Regarding the negative correlation between maternal blood and umbilical cord blood mercury levels on one side and each of vegetables, fruits and proteins rich foods on the other side, we can mainly ascribe these relations to their vitamins, and minerals content which mitigates mercury improper levels and effects.

Concerning vegetables whether green, yellow or orange, they all provide very respectful percentages of iron, folate, zinc, calcium, potassium, magnesium and vitamins A, C, E and K. Their functions have proved to oscillate between lessening mercury influences, and ant oxidation. The Quercetin bioflavonoid present in the green ones gives them their antioxidant and anti- inflammatory qualities. Carotenoids in the yellow and orange vegetables are also antioxidants and antigenotoxic [42].

We are mainly concerned with the antioxidant effect of vegetables because it boosts the immunity where mercury is condemned for jeopardising the immunological system. It inhibits the normal polymorph nuclear cells stimulation and even function hindering their capability to destroy foreign material [3].

Going on with our results research declared that regular vegetable eating caused a decrease in mercury levels in both mothers and newborns [19].

Concerning the proteins rich foods item, we would like to add that they (particularly red meat) are proper sources of selenium which antagonises mercury.

Finally, we have found a positive correlation between each of the maternal blood and cord blood mercury levels on one side and the dairy products on the other side. We can assume that this might have been due to nutritional causes and pollution ones.

Nutritionally, dairy products are good sources of calcium and poor sources of iron and zinc [43] [44]. Since mercury competes with iron to bind to haemoglobin, causing aplastic and hemolytic anaemia's, therefore it is rational that iron plays a role in overcoming toxic mercury levels. Accordingly, if the dairy products were not properly fortified with iron, therefore this can be a justification for our results [16] [44].

Similarly, as zinc has proved to have a protective role through altering mercury metabolism, therefore its low levels in dairy products can be another justification [21] [44].

Concerning the calcium content of dairy products, though it is known to be very good, still we suppose that this might not be the case if the dairy product is of a poor quality concerning the source and or the manufacturing brand [45].

Another burden here is the mercury itself because not only does it cause cellular oxidative stress but also mitochondrial mal-affection ending in intracellular calcium homeostasis disturbances and increased lipid peroxidation.

Therefore, we can suggest that any calcium content problem in dairy products could have an undesirable effect on mercury levels and hazards [3].

As regards the pollution causes, we can say that these might have augmented the dairy products contamination with mercury to a burden level which might have surpassed the capabilities of dairy nutrients to alleviate it. These include the manufacturing processes steps where they comprise many physical and chemical properties [46]. The packaging processes and materials, storage reservoirs and transportation containers have their share too [43].

Moreover, since cow bodies were found to be "biological filters" of heavy metals, therefore pollution of their farms from sewage, wastewater, industrial wastes exhaustion gases and soil are all possible blamed sources [43] [45].

Finally, cheese types, brands, and drainage processes performed to get white cheese and Labenah all impose their influences on dairy products heavy metals content [45].

In conclusion, as mercury is very detrimental particularly to the brain and its hazards can go on up till older ages. Therefore measures for its environmental decrements in parallel with the nutritional detoxification measures are recommended.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1659-1663.
https://doi.org/10.3889/oamjms.2018.366
eISSN: 1857-9655
Clinical Science

Investigation of Ondansetron, Haloperidol, and Dexmedetomidine Efficacy for Prevention of Postoperative Nausea and Vomiting In Patients with Abdominal Hysterectomy

Alireza Kamali[1], Lida Ahmadi[1], Maryam Shokrpour[2*], Shirin Pazuki[1]

[1]Department of Anesthesiology, Arak University of Medical Sciences, Arak, Iran; [2]Department of Gynecology, Arak University of Medical Sciences, Arak, Iran

Citation: Kamali A, Ahmadi L, Shokrpour M, Pazuki S. Investigation of Ondansetron, Haloperidol, and Dexmedetomidine Efficacy for Prevention of Postoperative Nausea ana d Vomiting In Patients with Abdominal Hysterectomy. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1659-1663. https://doi.org/10.3889/oamjms.2018.366

Keywords: Dexmedetomidine; Ondansetron; Haloperidol; Nausea; Vomiting; Abdominal hysterectomy

***Correspondence:** Maryam Shokrpour, Department of Gynecology, Arak University of Medical Sciences, Arak, Iran. E-mail: Maryam_shokrpour@yahoo.com

Received: 25-Jul-2018; **Revised:** 20-Aug-2018; **Accepted:** 06-Sep-2018; **Online first:** 24-Sep-2018

Funding: This research did not receive any financial support

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

Abstract

AIM: This study was aimed to compare the effects of ondansetron, haloperidol, and dexmedetomidine for reducing postoperative nausea and vomiting (PONV) after laparoscopic cholecystectomy.

METHODS: This randomised clinical trial study was performed on p.patients who were candidates for abdominal hysterectomy referring to Taleghani hospital in Arak. In this study, 114 patients with abdominal hysterectomy were randomly assigned to three groups (ondansetron, haloperidol, and dexmedetomidine) using the cubull randomisation method.

RESULTS: The results revealed a significant difference between the three groups of ondansetron, haloperidol and dexmedetomidine in terms of scorpion vomiting in recovery, 2 and 4 hours after surgery, and vomiting score was significantly lower in the ondansetron group compared with the other two groups (P = 0.04; P = 0.02; P = 0.001). There was a significant difference between the three groups of e ondansetron, haloperidol and dexmedetomidine regarding the mean dose of metoclopramide in mg for 24 hours after surgery. Therefore, the dosage of dexmedetomidine in the ondansetron group was less than the other two groups (P = 0.001).

CONCLUSION: these three drugs are effective in reducing PONV in patients undergoing a hysterectomy. However, the effect of ondansetron was found to be more than the other two drugs in reducing PONV.

Introduction

Despite the progression of the new and anti-vomiting technique, Postoperative nausea and vomiting (PONV) is still one of the common complications after general anaesthesia and surgery in the immediate 24 postoperative hours, which causes increased unpleasant side effects, prolonged recovery and delayed discharge, as well as increased hospital costs. PONV also leads to loss of appetite, dehydration, and electrolyte imbalance [1] [2]. Vomiting may lead to wound dehiscence and oesophagal rupture, and pneumothorax, etc. The average incidence of PONV is reported to be 53-70% in high-risk patients. Therefore, the prevention of PONV increases patient satisfaction and reduces the cost of treatment. The prevalence of PONV should be monitored effectively, especially in high-risk patients, depending on the female gender, type of surgery, duration of surgery, duration of anaesthesia, and carbon dioxide content, as well as some visitors in Recovery. The chemo trigger zone (CTZ) as a vomiting *trigger zone* is an *area* of the medulla oblongata that plays an important role in developing vomiting reflux [2] [3] [4]. Other risk factors for PONV include age over 50, female gender, infection, uremia, motion sickness, migraine, hypercalcemia, anxiety, etc. [3] [4]. Surgeries such as laparoscopic abdominal surgery with peritoneal inflammation, women's surgery, strabismus, and ear surgery can cause nausea and vomiting [3] [4] [5]. For the prevention of postoperative nausea and vomiting in patients with high-risk to moderate-risk (Scores 1 to 2), prophylactic single-drug administration is recommended, but combining treatment with two or more drugs from different classes of anti-nausea drugs is more effective than single medicine for high-risk patients [3].

It is worth noting that various drugs such as serotonin antagonists, anticholinergics, butyrophenones, Phenothiazines, steroids and histamine H2-receptor antagonists, etc. are used. One of these drugs is endometrin, haloperidol, and doxedetomidine, etc. [3]. Endonestrone is a serotonin receptor antagonist that is very important in preventing nausea and vomiting due to surgery and chemotherapy, and it has an anti-vomiting effect by inhibiting 5HT3 (5-Hydroxytryptamine type 3) receptors in the vomiting centre and chomicosterone [4]. These drugs include ondansetron, haloperidol, and *dexmedetomidine*, etc. [3]. Ondansetron is a serotonin receptor antagonist, which is very important in preventing nausea and vomiting due to surgery and chemotherapy; it exhibited an anti-vomiting effect by inhibiting *5-Hydroxytryptamine type 3* (*5-HT*3) receptors in the vomiting centre and the *compressor starting area* [4]. Haloperidol is a sedative a butyrophenone with an antagonistic effect on dopamine receptor 2, which can cause nausea and vomiting, by inhibiting dopamine receptor 2 in the *vomiting centre*. Due to its long half-life, its *one-time*-only *injection* can provide an appropriate coating within 24 hours [5]. Haloperidol is applied as anti-nausea in relieving nausea and vomiting, as well as in psychiatry and surgery for the management of delusions in people over 40 years of age [6].

Dexmedetomidine is strong, and a *highly selective α-2 agonist* that attaches to a *transmembrane G-protein* coupled receptor in the brain and spinal cord, and affects the function of the central nervous system and the central circulatory system to produce its analgesic and sympathetic effects [7]. *Dexmedetomidine* is potentially used in anaesthesia and intensive care units due to its anxiolytic, analgesic, and sympathetic effects as well as its hemodynamic constants [7] [8]. Regarding available evidence, PONV is one of the main complaints of surgery and anaesthesia and is one of the risk factors for vomiting after abdominal surgery, such as abdominal hysterectomy.

Therefore, the current study was aimed to compare the prophylactic effects of haloperidol, ondansetron and dexmedetomidine in reducing PONV in patients undergoing an abdominal hysterectomy.

Material and Methods

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards, and was approved by Arak University of Medical Sciences Institutional Review Board (Protocol number IR.ARAKMU.REC.2017.111; October 8, 2017).

This randomised clinical trial study was performed on a subset of patients undergoing abdominal hysterectomy referring to Taleghani Hospital of Arak, Iran. In this study, 114 patients with abdominal hysterectomy were randomly divided into three groups (haloperidol, ondansetron and dexmedetomidine) using cubull randomisation. Patients with non-emergency abdominal hysterectomy referred to Taleghani Hospital of Arak were entered into the study after obtaining informed consent. They were entered into the operating room after anesthesiologist's confirmation. All of the patients referred to complete monitoring including oxygen saturation (SpO_2), heart rate (PR), Blood pressure (BP), noninvasive blood pressure (NIBC), and body temperature measurements.

The patients were randomly assigned to 3 groups of 38 patients including the haloperidol group (2 mg IV, 1 cc), ondansetron group (4 mg IV, 1 cc) and dexmedetomidine group (1 µg/kg IV, 1 cc).

The injection of these drugs was performed after the abdomen pulled out of the uterus. Then, all patients received 3-5 cc/kg of crystalloid fluid as Compensatory Volume Expansion (CVE), and patients underwent general anaesthesia. All patients underwent general anesthesia with fentanyl (2 µg/kg), midazolam (0.3-0.5 µg/kg), atracurium (0.5-0.7 µg/kg) and propofol (2-2 mg/kg). Then, the patients were intubated and sub-ventilated. After the endotracheal fixation and hemodynamic stability of the patient, the surgeon was allowed to start surgery.

Patients entered the study after obtaining informed consent, but none of them was aware of the drug received. After the completion of surgery, the patients entered the recovery room, and the questionnaires including questions about the scour of nausea and vomiting, hemodynamic parameters of the patient were completed for the patients. All patients with vomiting scores of 2 and > 5 were treated with *metoclopramide*. Finally, the obtained data were analyzed by statistical software SPSS 23 and data were presented in the form of statistical tables and charts.

The sample size was determined as reported previously [8]. According to a randomised clinical trial, 114 patients were selected from patients with abdominal hysterectomy.

$$N = \frac{\left(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta}\right)^2 (\delta_1 + \delta_2)^2}{(\mu_1 - \mu_2)^2} = 38$$

$$Z_{1-\frac{\alpha}{2}} = 1/96 \ \mu_1 = 18 \ \delta_1 = 115$$

$$Z_{1-\beta} = 2.33 \ \mu_2 = 11 \ \delta_2 = 9$$

Inclusion criteria

1. All patients suffering from abdominal hysterectomy referred to Taleghani Hospital in Arak who have informed consent. 2. Patients with ASA Class I and II. 3. No history of psychiatric illnesses. 4. No Parkinson's disease, motion sickness or history of chemotherapy. 5. All patients with general anaesthesia. 6. Patients aged 35-60 years 7. A 150 min *maximum duration of surgery*

Exclusion criteria

1. ASA ≥ III and IV. 2. Patients outside the age range of 35-60 years. 3. Parkinson's disease. 4. The presence of patients without informed consent. 5. Psychiatric and psychiatric patients. 6. History of chemotherapy.

Results

Based on data presented in Table 1 and Figure 1, there was no significant difference between the three groups of haloperidol, ondansetron and dexmedetomidine regarding mean age, BMI and mean duration of surgery (P ≥ 0.05). The average age of patients in each group was 46 years, followed by an average BMI (BMI: 30) and an average duration of surgery in three groups (2 hours).

Table 1: Comparing the mean age, BMI and duration of surgery in patients undergoing an abdominal hysterectomy in the three groups of haloperidol, ondansetron and dexmedetomidine

Average/group	Ondansetron	Haloperidol	Dexmedetomidine	P-value
Mean age	45/8+/-6/8	47/1+/-5/4	46/9 +/-6/6	P=0.12 no significant
BMI average	30/9+/-7/6	30/6 +/- 8/8	31/1 +/- 7/8	P=0.31 no significant
Average duration of surgery	118/4 +/-9/6	117/6 +/-11/1	114/5+/-10/4	P=0.28 no significant

With regard to the Table 2, there was a significant difference between the three groups in terms of vomiting scores in recovery, 2 and 4 hours after surgery; the scores of vomiting was significantly lower in the ondansetron when comparing with the other two groups (P = 0.04 ; P = 0.02 ; P = 0.001).

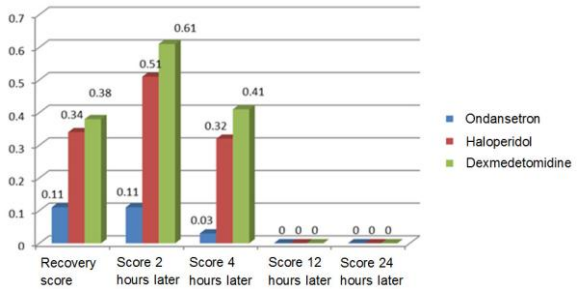

Figure 1: Comparison of vomiting and nausea scores in patients undergoing an abdominal hysterectomy in three groups

However, there was no significant difference between the two groups of haloperidol and dexmedetomidine regarding vomiting scores. Furthermore, 12 and 24 hours after surgery, the mean vomiting scores were identically equal in all three groups (P ≥ 0.05); therefore, no significant difference was observed between the three groups.

Table 2: Comparison of vomiting and nausea scores in patients undergoing an abdominal hysterectomy in three groups

Vomiting Scores / Group	Ondansetron	Haloperidol	Dexmedetomidine	P-value
Vomiting recovery score	0/11 +/-0/18	0/34 +/-0/41	0/32+/-0/38	P=0.04 significant
Vomiting score 2 hours later	0/11 +/-0/26	0/51+/-0/48	0/61+/-0/28	P=0.02 significant
Vomiting score 4 hours later	0/03 +/-0/08	0/32+/-0/21	0/41+/-0/48	P=0.001 Significant
Vomiting score 12 hours later	0	0	0	P≥0.05 no significant
Vomiting score 24 hours later	0	0	0	P≥0.05 no significant
Frequency Percentage of nausea	%10/1	%40/6	%48/3	P=0.001 Significant

There was a significant difference in the frequency of nausea among the three groups, and the incidence of nausea exhibited a significant decrease in the ondansetron group as compared to other two groups (P = 0.001).

Table 3: Comparison of the mean dose of *metoclopramide* consumed within 24 hours (mg) after operation in patients undergoing an abdominal hysterectomy in the three groups together with Average scoring for recovery

Average / group	Ondansetron	Haloperidol	Dexmedetomidine	P-value
the mean dose of metoclopramide (Mg)	1/06 +/-0/21	4/37 +/-1/1	3/54 +/-1/3	P=0.001 Significant
Average scoring for recovery	2/7+/-0/85	3/37+/-1/1	2/9+/-0/91	P=0.12 no significant

Our study revealed a significant difference between the three groups of ondansetron, haloperidol, and dexmedetomidine in terms of the mean dose of *metoclopramide* in mg for 24 hours after surgery; nevertheless, dosage of *metoclopramide* in the ondansetron group was less than the other two groups (P = 0.001; Table 3; Figure 2).

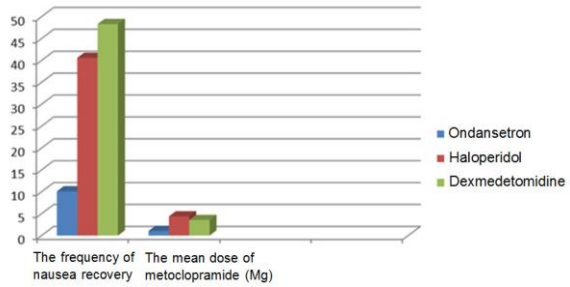

Figure 2: Comparison of the mean dose of metoclopramide consumed 24 hours after the operation (mg) and the frequency of nausea in patients undergoing an abdominal hysterectomy in the three groups

Based on Table 3 and Figure 3, no significant difference was found between the three groups regarding the average scoring in recovery, so that the

scoring average was determined as three in three groups (P = 0.12).

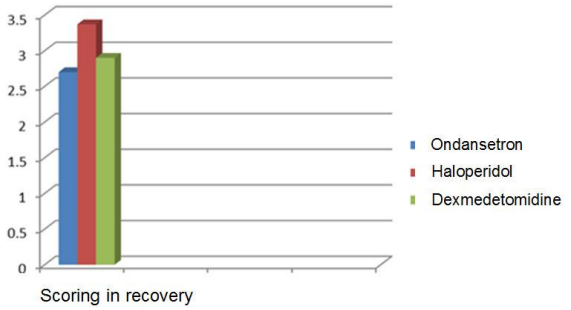

Figure 3: Comparison of average scoring in recovery in patients undergoing an abdominal hysterectomy in three groups

The frequency of hypotension and bradycardia did not exhibit any significant difference between the three groups (P = 0.0001). Furthermore, the incidence of shivering was not revealed to be significant in between the three groups and the incidence of shivering in the three groups was found to be approximately the same (13.5%) (Table 4).

Table 4: Frequency of hypotension, bradycardia and shivering in patients undergoing an abdominal hysterectomy in three groups

Average / group	Ondansetron	Haloperidol	Dexmedetomidine	P-value
Frequency of hypotension	0	%12/9	%9 /6	P=0.0001 Significant
Frequency of bradycardia	0	%3/1	%12/9	P=0.0001 Significant
Frequency of shivering	%12/5	%15/1	%12/9	P=0.11 no significant

Discussion

Achieving a suitable drug for controlling PONV is one of the important goals of anesthesiologists. Nausea is an unpleasant sensation that is perceived by the person who is aware of the imminent occurrence of vomiting after surgery and is one of the most common complications after surgery in women that occurs during the first 24 hours after surgery and anaesthesia [1]. Despite the advances in the new drugs for PONV, nausea and vomiting remain a common complaint after general anaesthesia, especially in women's surgeries [1] [2] [3]. This complication can occur between 20% and 30% of patients undergoing general anaesthesia after 24 hours of surgery. Several factors affect the amount of nausea and vomiting after surgery, including the type of surgery (gynaecological surgery, strabismus, Ear surgery), as well as the drugs used and the type of anaesthesia [9] [10] [11] [12] [13]. It is noteworthy that one of the important concerns of anesthesiologists over the years has been the availability of suitable drugs for controlling PONV. These drugs are used as prophylaxis or for the treatment of PONV [9]. The results of previous studies were in agreement with our study. In a study conducted by Haddadi et al., in Iran, the effects of ondansetron and metoclopyramide and dexmedetomidine in the prevention of PONV in children undergoing strabismus surgery have been investigated, where ondansetron was more effective than the other group in controlling PONV [14]. Of course, in our study, ondansetron was compared with haloperidol and dexmedetomidine, while ondansetron has been compared with *metoclopramide* and *dexamethasone* in the study above. Another study in 2009, evaluated the effect of ondansetron and the combination of ondansetron and *dexamethasone* in preventing PONV in patients with laparoscopic cholecystectomy. The results of this study indicated that the combination of ondansetron and dexamethasone was more effective in preventing nausea and vomiting Action [2]. The results of this study were in agreement with our findings, suggesting the effect of endonestrone on controlling PONV.

Pradeep Ret al. assessed the efficacy of haloperidol with ondansetron in preventing PONV after laparoscopic abdominal surgeries. They depicted that ondansetron 4 mg is not having a remarkable benefit over Haloperidol 2 mg for preventing PONV [3]. The results of the mentioned study were not consistent with our findings, because the current study depicted the effect of ondansetron in controlling postoperative nausea and vomiting. The cause of the difference in these two studies may be referred to the type of surgery. Another study demonstrated that oral ondansetron could be more favourable for women undergoing cesarean section with spinal anaesthesia [4]. The results of this study were consistent with our study, which our study has shown the effect of ondansetron on reducing PONV. Dexmedetomidine has been shown to reduce the incidence and severity of PONV, which is similar to dexamethasone. This is superior to dexamethasone in reducing postoperative pain and taking analgesic drugs over the first 24 hours after laparoscopic cholecystectomy [8]. The results of the mentioned study are not the same as our study, because we compared the effects of the three drugs of haloperidol, ondansetron and dexmedetomidine, but mentioned study did not compare the effects of dexamethasone and dexmedetomidine.

Furthermore, haloperidol has been equally demonstrated to be effective, like dexamethasone, for preventing PONV in patients undergoing gynaecological laparoscopic surgery [15]. Concisely, our findings also revealed that haloperidol, ondansetron and dexmedetomidine were effective in controlling postoperative nausea and vomiting that are in agreement with the aforementioned study. A study indicated that ondansetron has favourable effects on decreasing as a cost-effective and easily available medication for prevention of PONV, where endonestrone was nearly 30% more effective than the

combination of dexametasone-metocholopromide on PONV [16].

Our study also suggested a significant effect of ondansetron in controlling PONV. Studies have shown that exmedetomidine, haloperidol, and endonestrone drugs have been effective in controlling PONV [17] [18]. However, it should be noted that the effect of ondansetron is more than other drugs in most studies. Considering the negative effect of endonestrone on hemodynamic therapy, it is advised to use this drug [17] [19] [20]. The prophylactic use of this drug is recommended in women's surgery, especially abdominal hysterectomy.

In conclusion, our data suggested that these drugs are effective in reducing PONV in patients undergoing a hysterectomy. However, the effect of endonestrone on the reduction of postoperative nausea and vomiting is more than the other two drugs.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1664-1667.
https://doi.org/10.3889/oamjms.2018.340
eISSN: 1857-9655
Clinical Science

Value of Head CT Scan in the Emergency Department in Patients with Vertigo without Focal Neurological Abnormalities

Tomislav Pavlović[1,2]*, Marina Milošević[3], Sanja Trtica[1], Hrvoje Budinčević[2,3]

[1]Department of Radiology, Sveti Duh University Hospital, Zagreb, Croatia; [2]J. J. Strossmayer University of Osijek, Faculty of Medicine, Osijek, Croatia; [3]Stroke and Intensive Care Unit, Department of Neurology, University Hospital" Sveti Duh", Zagreb, Croatia

Citation: Pavlović T, Milošević M, Trtica S, Budinčević H. Value of Head Ct Scan in the Emergency Department in Patients with Vertigo without Focal Neurological Abnormalities. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1664-1667. https://doi.org/10.3889/oamjms.2018.340

Keywords: Vertigo; Emergency department; Computed tomography (CT)

***Correspondence:** Tomislav Pavlović. Department of Radiology, Sveti Duh University Hospital, Zagreb, Croatia. E-mail: tpavlovic2@gmail.com

Received: 20-May-2018; **Revised:** 14-Aug-2018; **Accepted:** 29-Aug-2018; **Online first:** 24-Sep-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Vertigo is a common symptom and reason for admission to the emergency department (ED).

AIM: This research aimed to determine the incidence of clinically significant findings on computed tomography (CT) in patients with vertigo without focal neurological abnormalities in the ED.

MATERIAL AND METHODS: The results of the native CT scans in the ED were retrospectively analysed. Exclusion criteria included: focal neurological abnormalities, underlying malignancy, brain metastasis, previous brain operation, headache, fever, nausea, vomiting, head trauma, coagulopathy. As a clinically significant finding, we took into an account tumour, haemorrhage and acute ischemic lesion. 72 patients fulfilled the set criteria, present vertigo, without focal neurological abnormalities. Out of 72 patients with a median age of 62 (23-87) years old, 54% of the patients were female, and 46% were male.

RESULTS: Normal CT findings were found in 44 patients (61.1%), 28 patients (38.9%) had pathological findings, out of that number 23 (31.9%) findings were clinically irrelevant and 5 (6.9%) were clinically significant. Out of the 5 clinically significant findings, tumour process was found in 3 (4.2%) patients, haemorrhage was found in 1 (1.4%) patient, and the ischemic lesion was found in 1 (1.4%) patient. Additional evaluation of five clinically significant findings showed a change of initial diagnosis in one case, but the significance of the finding remained the same.

CONCLUSION: Our study demonstrates a low diagnostic yield of head CT examination with 6.9% of clinically significant findings in patients with vertigo without focal neurological abnormalities.

Introduction

Vertigo is a common symptom and reason for admission to the emergency department (ED). Patients with vertigo constitute about 4% of all emergency visits [1] [2] [3] [4]. Vertigo is a false sensation of body movement or the environment around it. The patient usually senses rotation-rounding or spin. Vertigo represents one of the ten most common reasons for the ambulatory examination [5]. Treatment of patients with vertigo requires examinations from various specialists, including neurologists, ear nose throat (ENT) specialists and internists. Laboratory tests, imaging with brain computed tomography (CT) and brain magnetic resonance (MR) is often needed [2] [6]. Many patients with vertigo are released from the ED without a diagnosis [2] [7] [8]. CT scanning of the head is often performed in the evaluation of the patient with vertigo as a routine practice. This leads to the increased use of CT in ED [8] [9] especially in patients with vertigo [2]. CT has a limited value in the evaluation of the patient with vertigo [10] [11] [12], MR has a much greater sensitivity, but it is rarely available in the ED [11]. So far, published studies have revealed a small number of positive findings on head CT of the patients with vertigo, Lahwn-Heath et al., reported 2.2% positive findings on head CT scan in ED, Wasay et al., reported that head CT has a low

diagnostic yield for isolated vertigo [11]. Radiological investigations should be considered as elective diagnostic procedures, and they include CT, MR, MR angiography and digital subtraction angiography [13]. Evaluation of the vertigo is a significant financial load for the health system and also leads to the increased exposure to ionising radiation of the population [14]. Aetiology of vertigo may be related to the cardiovascular system or the central or peripheral nervous system. As a cause of vertigo, we can differentiate a central cause associated with the brain or peripheral cause associated with the diseases of the inner ear. The main reason for a head CT scan is the detection of potentially life-threatening disorders such as a stroke or a brain tumour [15]. Additional reasons for the CT scan examination are the legal aspects, patient's demand or pressure on the emergency physician.

This study aims to determine the incidence of positive CT scan findings in the emergency department in patients with vertigo, without focal neurological abnormalities.

Material and Methods

The results of the native CT scans of the brain performed in the emergency department (ED) of the University Hospital "Sveti Duh" in Zagreb from January 2017 till January 2018 were retrospectively analysed. Patients were older than 18 and younger than 87, and they presented to the ED because of vertigo. We took into the consideration only reports made by the selfsame radiologist with eight years of experience. We restricted our study to patients examined by residents of neurology or neurology specialists. All scans were obtained with 64-section multidetector CT scanner (Siemens). Exclusion criteria were: focal neurological abnormality, head trauma, underlying malignancy, brain metastases, previous brain surgery, headache, fever, nausea, vomiting, coagulopathy. Focal neurological abnormalities include impairments of the nerve, spinal cord or brain that affects a specific region of the body. CT scan reports were divided into three categories: V0-completely normal finding, V1-positive finding but clinically insignificant, V2-positive finding but clinically significant. As a clinically significant finding, tumour, haemorrhage and ischemic lesion were taken into consideration. All of the V2 reports were further evaluated by MR scan and/or CT angiography. Physicians requiring a CT scan were divided into two groups: N0-resident of neurology, N1-neurology specialist. For statistical analysis, MedCalc (16.2.0, MedCalc Software bvba, Ostend, Belgium) was used. Results were shown with descriptive statistics. Normality of the distribution of numeric variables was tested with the Kolgomorov-Smirnov test. Fisher's exact test was used for comparison of category variables differences. The results were considered statistically significant at $p < 0.05$. Ethical approval for this study was obtained.

Results

After data analysis, 72 patients fulfilled the set criteria, present vertigo, without focal neurological abnormalities. Out of 72 patients with median age of 62 (23-87) years old, 39 (54%) were women with median age of 63 (23-87) years old, and 33 (46%) of them were men with median age of 62 (27-86). Of the 72 patients who presented with vertigo, we found 6.9% clinically significant abnormal CT finding.

Table 1: CT scan reports according to the results

CT examination results	Male N = 33	Female N =39	All patients N = 72	p
VO	20; 27.8%	24; 33.3%	44; 61.1%	>0.99
V1	10; 13.9%	13; 18.1%	23; 31.9%	0.81
V2	3; 4.2%	2; 2.8%	5; 6.9%	0.66

Classification of the CT scan reports according to its results is shown in Table 1 and according to the physician requiring a CT scan in Table 2.

Table 2: CT scan reports according to the physician requiring a CT scan

	NO	N1	p
VO	22; 30.6%	22; 30.6%	0.15
V1	16; 22.2%	7; 9.7%	0.20
V2	3; 4.2%	2; 2.8%	> 0.99
All patients	41; 56.9%	31; 43.1%	

N0-resident of neurology; N1-neurology specialist.

Positive findings but clinically insignificant are shown in Table 3.

Table 3: Positive findings but clinically insignificant (V1)

Diagnosis N (%)
Microvascular changes and lacunas 11 (47.8%)
Parenchymal calcification 4 (17.4%)
Sinus changes 4 (17.4%)
Asymmetry of the ventricular system 3 (13.0%)
Cyst 1 (4.3%)

Positive findings but clinically significant depending on physician requiring a CT scan are shown in Table 4.

Table 4: Positive findings but clinically significant depending on the physician requiring a CT scan

V2	N0 N=3	N1 N=2	All patients V2 N=5 (6.9%)
Tumor	2	1	3 (4.2%)
Haemorrhage	0	1	1 (1.4%)
Ischaemia	1	0	1 (1.4%)

In three patients diagnosed with a tumour, MR showed expansive tumour of the cerebrum in two

cases and arteriovenous malformation of the cerebellum in one case. In a patient diagnosed with subarachnoidal haemorrhage, CT angiography and MR confirmed subarachnoidal haemorrhage. In case of the patient with the ischemic lesion, the finding was confirmed with MR scan. Additional evaluation of five clinically significant findings showed a change of initial diagnosis in one case, but the significance of the finding remained the same. Of the 72 patients who presented with vertigo 20 (27.8%) of them were admitted to the hospital. Of the 5 patients (6.9%) with V2 clinically significant CT finding, all 5 admitted to the Hospital. There was a statistically significant higher admission rate for patients with vertigo who also had a clinical significant CT finding than those who had normal head CT finding (p = 0.001). Classification of the CT scan reports according to the admission to the hospital is shown in Table 5.

Table 5: Classification of the CT scan reports according to the admission to the hospital

CT examination results	No admitted no.(%)	Admitted no.(%)	p
VO	36; 50.0%	8; 11.1%	0.03
V1	16; 22.2%	7; 9.7%	0.81
V2	0; 0%	5; 6.9%	0.001
All patients	52; 72.2%	20; 27.8%	

Discussion

Vertigo is a common symptom in medical practice and a common reason for presentation in ED [3] [4] [16]. Diagnostic evaluation of vertigo with CT scan remains controversial; there are some studies that have confused the importance of CT scan in vertigo without focal neurological abnormalities and recent head trauma [10] [17]. Previous studies in the diagnostic of clinically significant findings in CT scans of the head in patients with vertigo but without recent head trauma have various results. Mitsunaga et al., [18] showed 7.1% of clinically significant findings; the study included patients with focal neurological deficit and patients with head trauma. Lawhn-Heath et al., [19] reported 2.2% positive findings for head CT scan in ED; they included patients with the focal neurological deficit, headache and trauma. Ahsan et al., [1] found 6.17% positive findings and 0.74% clinically significant findings for patients who were examined with CT or MR; they included patients with vertigo and dizziness, excluded patients with a history of stroke, brain tumour, brain surgery and other neurological disorder. Fakhran et al., [20] reported 3% positive findings on CT and MR examination performed with contrast; they excluded patients with the focal neurological deficit. Our research revealed 6.9% of clinically significant emergency examinations in patients with vertigo, without focal neurological abnormalities. Analysing the incidence of clinically significant findings in this study, screening methods should be taken into account, considering that all of the patients were examined by residents of neurology or neurology specialist unlike in other studies where CT scan was required by emergency physicians, general practitioner, internal medicine specialists and other. Navi et al., [21] reported 7% of relevant abnormal head CT scans in the ER what is consistent with our results. Grossman et al., [22] reported 5% of abnormal head CT findings after excluding patients with trauma, altered mental status, seizure and hypoglycaemia what is also similar to our results. Similar to our results, Chase et al., [23] reported 8.3% abnormal finding on MR scan in ED and non-ED patients with vertigo.

According to the results of the requested CT examinations, we found that there is no statistically significant difference between residents of neurology and neurology specialists, according to that, we can conclude that the competences of residents of neurology after education meet the compliance with the clinical guidelines for the implementation of the screening of patients with vertigo. In our study incidence of ischemia is 1.4%. The result is consistent with prior studies, Kerber et al. reported that about 3% of the patients with dizziness had a stroke aetiology, and less of 1% of patients with isolated dizziness had a stroke as the aetiology [7] [8]. Kim et al., [24] estimate that the overall stroke risk for patients with dizziness presenting to the ED is 2.4%. Further evaluation of the clinically significant findings with MR scan or CT angiography showed a change in diagnosis in one case, but the finding remained clinically significant, and there was no change in the outcome category. Mitsunaga et al., [16] reported 18.6% admission to the hospital for the patients with vertigo. We also report an admission rate of 27.8% for patients with vertigo, which is similar to the 22% of patients admitted to the hospital from the ED in the study Navi et al., [21]. In our study patients with vertigo with positive clinically significant CT findings had higher admission rate which is consistent with study Mitsunaga et al., [18].

In conclusion, our study demonstrates a low diagnostic yield of head CT examination for patients with vertigo without focal neurological abnormalities. Routine CT scan cannot be recommended in these patients. Adequate guidelines need to be developed for the diagnostic treatment of patients with vertigo without neurological symptoms and signs.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1668-1673.
https://doi.org/10.3889/oamjms.2018.344
eISSN: 1857-9655
Clinical Science

Evaluation of Diagnostic Efficiency of Alpha-Fetoprotein in Patients with Liver Cirrhosis and Hepatocellular Carcinoma: Single-Center Experience

Lejla Mehinovic[1], Erna Islamagic[1]*, Azra Husic-Selimovic[2], Amina Kurtovic-Kozaric[3], Zora Vukobrat-Bijedic[4], Damir Suljevic[1]

[1]Department of Biology, Faculty of Science, University of Sarajevo, Sarajevo, Bosnia and Herzegovina; [2]Clinic for Gastroenterohepatology, Clinical Center of the University of Sarajevo, Sarajevo, Bosnia and Herzegovina; [3]Department of Clinical Pathology, Cytology and Human Genetics, Clinical Center of the University of Sarajevo, Sarajevo, Bosnia and Herzegovina; [4]Polyclinic Al-Tawil, Sarajevo, Bosnia and Herzegovina

Citation: Mehinovic L, Islamagic E, Husic-Selimovic A, Kurtovic-Kozaric A, Vukobrat-Bijedic Z, Suljevic D. Evaluation of Diagnostic Efficiency of Alpha-Fetoprotein in Patients with Liver Cirrhosis and Hepatocellular Carcinoma: Single-Center Experience. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1668-1673. https://doi.org/10.3889/oamjms.2018.344

Keywords: AFP; Hepatocellular carcinoma; Liver cirrhosis

***Correspondence:** Erna Islamagic. Department of Biology, Faculty of Science, University of Sarajevo, Sarajevo, Bosnia and Herzegovina. E-mail: erna.islamagic@hotmail.com

Received: 31-May-2018; **Revised:** 22-Aug-2018; **Accepted:** 28-Aug-2018; **Online first:** 24-Sep-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: AFP serum levels are considered as diagnostic and specific for hepatocellular carcinoma (HCC) in patients with liver cirrhosis (LC).

AIM: This study aimed to examine the diagnostic value of AFP in the distinguishing of patients with HCC from patients with LC, and to analyse the potential correlation between AFP levels and liver disease stages.

MATERIAL AND METHODS: Fifty patients with LC and fifty patients with HCC were included in this study. The majority of the patients were males, while the HBV aetiology was dominant.

RESULTS: Significant differences between LC and HCC patients were detected for AST, ALT, GGT, bilirubin, AFP and AP. Patients with HCC had higher AFP values compared to LC. There was no significant correlation between the size of the tumour lesion and serum AFP levels. A positive correlation between AFP concentration and GGT activity was determined, as was the negative correlation between AFP and age of the subjects. The AFP value of 23.34 ng/m showed high sensitivity (84%) and specificity (82%).

CONCLUSION: The size of the surface below the ROC curve (AUC) was 0.877 (0.80-0.95), which makes AFP a good biomarker and this diagnostic test is sufficient to separate patients with HCC and LC.

Introduction

Hepatocellular carcinoma (HCC) is a malignant epithelial neoplasm with hepatocellular differentiation, and it is the most common primary malignant liver neoplasm [1]. HCC is ranked sixth in the world among all malignant neoplasms, and due to its rapid progression and poor outcomes it is ranked third regarding mortality from malignant neoplasms: among men, it is the fifth most common malignancy and the third most common the cause of death from malignant neoplasms [1] [2]. Men are three times more likely than women to develop HCC, which is ranked as the eight most common type of cancer [1]. Over the past 20 to 30 years, the occurrence of newly diagnosed HCC has more than doubled in the United Kingdom and the United States, and the mortality rate will rise by 14% among men in the UK for the next 20 years [1] [2] [3].

The cause of higher incidence is an increase in the number of HCV infections as well as migration from regions where HBV is highly represented, such as developing countries, which is also considered to be a biological carcinogen [4] [5]. The risk for HCC disease was additionally increased in patients with higher levels of HBV replication, indicating the presence of hepatitis B antigen (HBeAg) and high levels of HBV DNA [6] [7] [8].

Many studies pointed out serum levels of AFP and GGT as significant prognostic factors in predicting the prognosis of HCC patients [9] [10]. Several studies have shown that younger patients with diagnosed HCC have higher levels of serum AFP compared to older patients with the same pathological condition [11] [12] [13]. Furthermore, recent studies have shown

that high serum AFP concentration correlates with poor prognosis in patients with HCC. According to previous studies, elevated serum concentrations of AFP in patients with LC are a risk factor for the development of HCC because increased AFP production in patients with LC is a reflection of large and abnormal hepatic regeneration [14] [15].

AFP serum levels > 400 ng/ml are considered as diagnostic and specific for HCC in patients with liver cirrhosis [16]. According to Gomez Senenta et al., the high specificity of AFP that can be obtained by increasing its diagnostic thresholds and allows the use of this biomarker as a confirmatory test for the diagnosis of HCC [17]. Furthermore, previous studies have shown that AFP values > 200 ng/ml in patients with cirrhosis of HBV and/or HCV etiology are highly predictive for HCC indicating that using higher serum AFP concentration as a limit value of the diagnostic test progressively reduces the number of detected HCC cases, i.e. reduces its sensitivity, but in favor of its specificity [18] [19] [20] [21] [22] [23].

This study aimed to examine the diagnostic value of alpha-fetoprotein in the distinguishing of patients with HCC from patients with liver cirrhosis, i.e. to determine its sensitivity and specificity in the detection of hepatocellular carcinoma in the examined sample. Additionally, the aim was to analyse the potential correlation between AFP levels and liver disease stages.

Material and Methods

In this study 100 patients (57 men and 43 women) were included and categorised into two groups. The first group (n = 50) consisted of patients with diagnosed liver cirrhosis (LC) of HBV and /or HCV viral aetiology, and the average age of patients was 62.70 ± 10.49 years. The second group consisted of patients (n = 50) with proven hepatocellular carcinoma (HCC), and the average age of patients was 63.76 ± 10.92 years. In all patients, total bilirubin and AFP (alpha-fetoprotein) concentrations, AST (aspartate aminotransferase), ALT (alanine aminotransferase), γGT (gamma-glutamyl transferase), AP (alkaline phosphatase) activity were analysed. Biochemical parameters were analysed using the VITROS 5600 Integrated System (Ortho-Clinical Diagnostics, USA) analyser and chemiluminescent microparticle immunoassay ARCHITECT AFP assay (CMIA, Ireland) was used for AFP detection. EHO and computerised tomography (CT) were used to detect and measure the size of this lesion (HCC).

All data were analysed using IBM SPSS Statistics ver. 20 (USA). Variance analysis (ANOVA) was used to test differences. Survival Kaplan-Meier test was used to analyse the coherence between sensitivity and specificity through all possible limit values that determine the positive pathological condition or presence of the disease. The area under the ROC curve (AUC) is the measure of the discriminating power of the diagnostic test.

Results

The percentage of patients diagnosed with LC and HCC, patients' sex and age, aetiology of disease (HCV, HBV) and significant differences (p values) are presented in Table 1. The group of patients with a diagnosis of liver cirrhosis (LC) had 50 patients (26 males and 24 women), the average age of 62.70 years. In the HCC group (n = 50) consisted of 31 males and 19 women average age was 63.73 years. No significant differences were found between men and women when comparing both diseases; also no significant difference in age and viral aetiology was established. HBV is a dominant viral agent present in 62% of the patients.

Table 1: Demographic and clinical characteristics of patients with LC and proven HCC

Characteristics		Patients with LC[1]	Patients with HCC[2]	Total	p values
Gender	Males	26 or 52 %	31 or 62 %	57	> 0.05
	Females	24 or 48 %	19 or 38 %	43	> 0.05
Age	Mean	62.70±10.49	63.73±10.92	63.29±10.67	> 0.05
	Range	30-81	29-81	29-81	-
Etiology	HBV[3]	28 or 56 %	34 or 68 %	62	> 0.05
	HCV[4]	21 or 42 %	16 or 32 %	37	> 0.05
	HBV+HCV	1 or 2 %	-	1	-

[1]LC - liver cirrhosis; [2]HCC - hepatocellular carcinoma; [3]HBV- hepatitis B virus; [4]HCV- hepatitis C virus.

Table 2 shows the values of biochemical parameters in LC and HCC patients, and significant differences between them. All patients had high values of biochemical parameters compared to the reference range, while HCC patients had higher values compared to LC patients. Significant differences between these two groups were observed for AST, γGT, AP and AFP values.

Table 2: Differences in biochemical parameters in LC and HCC patients

Parameters	Patients with LC[1] (n = 50)	Patients with HCC[2] (n = 50)	P values
Total bilirubin	43.63 ± 34.17	60.82 ± 99.41	> 0.05
AST	109.24 ± 145.02	160.42 ± 138.48	< 0.05*
ALT	75.50 ± 91.57	78.08 ± 60.77	> 0.05
γGT	110.98 ± 163.67	192.74 ± 188.47	< 0.05*
AP	123.00 ± 88.03	199.56 ± 149.47	< 0.05*
AFP	256.82 ± 1634.03	9129 ± 17547.01	< 0.05*

* Significant differences at the level of 0.05.

Table 3 shows AFP serum concentrations in LC and HCC patients. Also, AFP values for HCC patients with viral aetiology were presented as well as Spearman correlation between AFP and age of patients. Males in the group of LC and HCC patients

had significantly higher values compared to females. Among HCC patients, very high values of AFP (12465.08 ng/l) were determined for patients with HBV aetiology in comparison to patients with HCV aetiology, but these differences were not significant. A negative correlation (-0.053) but not statistically significant was found between the serum levels of AFP concentration and age of the patient (younger patients had more serum AFP values).

Table 3: Differences in serum concentrations of AFP according to aetiology and gender

Diagnosis	HBV/HCV	Mean ± SD	p values	Correlation between AFP and age
HCC patients (Males/females)	HBV	12465.08	< 0.05	
	HCV	2040.92		
HCC patients	Males	10790 ± 19757.43	> 0.05	
	Females	6419 ± 13231.46		-0.053
LC patients	Males	468.60 ± 2265.50	> 0.05	
	Females	27.40 ± 62.82		

Table 4 shows the distribution of serological AFP values between LC and HCC patients. Both groups had a very high percentage of patients with increased serum AFP (> 7 ng/ml). Only 8% of HCC patients had serum AFP values within the reference range.

Table 4: Distribution of serological AFP values between LC and HCC patients

AFP concentrations	LC patients	HCC patients	Total
> 7 ng/ml	32 or 64 %	46 or 92 %	78 %
< 7 ng/ml	18 or 36 %	4 or 8 %	22 %

The sensitivity of the medical diagnostic test (true positive rate) gives the ability to detect the presence of the disease; however, the specificity of the medical diagnostic test (true negative rate) gives the ability to determine the absence of the disease. The ROC curves show the relationship between sensitivity and 1-specificity through all possible limit values that determine the positive pathological condition or presence of the disease. The area below the ROC curve (AUC) is the measure of the discriminating power of the diagnostic test [24].

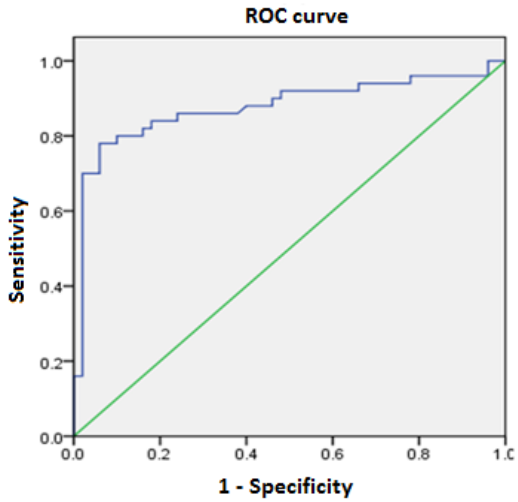

Figure 1: ROC curve of serum alpha-fetoprotein for the diagnosis of hepatocellular carcinoma. The green line corresponds to the 1:1 correlation between two parameters

The ROC curve (receiver-operating characteristic curve) is, therefore, a graphical representation of the proportion of successfully identified and falsely identified cases of hepatocellular carcinoma. Figure 1 shows an AUC of 0.877 (0.80-0.95), making this biomarker a good distinguishing parameter for patients with HCC and those with LC.

Table 5 shows AUC values for ROC curve. Also, ROC analysis implies testing all AFP serum values, and for each of the sensitivity and specificity were detected. It has been determined that the optimal limit value of AFP serum concentrations for distinguishing of patients (with and without HCC) within our population was 23.34 ng/ml. At this serum concentration, this test has approximate and high sensitivity and specificity (84% vs 82%) indicating the diagnostic significance of this tumour marker for early diagnosis of hepatocellular carcinoma in LC patients.

Table 5: Area of measurements under ROC curves (AUC), AFP sensitivity and specificity

Variable	AUC	STD Error[1]	Asympt sig.[2]	UCL	LCL	Cut-off	Sens.	Specify.
AFP	0.877	0.038	0.000	0.802	0.0952	23.34	84%	82%

[1] Under a non-parametric assumption; [2] Null hypothesis: true area = 0.5; UCL – upper control limit; LCL – lower control limit; Sens. – sensitivity; Spec. – specificity; Optimal cut-off: optimal limit value.

Since our limit value has similar sensitivity and specificity, the limit values with the best performance regarding sensitivity and specificity are shown in Table 6.

Table 6: Specificity and sensitivity of the different AFP limit values for the diagnosis of hepatocellular carcinoma

AFP (ng/ml)	Sensitivity %	Specificity %
6.27	92 %	34
12.38	88	60
19.14	84	76
58.49	80	90
81.58	78	94
149.77	74	94
219.96	72	98
341.70	70	98
420.67	66	99

Table 7 shows a grouping of patients with HCC in comparison to tumour lesion size and serum AFP level.

Table 7: Tumor size in HCC patients and correlation of AFP about lesion size

Tumour size (cm)	N (%)	AFP (ng/ml)		
		≤ 20	21-399	≥ 400
< 3	2 or 4 %	1	1	0
3-5	11 or 22 %	1	3	7
> 5	37 or 74 %	6	4	27

According to the results shown in Table 7, 37 patients with proven HCC (74%) had a tumour> 5 cm, and six patients had normal serum AFP levels, four moderately elevated, and 27 patients with tumour size > 5 cm had significantly elevated values serum AFP. In the group of 11 patients (22%) with proven HCC with tumour size from 3-5 cm, one patient had a normal serum AFP level, in three patients this

biomarker was moderately elevated and in 7 patients it was elevated (≥ 400 ng/ml). Only two patients (4%) with proven HCC had tumour size < 3 cm; one of them had a normal serum AFP level, and the other one had moderately elevated AFP concentration.

Discussion

Our research has shown a higher percentage of male patients with LC and HCC diagnosis, while the average age is not different in comparison to women. Most patients had HBV viral aetiology with dominant prevalence in our country [25]. Patients with hepatocellular carcinoma developed after HBV infection have significantly higher mean levels of AFP than patients with proved hepatocellular carcinoma developed in the background of HCV infection. Therefore, a type of viral infection affects serum levels of alpha-fetoprotein in patients with hepatocellular carcinoma.

Due to more progressive disease, HCC patients had higher values of all analysed parameters compared to LC patients. The largest variations were detected for AFP concentrations. The serological values of AFP and γGT correlate, as previously confirmed in the study of Ertle et al., [26]. This correlation shows parallel reexpression of AFP with reexpression of γGT during the development of HCC, otherwise present in embryonic liver and suppressed after birth. Younger patients tend to have higher levels of serum AFP compared to older patients [12]. The results of our study showed a significant reverse correlation between serum AFP levels and age of patients. 84% of our patients with HCC had an elevated level of this biomarker, similar to results in previous studies [27] [28].

This is the first study in Bosnia and Herzegovina that by analysing the ROC curve evaluates the diagnostic performance of serological tests. The AUC value > 0.96 suggests, an excellent discriminatory ability of the diagnostic test [29]. In our study, measurement of AFP has shown a significant ability to discriminate between HCC and LC diagnosis due to its AUC that was very close to the specified value. In previous studies, the ROC curve of the alpha-fetoprotein used as a diagnostic test suggests that the serum AFP level of about 20 ng/ml provides the optimal balance between sensitivity and specificity (optimal cut-off/limit value). According to Trevisiani et al., the best diagnostic cut-off serum AFP level ranged from 16 to 20ng/ml, with a high specificity of 89.4% and a sensitivity of 60% [30]. In our case, this diagnostic test has omitted 40% of HCC cases. If a higher limit value were used, it would progressively reduce the number of detected HCC cases, i.e. would reduce the sensitivity of the diagnostic test. Recent results of Daruzo et al. and El-Hussein et al. indicated a sensitivity of 69% and 68.2% at the limit of 19.2 ng/ml in the first and 25 ng/ml in the second study [31] [32].

Our research has confirmed results from earlier studies where the best sensitivity and specificity balance of the AFP serum level was at the value of 20 ng/ml; in our case, it was 23.34 ng/ml. Optimal AFP serum level for our study had a high sensitivity of 84% and specificity of 82%. This value of sensitivity was higher than values obtained by Arietta et al. where the optimal cut-off value at 21ng/ml had a 76% of sensitivity [33]. However, our sensitivity (84%) and specificity (82%) values were within sensitivity, and specificity ranges from 39% to 97%, and 76% to 95%, respectively, and they are similar to the results from previous studies [19] [20] [21] [22] [23]. However, our results regarding the sensitivity and the specificity of the optimal AFP limit value were more favourable in comparison to results reported by Trevisiana et al., Durazo et al., El-Hussain et al. and Hernandez et al., which were taken as reference in current scientific frameworks in this field [18] [30] [31] [32]. One explanation for this phenomenon would be that in our study only patients with viral aetiology were involved, whereas in the mentioned studies viral aetiology was dominant but not excluded. Accordingly, this biomarker has significantly better performance in hepatocellular carcinoma with viral aetiology compared to other HCC etiologies, as it was demonstrated in the recent study by Ertel et al., [26]. The surface area under the ROC curve (AUC) was 0.877 (0.80-0.95), making this biomarker a good diagnostic test for distinguishing HCC and LC patients.

In the case of our patients, the value of 0.877 is at the very limit so that a diagnostic test could be characterised as excellent, given this value was close to 0.90. Our results were similar to results obtained by Trevisiani et al., where AFP serum concentrations higher than 20 ng/ml had a specificity of 98.4% or less than 2 % of patients without HCC had serological AFP levels higher than 20 ng/ml [30]. Also, the results from our study confirmed previous serum levels of AFP > 400 ng/ml as diagnostic, highly specific for HCC, i.e. that 1% or less of our patients had high AFP concentrations and did not have HCC [34]. It is important to note that high limit value is not useful for detecting early stage of HCC [34]. In these cases, patients with primary liver cancer may have normal levels of serum AFP; however normal or moderately elevated AFP levels do not exclude the HCC diagnosis.

We found that 74% of HCC patients had tumour lesion size more than > 5 cm as it was reported earlier by Arietta et al., (75%) [33]. However, previous studies have found that two-thirds of patients with hepatocellular lesion size < 4 cm have serum AFP levels < 200 ng/ml. Also, it has been reported that up to 20% of HCC patients do not produce elevated levels of AFP [35]. These data are consistent with the results of our study where 16% of HCC

patients did not have elevated levels of AFP. Although it may be inferred that smaller tumour size is related with lower serum AFP concentrations in comparison to tumours with larger diameter, our results showed that there was no significant correlation between the size of the tumour lesion and the level of AFP, which is similar to results from previous studies except in case of results obtained by Abbasi et al. who have established the existence of correlation [36] [37]. The absence of correlation between the serum concentration and the size of the hepatocellular lesion could be explained by the fact that tumour differentiation and its AFP secretion ability are more important in determining the level of serum AFP produced by primary liver cancer, than tumour size.

Adequate sensitivity and specificity allow the use of serum AFP values as an extremely useful tool in preclinical HCC detection in liver cirrhosis patients. We believe that the results of our research gave a complete picture of the role of AFP in HCC pathogenesis and suggested the justification of using this parameter in determination and monitoring.

Serum levels of liver biomarkers are significantly higher in HCC patients compared to LC patients. The serum AFP level could not be used as a reliable indicator of tumour lesion size; tumour differentiation and its AFP secretion ability are more important for determining serum AFP levels produced by primary liver cancer than its size alone. The size of the AUC under ROC curve of 0.877 suggests that the determination of AFP serological concentration may be considered as a good diagnostic test for the distinguishing patients with hepatocellular carcinoma and those with cirrhosis (with viral B and C aetiology). Based on the obtained ROC curve, the optimal serum concentration of AFP for discrimination between patients with and without HCC within our population was 23.34 ng/ml. This value achieved a balance of sensitivity and specificity of the serum AFP level, used as a diagnostic test.

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https://doi.org/10.3889/oamjms.2018.390
eISSN: 1857-9655
Clinical Science

High Il-1β Serum as a Predictor of Decreased Cognitive Function in Mild Traumatic Brain Injury Patients

Dewa Putu Gede Purwa Samatra*, Ni Made Dwita Pratiwi, I Putu Eka Widyadharma

Department of Neurology, Faculty of Medicine, Udayana University, Sanglah General Hospital, Bali, Indonesia

Citation: Samatra DPGP, Pratiwi NMD, Widyadharma IPE. High Il-1β Serum as a Predictor of Decreased Cognitive Function in Mild Traumatic Brain Injury Patients. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1674-1677. https://doi.org/10.3889/oamjms.2018.390

Keywords: IL-1β serum; Cognitive dysfunction; Traumatic brain injury

***Correspondence:** Dewa Putu Gede Purwa Samatra. Department of Neurology, Faculty of Medicine, Udayana University/Sanglah General Hospital, Bali, Indonesia. E-mail: purwa_bali@yahoo.com

Received: 05-Jul-2018; **Revised:** 06-Sep-2018; **Accepted:** 07-Sep-2018; **Online first:** 24-Sep-2018

Funding: This study was financially supported by a grant from the Research and Development Faculty of Medicine, Udayana University

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

Abstract

BACKGROUND: Traumatic brain injury (TBI) exerts a significant impact on society with regards to physical, affective, and cognitive impairment. The consequent cognitive sequelae include a problem in memory, attention, concentration, and processing speed. Following traumatic brain injury, inflammatory response developed, characterised by increased interleukin 1-β (IL-1β) levels in the blood. IL 1-β at pathophysiological concentration has been reported to cause an inhibition of the expression of long-term potentiation (LTP) in the areas CA1, CA3, and dentate gyrus of the hippocampus.

AIM: This study aims to determine whether high IL-1β serum is a predictor of decreased cognitive function in mild TBI.

METHODS: This is a prospective cohort study conducted at the emergency room, surgical and neurologic ward at Sanglah Hospital from November 2017 until January 2018. As many as thirty-five mild TBI with normal IL-1β serum (< 0.0565 pg/ml) and thirty-five of those with high IL-1β serum (≥ 0.0565 pg/ml) subjects were included within the corresponding period. The decrease of cognition after trauma was measured seven days later.

RESULTS: This study demonstrated that group with high IL-1β serum levels were at higher risk of suffering from cognitive impairment after TBI when compared with the group with normal IL-1β serum levels (RR = 2.6; 95% CI 1.49-4.55, p < 0.001).

CONCLUSION: Mild TBI with high serum IL-1β levels were more than twice likely to experience decreased cognitive function than those with normal IL-1β levels.

Introduction

Traumatic brain injury (TBI) is a neuro-emergency situation which has a significant primary impact (i.e. cost of treatment) and also secondary impact (i.e. loss of productivity) in the patient. There are various TBI complications including physical, affective, and cognitive impairment. Furthermore, cognitive impairment comprises memory dysfunction, poor concentration and thinking process [1]. Meanwhile, physical impairment may occur as a headache, dizziness, and lethargy, whereas affective impairment includes emotional disturbance such as irritability, anxiety disorder, and depression. Those impairments may potentially affect the ability of patients to do work as usual and their role in society [2]. TBI is the third leading cause of death related to traumatic cases in the U.S.; it is estimated that 1.7 million American citizens suffered from TBI every year [3].

The resulting inflammatory response after TBI occurs both locally at the injured area (so-called neuroinflammation) and systemically. After the initial injury, complement is activated which subsequently followed by neutrophil, lymphocyte, and monocyte infiltration via blood-brain barrier. Also, secretion of pro-inflammatory cytokines and mediators, prostaglandin, reactive agents, and other inflammatory molecules also occur simultaneously. These processes subsequently lead to increased chemokine and adhesion molecule expressions, enabling immune cells and microglia to infiltrate brain parenchyma which initiates secondary brain injury [4] [5].

Interleukin 1-β (IL-1β) is an inflammatory marker that can be potentially used as a predictor of

TBI. IL-1β is produced by the central nervous system as a response to several stimuli, such as administration of peripheral lipopolysaccharides, TBI, acute stress, anorexia, and administration of β-α adreno-receptor agonists. In the brain, IL-1β levels are particularly high in the hippocampus and hypothalamic area. High IL-1β levels to the extent of its pathophysiologic concentration (0.1-10 ng/mL) as found in post-mortem tissue and cerebrospinal fluid in a chronic disease like Alzheimer have been reported to inhibit long-term potentiation (LTP) in CA1, CA3, and dentate gyrus of the hippocampal area [6].

A study conducted by Coogan et al., [6] also found that chronic high IL-1β levels were found to inhibit LTP induction process. Furthermore, another study by Ross et al., [7] concluded that IL-1β at 1 and 10 ng/mL could decrease the post-synaptic excitatory potential by 25% in CA1 area of the rat hippocampus. However, further studies are required to profile IL-1β levels increment if it is to be used as a predictor of cognitive function in TBI.

Therefore, this study aims to determine whether high IL-1β serum is a predictor of decreased cognitive function in mild TBI.

Material and Methods

This was an analytic observational prospective cohort study, with consecutive non-random sampling method. This study had been approved by the Ethical Committee for Human Study, Faculty of Medicine, Udayana University and all of the investigators had ensured that the study adhered to the WMA Declaration of Helsinki [8]. Also, every patient who participated in this study had agreed and signed informed consent paper.

This study was conducted at the emergency room, surgical and neurologic ward at Sanglah Hospital from November 2017 until January 2018. During the corresponding period, as many as thirty-five subjects suffering from mild TBI with normal IL-1β serum (< 0.0565 pg/mL) and thirty-five mild TBI subjects with high IL-1β serum (≥ 0.0565 pg/mL) were included.

The cognitive state examination pre-TBI, depression, and cognitive evaluation post-TBI were done using Short Form of the Informant Questionnaire on Cognitive Decline in the Elderly (Short IQCODE), Hamilton Rating Scale for Depression, and Indonesian Version of Montreal Cognitive Assessment (MoCA-INA) questionnaire, respectively. Mild TBI was diagnosed based on the subject's history, physical and neurological examinations and confirmed by non-contrast CT scan. IL-1β levels were measured during admission, i.e. at the onset of initial brain injury, before 24 hours using ELISA-based Quantikine HS Human IL-1/beta-1F2 Immunoassay (R&D system, USA). The protocol was conducted according to the procedure written at the packaging.

The collected data were subsequently analysed using IBM SPSS version 20 for Windows (IBM Inc, USA). Data were analysed in two phases, i.e. descriptive and analytical phases. Analytical statistics were performed in order to obtain the relative risk of cognitive impairment incidence post mild TBI between two groups, along with test for significance using Chi-square test. *P* value of < 0.05 was considered significant with confidence interval of 95%.

Results

70 subjects were involved in this study, whom further divided into two groups (normal vs. high IL-1β), each with 35 subjects. IL-1β serum levels from all subjects were within 0.028 to 2.315 pg/mL. An ROC curve was then determined in order to define the ability of IL-1β serum levels as a predictive factor of cognitive impairment. The obtained AUC of 86.9% (95% CI 78.5%-95.3%, p < 0.001) was considered adequate statistically (Figure 1). The results of ROC coordinate had showed that IL-1 β cut-off point of ≥ 0.0565 pg/mL which was used in this study had a 88.9% sensitivity and 64.7% specificity.

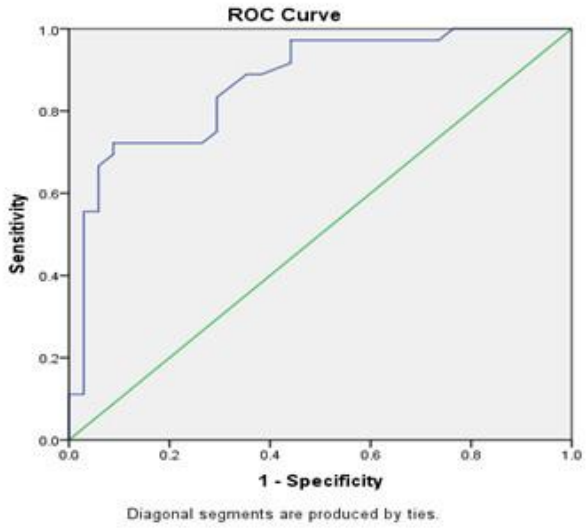

Figure 1: ROC of IL-1β serum levels toward cognitive decline with 86.9% AUC

Accordingly, all subjects were divided into two groups, i.e. those with high IL-1β as defined by serum levels of ≥ 0.0565 pg/mL and low IL-1β defined by serum levels of < 0.0565 pg/mL.

The characteristics of subjects based on IL-1β levels were presented at the Table 1.

Table 1: Subject's baseline characteristics

Variables	Normal IL-1β value	High IL-1β value	p
	n (%)	n (%)	
Gender			
Male	16 (45.7)	27 (77.1)	0,007[a]
Female	19 (54.3)	8 (22.9)	
Age			
17-25 years old	15 (42.9)	15 (42.9)	1,000[a]
26-35 years old	20 (57.1)	20 (57.1)	
Education			
Middle School	32 (91.4)	32 (91.4)	1,000[b]
Bachelor degree	3 (8.6)	3 (8.6)	

[a]*Chi-square test;* [b]*Fisher's Exact test.*

As many as 16 (45.7%) male and 19 female (54.3%) subjects had normal IL-1β levels, whereas 27 male (77.1%) and 8 female (22.9%) subjects had high IL-1β serum levels. Within normal IL-1β group, 15 (42.9%) subjects were of late adolescents and 20 (57.1%) were of early adults, while in high IL-1β group, 15 (42.9%) subjects were of late adolescents category and 20 (57.1%) subjects were of early adults (p = 1).

Similarly, education levels within group were alike, i.e. 32 (91.4%) vs. 32 (91.4%) subjects from normal and high IL-1β serum groups, respectively, graduated from middle school, whereas as many as 3 (8.6%) subjects from both groups graduated from university.

Table 2: Bivariate analysis of serum IL-1β levels with Cognitive Function

Variables		Cognitive Function		RR (IK 95%)	P
		Decreased n (%)	Normal n (%)		
Serum IL-1β levels	Normal	10 (28.6%)	25 (71.4%)	2.60 (1.49-4.55)	<0.001
	High	26 (74.3%)	9 (25.7%)		

The incidence of cognitive function impairment in mild TBI subjects with normal serum IL-1β levels was 28.6%, significantly lower when compared with those of high serum IL-1β group (74.3%). Accordingly, subjects with high serum IL-β levels were more prone to suffer from the decreased cognitive function as opposed to a normal group (RR 2.60; 95% CI 1.49-4.55, p < 0.001).

Table 3: Bivariate analysis of other variables with cognitive function impairment

Other Variables	Cognitive Function		RR (IK 95%)	p
	Decreased n (%)	Normal n (%)		
Male	26 (72.2)	17 (50.0)	1.63	0.056[a]
Female	10 (27.8)	17 (50.0)	(0.94-2.82)	
Late adolescent	11 (30.6%)	19 (55.9%)	0.59	0.032[a]
Early adult	25 (69.4%)	15 (44.1%)	(0.35-0.99)	
Middle School	33 (91.7%)	31 (91.2%)	1.03	1.000[b]
Bachelor Degree	3 (8.3%)	3 (8.8%)	(0.45-2.38)	

[a]*Chi-square Test;* [b]*Fisher's Exact Test.*

Further analyses revealed that decreased cognitive function was more commonly found among males (72.2%) across all groups. Indeed, males were more likely to suffer from decreased cognitive function than females, despite statistically non-significant (RR = 1.63; 95% CI 0.94-2.82, p = 0.056). In regards to age group, fewer late adolescent suffered from decreased cognitive function as opposed to early adult (30.6% vs. 69.4%, respectively; RR 0.59, 95% CI 0.35-0.99, p = 0.032). Furthermore, with respect to education levels, decreased cognitive function did not differ significantly in middle school- vs. university graduates (RR = 1.03, 95% CI 0.45-2.38, p = 1.00).

Discussion

This study showed that there were 27 males (77.1%) and 8 females (22.9%) who suffered from TBI had high serum IL-1β levels. These results were in accordance to a study conducted at Hasan Sadikin Hospital during the 2008-2010 period, in which as many as 79.8% males and 20.2% females from 3,578 subjects suffered from TBI [9]. This significant proportional difference of sex-based TBI was not unfamiliar, as males ride 4.5 times more frequently than females and tend to engage in physical conflict, thus potentially result in head injuries [10].

In normal serum IL-1β group, as many as 15 subjects were of late adolescent (42.9%), and 20 subjects were of early adult (57.1%). In this study, age was focused on these two groups since according to Indonesian national health statistics, TBI occurs most often among 15-44 years old age group. The decreased cognitive function was found more commonly among early adult with statistical significance. The result was apparently by another study which found that older subjects demonstrated a greater cognitive decline than younger one over a 5-year period [11]. Herein, we demonstrated a higher incidence of an acute decrease in cognitive function in older individuals shortly after TBI. These results may in part be explained by the age-related difference in synaptic plasticity and cortical volume. It is still to be determined, however, if the cognitive decline persists over a longer period, and if there is any difference in the status and rate of meaningful cognitive recovery among different age groups.

In both normal and high serum IL-1β groups, decreased cognitive function did not seem affected by educational level. This is contrary to the common belief and clinical findings that a higher educational level may be protective to cognitive decline post-TBI, with one of the most important factors is having higher cognitive reserve [12]. Our study results may be limited by the scarcity of subjects who had attained university degree (3 subjects in each group), thus providing inadequate power to the results.

In this study, serum IL-1β levels among all subjects were within 0.028 pg/mL to 2.315 pg/mL. Furthermore, AUC derived from ROC method was 86.9% (95% CI 78.5%-95.3%, p < 0.001). Statistically, AUC of 86.9% had an adequate diagnostic value

(Figure 1). ROC coordinate showed that IL-1β cut-off point of ≥ 0.0565 pg/mL used in this study had 88.9% sensitivity and 64.7% specificity. In our study, IL-1β levels among all groups were markedly lower than previous studies. Several reasons might account for this discrepancy, i.e. firstly, we included relatively younger subjects (15-44 years old) as opposed to a study with a wide age range (18-74 years old) [13]. IL-1β is not specific for TBI. Thus various diseases, including infection or metabolic/degenerative can obscure the result. Secondly, even when there are comorbidities, IL-1β levels can still surprisingly detected at minute levels, ranging from 0.5 pg/mL in the hip fracture to 1.39 pg/mL in subjects with congestive heart failure [14]. Given the wide variations of IL-1β levels, it should be ideally measured periodically and assessed for its elevation/decline rather than its absolute levels per se.

Most importantly, this study found a markedly increased risk of experiencing decreased cognitive function among subjects with high IL-1β levels. Our study thus confirmed the previous findings, for instance, one which was conducted by Coogan et al., [6] that abnormally high IL-1β levels had been shown to inhibit LTP. IL-1β at 1 and 10 ng/mL can decrease the post-synaptic excitatory potential by 25% in CA1 area of the rat hippocampus, thus potentially affecting memory formation, or even its storage and retrieval.

In conclusion, mild TBI with high serum IL-1β levels was more than twice likely to experience decreased cognitive function than those with normal IL-1β levels.

Acknowledgement

This study was financially supported by a grant from the Research and Development Faculty of Medicine, Udayana University. Authors in this study thanked all of the patients who participated in this study, as well as to Putri Rossyana Dewi, M.D. and Andreas Soejitno, M.D. for their assistance in translating this manuscript into English.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1678-1681.
https://doi.org/10.3889/oamjms.2018.306
eISSN: 1857-9655
Case Report

Non Catether Induced Renal and Inferior Vena Cava Trombosis in a Neonate: A Case Report

Natasha Aluloska[1*], Snezana Janchevska[2], Velibor Tasic[3]

[1]Neonatology Department, University Children Hospital, Skopje, Republic of Macedonia; [2]NICU, University Clinic for Gynecology and Obstetrics, Skopje, Republic of Macedonia; [3]Nephrology Department, University Children Hospital, Skopje, Republic of Macedonia

Citation: Aluloska N, Janchevska S, Tasic V. Non-Catheter Induced Renal and Inferior Vena Cava Thrombosis in a Neonate: A Case Report. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1678-1681. https://doi.org/10.3889/oamjms.2018.306

Keywords: Renal thrombosis; Venous thrombosis; Newborn; Hematuria; Ultrasound

***Correspondence:** Natasha Aluloska. Neonatology Department, University Children Hospital, Skopje, Republic of Macedonia. E-mail: aluloska@gmail.com

Received: 18-Jun-2018; **Revised:** 23-Jul-2018; **Accepted:** 27-Jul-2018; **Online first:** 23-Aug-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Neonatal renal vein thrombosis is the most common vascular condition in the newborn kidney, which could lead to serious complication in infants.

CASE REPORT: We report a case of the unilateral renal vein and inferior vena cava thrombosis, presented with gross hematuria and thrombocytopenia in a neonate. The neonate was a macrosomic male born to a mother with hyperglycemia in pregnancy. The baby was born with perinatal asphyxia and early neonatal infection and massive hematuria. Clinical and laboratory examination showed enlarged kidney having corticomedullary differentiation diminished and azotemia. Diagnosis of renal vein thrombosis was suspected by renal ultrasound and confirmed by magnetic urography. Prothrombotic risk factors were evaluated. The child is being managed conservatively. Measures aimed at the prevention of end-stage renal disease because of its poor outcome were highlighted. Despite anticoagulant therapy, the right kidney developed areas of scarring and then atrophy.

CONCLUSION: In this work, we present a patient with multiple entities in the aetiology of non-catheter induced renal and vena cava thrombosis in a neonate. Clinicians should suspect renal vein thrombosis in neonates when presented with early postnatal gross hematuria, palpable abdominal mass and thrombopenia.

Introduction

Renal vein thrombosis is a rare condition in neonates, usually as a consequence of perinatal asphyxia, maternal diabetes mellitus, hypovolemia, hyperosmolarity and coagulopathies. Less often the cause of renal vein thrombosis is congenital heart disease, disseminated coagulopathy, prematurity and infection. External risk factors include central arterial and venous catheters. Neonates are most susceptible age group for renal vein thrombosis; premature infants are a most susceptible neonatal group.

Neonates are most susceptible for renal vein thrombosis due to the physiology of neonatal hemostasis and lower levels of anticoagulants also low levels of fibrinolytic components. The prevalence is 2.2 per 100,000 lives and around 0.5 per 1000 newborn babies treated in NICU. The prevalence of inferior vena cava thrombosis in neonates is the main symptoms are gross hematuria, papabile abdominal mass due to enlarged kidney and thrombocytopenia.

Diagnosis is suspected based on specific symptoms, ultrasonography and additional imaging studies such as venography, CT and MR urography confirm the diagnosis. Aetiology of hypercoagulable state should be searched for and treated. Inherited prothrombotic anomalies have been reported in association with neonatal renal vein thrombosis. Signs and symptoms of acute kidney injury should be monitored, and long-term follow up is mandatory. We present a patient with multiple entities in the aetiology of non-catheter induced renal and vena cava thrombosis in a neonate.

Case report

We report a case of the unilateral renal vein and inferior vena cava thrombosis, presented with gross hematuria and thrombocytopenia in a neonate. The neonate was a macrosomic male, born to a mother with hyperglycemia in pregnancy. The family history was negative for coagulopathies. He was born in 40th-week gestation with perinatal asphyxia, Apgar scores were 6/7/7. The baby was large for gestational age; the birth weight was 5270 gr. He was plethoric, had hemoconcentration, polycythemia and had signs early neonatal sepsis (leukocytosis, thrombopenia) with massive hematuria. Fluid management and antibiotic treatment were started. Initial treatment included broad-spectrum antibiotics and anticoagulant therapy, with careful attention to fluid balance and nutrition.

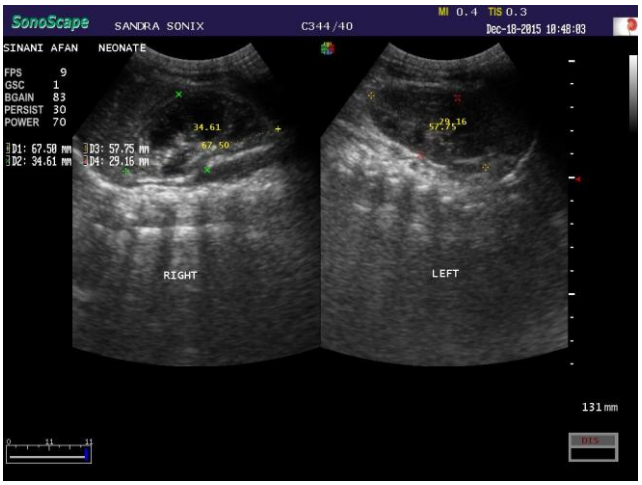

Figure 1: Swollen right kidney and normal sized left kidney in the initial phase of renal vein thrombosis

Diagnosis of renal vein thrombosis was suspected by renal ultrasound and confirmed by magnetic urography. Abdominal ultrasound showed enlarged right kidney having corticomedullary differentiation diminished (right kidney 70 x 48 mm, left kidney 58 x 30 mm), normal values of serum creatinine (49 micromoles/l) and uric acid (179.5 micromole/l). Magnetic urography showed enlarged edematous right kidney with a subcapsular hematoma. Thrombus is seen in the right renal vein and vena cava inferior (25 mm) distally from the renal vein. Prothrombotic risk factors were evaluated.

Protein C and antithrombin III activity were normal for the age. Thrombophilic genes were tested, and heterozygosity of *FBG 455 G/A, MTHFR C677T* and *MTRR A66G* polymorphisms was found. Mutations causing arterial and thrombotic anomalies were tested. Heterozygous mutation *C677T* was found in the methylene tetra hydro folate reductase gene (*MTHFR*), a heterozygous mutation in the *LTA* and *455 G>A* in the B-fibrinogen gene were found.

The child is being managed conservatively. Anticoagulant therapy continued with subcutaneous low molecular mass heparin (LMWH) twice a day. Laboratory tests of kidney function showed a transient rise in serum creatinine and urea, proteinuria, and hematuria. Inflammatory parameters gradually normalised and the subsequent blood culture was negative.

Figure 2: Longitudinal ultrasound scan shows a swollen right kidney with a suprarenal anechoic cyst-like lesion and hyperechoic intramedullary streaks in the initial phase of renal vein thrombosis

Afterwards, the boy has been attending the outpatient clinic. Anticoagulant therapy continued with subcutaneous low molecular mass heparin (LMWH) for three months and subsequently discontinued when the coagulation profile was normal. Despite anticoagulant therapy, the right kidney developed areas of scarring and then atrophy. Kidney volumes after 4 months of treatment showed right kidney 34 x 20 mm and left kidney measuring 68 x 35 mm). His follow-up laboratory results showed normal kidney function. At the age of 8 months, DMSA scan showed no renal tissue on the right side, as a consequence of renal atrophy.

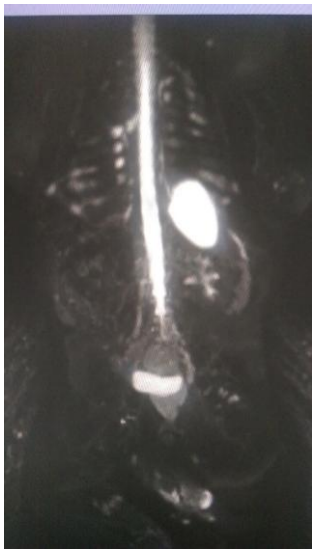

Figure 3: Magnetic resonance- magnetic urography in the early phase of renal and inferior vena cava thrombosis

Discussion

We present a rare case of non-catheter induced inferior cava and renal vein thrombosis in a neonate. Factors predisposing this neonate to RVT include maternal diabetes, early onset infection and complicated labour. The neonate was with no family history of thrombotic disorders or fetal losses, although the child was found to have prothrombotic risk factors.

Figure 4: Small right kidney and compensatory hypertrophied left kidney, five months after birth

Neonates are prone to thrombosis in comparison to other age groups. This is because they have decreased levels of anticoagulants and fibrinolytic components. Factors predisposing to neonatal venous thrombosis are genetic and environmental. Maternal factors predisposing vein thrombosis in neonates are maternal hyperglycemia and diabetes, preeclampsia, chorioamnionitis and autoimmune diseases. Early neonatal infection, perinatal asphyxia, hypovolemia and hemoconcentration due to dehydration, polycythemia, hypercoagulability lead to renal vein thrombosis [1]. The most important cause is central arterial and venous catheters, especially umbilical venous catheterisation, reporting 75-95% of thrombotic events were catheter-related [2].

A Dutch study refers to 66% of all thrombi in catheter-induced thrombosis was found in the inferior vena cava, but they didn't find inferior vena cava thrombi in the non-catheter related thrombosis [3]. Studies reporting non-catheter induced inferior vena cava thrombosis are rare and usually report an association with extensive renal vein thrombosis, inferior vena cava anomalies or external pressure. Inherited prothrombotic anomalies have been reported in association with neonatal renal vein thrombosis. Studies report prothrombic anomalies associated with renal vein thrombosis but less often with inferior vena cava thrombosis [4]. Our patient had a rare set of genetic and external factors leading to renal vein thrombosis but also inferior vena cava thrombosis.

Symptoms usually include gross hematuria, thrombopenia, palpable abdominal mass and renal failure. They frequently overlap with the signs and symptoms of neonatal infection and shock. Clinicians need to monitor closely to prevent renal damage. The diagnosis is suspected on renal ultrasonography (showing renal enlargement) but proven with computed tomography or magnetic urography and radioisotope scans [5] [6]. Treatment of neonatal vein thrombosis remains controversial, and the benefits and the possible side effects need to be measured. Surgical intervention is not indicated in the early phases of renal vein thrombosis [7]. Thrombectomy is rarely possible due to the small blood vessel calibre. Treatment modalities include thrombolysis using heparin, urokinase and recombinant plasminogen activator (r-tPA) [8] [9] [10] [11]. Large randomised controlled studies using anticoagulant treatment in newborns are still missing. Low molecular weight heparin (LMWH) is mostly used as an anticoagulant agent in infants due to fewer side effects and the easy way of subcutaneous administration [12] [13] [14].

Although different modalities have been used treating neonatal vein thrombosis, the outcome is often unsatisfactory, and renal damage and renal loss are what remains afterwards [15] [16] [17].

In conclusion, in this work, we present a patient with multiple entities in the aetiology of non-catheter induced renal and vena cava thrombosis in a neonate. Clinicians should suspect renal vein thrombosis in neonates when presented with early postnatal gross hematuria, palpable abdominal mass and thrombopenia. Measures aimed at prevention of end-stage renal disease must be overtaken because of its poor outcome despite the anticoagulant treatment used.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1682-1684.
https://doi.org/10.3889/oamjms.2018.261
eISSN: 1857-9655
Case Report

Unilateral Primary Congenital Lymphedema of the Upper Limb in an 11-Month-Old Infant: A Clinical and Pharmacological Perspective

Girish Gulab Meshram[1*], Neeraj Kaur[2], Kanwaljeet Singh Hura[3]

[1]Department of Pharmacology, Postgraduate Institute of Medical Education and Research and Dr Ram Manohar Lohia Hospital, New Delhi, India; [2]Department of Radiology, The University of Texas Health Science Centre, San Antonio, Texas, USA; [3]Department of Paediatrics, Richmond University Medical Centre, Staten Island, New York, USA

Citation: Meshram GG, Kaur N, Hura KS. Unilateral Primary Congenital Lymphedema of the Upper Limb in an 11-Month-Old Infant: A Clinical and Pharmacological Perspective. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1682-1684. https://doi.org/10.3889/oamjms.2018.261

Keywords: Congenital lymphedema; Decongestive therapy; Pharmacotherapy

***Correspondence:** Dr. Girish Gulab Meshram M.D., Department of Pharmacology, Postgraduate Institute of Medical Education and Research and Dr. Ram Manohar Lohia Hospital, New Delhi 110001, India. Email: drgirish23@yahoo.co.in

Received: 06-Mar-2018; **Revised:** 21-Aug-2018; **Accepted:** 30-Aug-2018; **Online first:** 18-Sep-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Lymphedema is the accumulation of a protein-rich fluid in the interstitial space due to reduced lymph transport capacity. Congenital primary lymphedema affecting only one of the upper limbs is a rarity.

CASE REPORT: We present a case of an 11-month-old infant presenting with swelling of the right upper limb, which had gradually progressed over the past five months. Lymphoscintigraphy was suggestive of lymphatic blockade in the right upper limb. All other investigations were within normal limits. A diagnosis of primary congenital lymphedema affecting the right upper limb was made. The patient was managed conservatively with complex decongestive therapy and was requested regular follow-up. The lymphedema did not increase within four months of follow-up.

CONCLUSIONS: Complex decongestive therapy is the cornerstone of the management of primary congenital lymphedema. New investigational therapies such as leukotriene B4 antagonists hold a promise for patients with lymphedema.

Introduction

Lymphedema is a chronic localised swelling caused due to excessive accumulation of lymphatic fluid in the interstitium and resulting from defective lymphatic drainage [1]. Lymphedema can be primary or secondary. Primary lymphedema results from developmental abnormalities leading to pathological lymphatics. Secondary lymphedema is an acquired dysfunction of normally developed lymphatics [1] [2]. Congenital subtype of primary lymphedema is defined as swelling that has an onset at birth up to two years [3]. Primary congenital lymphedema affects the lower limbs in the majority of the cases, and unilateral involvement of one of the upper limbs is a rarity [4]. We present a case of an 11-month-old infant with primary congenital lymphedema affecting the right upper limb in isolation.

Case report

An 11-month-old infant presented to the department of paediatrics with a swelling of the right upper limb. The swelling was first noticed by the parents at around seven months after birth and had been gradually growing since then (Figures 1 and 2).

No history of fever, trauma, pain, surgery, rash, drug intake or weight loss was present. The parents of the patient did not have a consanguineous marriage. The family history of the presence of similar swellings in other close family members was absent. The height and weight of the infant were appropriate for his age. The general examination did not reveal any abnormality. Local examination of the right upper limb showed nonpitting, nontender oedema without any signs of inflammation. Radial, ulnar, and brachial pulses were palpable. No abnormal growth was found in the axillae or breasts. Generalized lymphadenopathy was absent. Examination of the left upper limb, both lower limbs, and genitalia did not reveal any abnormality. Routine blood and urine tests were within normal limits. Liver, kidney, and thyroid function tests were within normal ranges.

Figure 1: An 11-month-old infant with unilateral lymphedema of the right upper limb

Peripheral blood smear and antigenic testing for microfilariae were negative. Duplex ultrasound of the right upper limb, chest X-ray, HRCT of the chest did not reveal any abnormalities. Lymphoscintigraphy was suggestive of lymphatic blockade in the right upper limb. Based on the clinical features, and investigations a diagnosis of primary congenital lymphedema affecting the right upper limb was made.

The patient was treated conservatively and was advised regular follow-up. Conservative management included elevation of the affected limb, compression bandages, and skin care. The lymphedema did not show any progression in the next four months of follow-up.

Figure 2: Unilateral swelling of the right upper limb

Discussion

Primary lymphedema has an estimated prevalence of 1.15 per 100000 [5]. It is categorised according to the age of onset as congenital (before two years), praecox (before 35 years), and tarda (after 35 years) [1] [2]. Isolated involvement of one of the upper limbs is one of the rarest forms of congenital lymphedema as 92 % of cases of congenital lymphedema involve the lower extremities [4] [6].

Around 20 genes have been identified to be associated with primary lymphedema. Some of these genes include FLT4, VEGFR3, FOXC2, CCBE1, GCJ2, SOX18, GATA, and PNPN14 [7]. Genetic mutations explain only around one-third of the cases of primary lymphedema, mostly of inherited forms. These genes encode/regulate proteins of the vascular endothelial growth factor receptor-3, and other tyrosine kinase receptors and exert their effects via the RAS/MAPK and the PI3/AKT pathways [7] [8]. Genetic analyses for truncating the affected gene were not carried out in our patient due to his financial constraints. Some diseases such as filariasis, soft-tissue tumours, Kippel-Trenaunay syndrome, and chronic venous insufficiency mimic isolated primary lymphedema of the upper limb. In each of these conditions, the swelling progresses gradually and painlessly over a period of weeks or months [9]. Each of these conditions were systematically ruled out in our patient through the clinical history, examination, and appropriate investigations.

Management of primary lymphedema is done via decongestive lymphatic therapy, which includes an intensive and maintenance phase. Both these phases include the use of compression bandages, therapeutic exercises, skin care, manual lymphatic drainage, and patient education [10]. Our patient did not show any

increase in lymphedema after undergoing a month of intensive and three months of maintenance decongestive therapy. However, the parents of the child were advised monthly follow-ups and look out for an increase in the size of the swelling, signs of inflammation, hyperkeratotic skin lesions, and verrucous growths.

Currently, there is no effective pharmacotherapy for primary lymphedema. Short-term administration of diuretics is found to be effective in some patients in the initial stages of lymphedema. Long-term administration of benzopyrones increases hydrolysis and absorption of tissue proteins at the site of lymphedema. Intra-arterial autologous lymphocyte injection is suggested to enhances the proteolysis of extracellular proteins and reduce lymphedema in some reports. Zinc supplementation and low-fat diet were found to effective in reducing lymphedema in a few reports. Use of propranolol has been suggested to improve some symptoms of lymphedema in a report. Surgical interventions such as debulking procedures, resection approach, buried-dermal flaps, microsurgical techniques, and liposuction are rarely indicated due to their low success rates. Surgical therapy is usually tried only when conservative management has failed [1] [11].

Preclinical evidence suggests that exogenous injection of human recombinant vascular endothelial growth factor C restores lymphatic flow. Also, adipose-derived stem cells have shown potential in lymphedema therapy [12]. Drugs targeting the PI3/AKT/mTOR signalling pathway such as mechanistic target of rapamycin inhibitors are also being evaluated for reducing lymphedema in some somatic overgrowth disorders [2]. Leukotriene B4 affects lymphangiogenesis in animal models and is elevated in patients with lymphedema. Hence, drugs antagonising the effect of leukotriene B4 such as ketoprofen, and bestatin are presently being evaluated in Phase-2 of clinical trials [13].

In conclusion, primary congenital lymphedema affecting only one of the upper limbs is an extremely rare disorder. Conservative management via decongestive therapy prevented further progression of the lymphedema in our patient. Investigational therapeutic agents such as leukotriene B4 antagonists provide an exciting prospect for patients with lymphedema.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1685-1687.
https://doi.org/10.3889/oamjms.2018.379
eISSN: 1857-9655
Case Report

Acupuncture Treatment for Fertility

Jihe Zhu[1], Blagica Arsovska[1,2], Kristina Kozovska[1*]

[1]Faculty of Medical Sciences, University Goce Delchev, Shtip, Republic of Macedonia; [2]Institute of Biology, Faculty of Natural Sciences and Mathematics, Skopje, Republic of Macedonia

Citation: Zhu J, Arsovska B, Kozovska K. Acupuncture Treatment for Fertility. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1685-1687. https://doi.org/10.3889/oamjms.2018.379

Keywords: Fertility; Couple; Treatment; Acupuncture; Traditional Chinese Medicine

***Correspondence:** Kristina Kozovska. Faculty of Medical Sciences, University Goce Delchev, Shtip, Republic of Macedonia. E-mail: tongdatang-tcm@hotmail.com

Received: 16-Jul-2018; **Revised:** 07-Sep-2018; **Accepted:** 09-Sep-2018; **Online first:** 19-Sep-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Infertility is considered an inability to conceive in a sexually active couple who are trying to get pregnant for one year. About 8-12% of the couples in the world are facing problems with infertility, and in this modern times the per cent is getting bigger, and 20% of the cases are due to both male and female factor. By the theory of Traditional Chinese Medicine (TCM) infertility occurs due to disrupted vital balance and blockages in the Qi energy and Blood circulation flow.

CASE REPORT: We present a case of a young couple, 28 years old man and woman who were unable to conceive after trying for a year. Both were treated with acupuncture treatment in a clinic for TCM and acupuncture in Skopje, Macedonia. Treatments were with duration of 35-40 minutes on both sides of the body. Treatments were done indoors, at room temperature, once a week in three months. The man has done 4 treatments to improve the sperm quality. The woman had ovarian cysts which were gone after 7 treatments and succeeded to get pregnant. Acupoints that were used in the treatment in both patients are: HN1 (SiShenCong), LI4 (HeGu), ST36 (ZuSanLi), GB34 (YangLingQuan), SP9 (YinLingQuan), SP6 (SanYinJiao), LR3 (TaiChong), RN13 (ZhongWan), RN7 (QiHai), RN4 (GuanYuan), RN2 (QuGu), ST29 (GuiLai), ST25 (TianShu), GB20 (FengChi), DU14 (DaZhui), BL18 (GanShu), BL19 (DanShu), BL20 (PiShu), BL21 (WeiShu), BL25 DaChangShu) and BL32 (CiLiao).

CONCLUSION: Acupuncture as a treatment for infertility shows great results both in man and women. Acupuncture can be considered as successful treatment in restoring fertility in the patients, by improving the sperm quality and ovaries function and balancing the endocrine system and hormones.

Introduction

Infertility is considered as the inability to conceive in a sexually active couple who are trying to get pregnant for one year. 8-12% of the couples in the world are facing problems with infertility, and in these modern times, the per cent is getting bigger. 20% of the cases are due to both male, and female factor [1] [2] Problems that are possible to arise in men are abnormal sperm, low sperm motility or low sperm count. Causes can be genetic factors, long-term use of steroids, age, obesity, stress, and inflammation of the testicles, cystic fibrosis, diabetes and other diseases. In women problems that can arise are a polycystic ovarian syndrome, hyperprolactinemia, thyroid problems, hormonal imbalance, poor egg quality, cancer, AIDS, etc. Risk factors and causes are age, obesity, eating disorders, smoking, alcohol, stress, infections and other [1].

By the theory of Traditional Chinese Medicine (TCM) infertility occurs due to disrupted vital balance and blockages in the Qi energy and Blood circulation flow. When the free flow of the energy is blocked, it can cause deficiency, stagnancy or heat syndrome [3].

Deficiency syndrome is blocking and disrupting the sexual and reproductive function both in men and women.

The stagnancy syndrome disables the free flow of the energy and Blood and restricts it from circulation to the tissues in the reproductive organs.

The heat syndrome is connected to inflammation processes which have an impact on semen quality and gynaecological infections.

All three syndromes can be treated with Traditional Chinese Medicine, concretely with acupuncture and herbs [4] [5]. When it is known which syndrome to treat, and the problem is solved, the body returns to health and conception may happen naturally. TCM sees every patient as unique, and the treatment is made by their current condition and constitution. With such a long history for more than 5000 years, TCM is highly effective with no side effects. Acupuncture as part of the TCM as a treatment for infertility is used for a very long time. With the insertion of tiny sterile needles into acupoints located at specific points on the body the way the body functions can be regulated and the problem that affects fertility can be addressed. Acupuncture treatment can balance the hormones, strengthen the immune system, regulate the energy and Blood flow, remove the stasis and phlegm and stimulate the nervous system. With a combination of acupuncture and herbs, it is possible to improve the ovarian and follicular function and increase the blood flow to the endometrium [3] [6] [7].

Material and Methods

In this research is presented a case of a young couple, 28 years old man and woman who were unable to conceive after trying for a year. The male patient is with normal findings, normal blood pressure, blood sugar and appetite. Before starting the treatments, the patient was taking E vitamin and Tribestan tablets for improving fertility and to stimulate testosterone excretion. The acupuncture treatment was made to improve the sperm quality. The female patient is diagnosed with endometriosis on both ovaries. On the scans done before the treatment are visible 'chocolate' cysts on both ovaries. Since the patient was diagnosed, she was taking hormonal therapy for one year and one month before starting the acupuncture treatment the hormonal therapy was stopped. The menstrual cycle is regular but very painful. The patient was also struggling with hormonal acne on the face. Both of the patients were treated with acupuncture treatment in a clinic for TCM and acupuncture in Skopje, Macedonia by a doctor specialist in acupuncture. Treatments were with duration of 35-40 minutes on both sides of the body. Treatments were done indoors, at room temperature, once a week in three months. In the treatment were used fine sterile disposable needles 0.25 x 25mm manufactured by Wuijuiang City Medical & Health Material Co., LTD. Points that were used in the treatment in both patients are: HN1 (SiShenCong), LI4 (HeGu), ST36 (ZuSanLi), GB34 (YangLingQuan), SP9 (YinLingQuan), SP6 (SanYinJiao), LR3 (TaiChong), RN13 (ZhongWan), RN7 (QiHai), RN4 (GuanYuan), RN2 (QuGu), ST29 (GuiLai), ST25 (TianShu), GB20 (FengChi), DU14 (DaZhui), BL18 (GanShu), BL19 (DanShu), BL20 (PiShu), BL21 (WeiShu), BL25 DaChangShu) and BL32 (CiLiao).

Results

When treating infertility both male and female factor is very important. Therefore both patients have done the treatment at the same time. On both patients were used the same points. The male patient has done 4 treatments to improve the sperm quality. Before the treatment, the patient has done one sperm test. Results from the test show a small amount of progressively moving sperm cells (5%) and a limited amount of immobile sperm cells (50%). The results from the sperm test done before the treatment are shown in Table 1.

Table 1: Results from the sperm test analysis

Test	Results	Normal Range
Ejaculate	49.50 x 10^6/ml	>39 x 10^6/ml
Processed after	40 min	30-120 min
Viscosity	Normal	
Volume	3.3 ml	2-5 ml
pH	7.5	7.2-7.8
No agglutination		
Total number of sperm cells in 1 ml.	15.00 x 10^6/1 ml	>15.0 x 10^6/1 ml
% of progressively moving sperm cells	5%	>40%
% of non-progressively moving sperm cells	45%	10%
% of immobile sperm cells	50%	<50%
Normal liquefaction		

The female patient had ovarian endometriotic cysts or 'chocolate' cysts on both ovaries. After 7 treatments the cysts were gone, and the patient got pregnant spontaneously successfully. Figure 1, is shown ultrasound image done before the treatment showing the cysts on both ovaries.

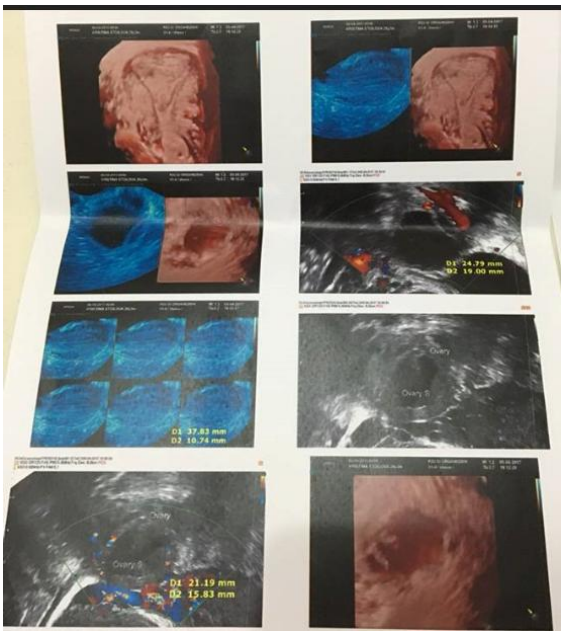

Figure 1: Ultrasound image report before the treatment

Figure 2 is shown ultrasound image done after the treatment showing the cysts are gone from both ovaries.

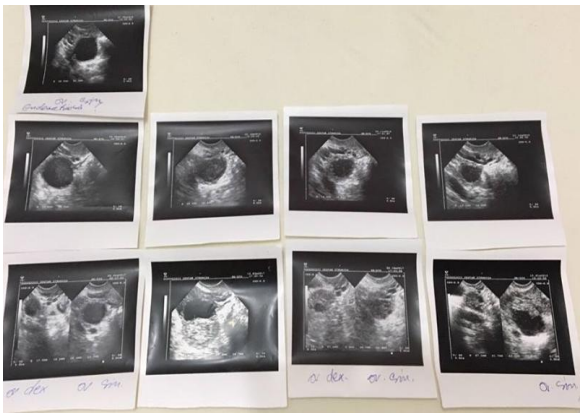

Figure 2: Ultrasound image report after the treatment

Table 2 are shown the results from the blood test analysis done after the treatment. Results are showing normal hormone levels of Prolactin, Estradiol, TSH, LH, FSH and Testosterone, low tumour markers levels (CEA, OMMA, GIMA) and increased HCG hormone level indicating that the patient is pregnant in a second/third gestational week.

Table 2: Blood test results

Test	Results	Normal Range
HCG	452 mIU/ml	16-156 mIU/ml (1-2 gest. week) 101-4870 mIU/ml (2-3 gest. week) 1110-31500 mIU/ml (3-4 gest. week) 2560-82300 mIU/ml (4-5 gest. week) 23100-151000 mIU/ml (5-6 gest. week) 27300-233000 mIU/ml (6-7 gest. week)
CEA	< 0.20 ng/mL	0.80-2.50 ng/mL
OMMA	10.1 U/mL	1.00-21.0 U/mL
GIMMA	< 2.50 U/mL	2.50-37.0 U/mL
FSH	4.54 mIU/mL	1.20-9.0 mIU/mL
LH	3.16 mIU/mL	0.38-16.9 mIU/mL
Estradiol	44.6 pg/mL	26.0-161 pg/mL
Prolactin	8.06 ng/mL	3.20-24.0 ng/mL
TSH	1.39 uIU/mL	0.400-4.0 uIU/mL
Testosterone	1.69 nmol/L	< 2.0 nmol/L

Discussion

The points used in the treatment were chosen with the aim to help the patients to improve the energy flow to the reproductive organs, warm the Blood for better circulation and remove the blockages that cause stagnation in the flow. Ovarian cysts can occur because of stagnation in the Liver Qi, caused by excessive dampness. Excessive dampness is caused by blood stasis and fluids accumulated in the abdomen which slowly becomes phlegm. Accumulation of phlegm and fluids in the lower body is a problem of the Kidney Yang, i.e. a failure of the Kidney to send the water upward. When the water stays in the lower body, then it transforms into phlegm. Therefore the Kidney fails to support the Liver. The main function of the Liver and Kidney meridians is to remove the toxins from the body. If this function is weakened, then the immune system can be disturbed, and abnormal immune reactions may be triggered by the growth of abnormal tissue in the female reproductive organs. In TCM, if there is no good and free blood circulation in the body, blood stagnation can occur and lead to disease [3] [5] [6] [8] [9].

When the cysts are formed because of accumulated water and fluids, the three principal methods of treatments are: (1) yo warm the Kidney with the aim to prevent further accumulation; (2) resolve the phlegm masses, and (3) restore normal blood circulation to the affected area [5].

In TCM, infertility in male patients is also a problem of Liver Qi stagnation, Kidney deficiency and accumulation of dampness and heat in the lower abdomen. Therefore the treatment protocol is the same in both patients [10] [11]. The treatment in both patients aims to remove the liver toxins, dissolve blood stagnations in the liver, nourish the Kidney Qi, invigorate the Blood, lower stress and stimulate the whole body to function better [3] [11].

In conclusion, acupuncture as a treatment for infertility shows great results both in man and women. Acupuncture can be considered as successful treatment in restoring fertility in the patients, by improving the sperm quality and ovaries function and balancing the endocrine system and hormones.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1688-1693.
https://doi.org/10.3889/oamjms.2018.354
eISSN: 1857-9655
Case Report

A Series of Patients with Kaposi Sarcoma (Mediterranean/Classical Type): Case Presentations and Short Update on Pathogenesis and Treatment

Ivanka Temelkova[1], Michael Tronnier[2], Ivan Terziev[3], Uwe Wollina[4], Ilia Lozev[5], Mohamad Goldust[6], Georgi Tchernev[1,7*]

[1]Medical Institute of Ministry of Interior (MVR), Department of Dermatology, Venereology and Dermatologic Surgery, General Skobelev Nr 79, Sofia, Bulgaria; [2]Helios Klinikum GMBH-Dermatology, Venereology and Allergology Senator-Braun-Allee 33 Hildesheim, Hildesheim, Germany; [3]Universitetska Mnogoprofilno Bolnitsa za Aktivno Lechenie "Tsaritsa Yoanna"-Common and Clinical Pathology, Sofia, Bulgaria; [4]Städtisches Klinikum Dresden-Department of Dermatology and Allergology, Friedrichstrasse 41, Dresden, Germany; [5]Medical Institute of the Ministry of Interior-Surgery, Sofia, Bulgaria; [6]Department of Dermatology, Mazandaran University of Medical Sciences, Sari, Iran; [7]Onkoderma-Policlinic for Dermatology and Dermatologic Surgery, General Skobelev 26, Sofia, Bulgaria

Citation: Temelkova I, Tronnier M, Terziev I, Wollina U, Lozev I, Goldust M, Tchernev G. A Series of Patients with Kaposi Sarcoma (Mediterranean/Classical Type): Case Presentations and Short Update on Pathogenesis and Treatment. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1688-1693. https://doi.org/10.3889/oamjms.2018.354

Keywords: Kaposi; Pseudo-Kaposi; Stewart Treves syndrome; Radiation therapy; Treatment choice; Morbus Mali

***Correspondence:** Georgi Tchernev. Medical Institute of Ministry of Interior (MVR), Department of Dermatology, Venereology and Dermatologic Surgery, General Skobelev Nr 79, Sofia, Bulgaria; Onkoderma-Policlinic for Dermatology and Dermatologic Surgery, General Skobelev 26, Sofia, Bulgaria. E-mail: georgi_tchernev@yahoo.de

Received: 26-Aug-2018; **Revised:** 30-Aug-2018; **Accepted:** 01-Sep-2018; **Online first:** 20-Sep-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Kaposi's sarcoma was first described in 1872 by Moritz Kaposi. To date, it is considered a malignant disease is originating from the endothelial cells of the lymphatic vessels believed to be infected with HHV-8. The current classification defines four major epidemiological forms of Kaposi's sarcoma: classical, endemic, AIDS-associated, and iatrogenic.

CASE REPORT: A 90-year-old male is presented with multiple plaques- and tumour-shaped brown-violet formations located on an erythematous-livid base in the area of both feet and both shanks. Two samples were taken from the lesions on the skin of the shanks, with histopathological examination and the subsequent immunohistochemistry showing Kaposi's sarcoma.

CONCLUSIONS: Kaposi sarcoma is a disease that causes difficulties both in diagnostic and therapeutic respect. The only sure way to determine the correct diagnosis is immunohistochemical staining with the anti-HHV8 antibody. Despite the wide range of systematic and local treatment options, there is still no unified algorithm and a unified strategy for the treatment of Kaposi's sarcoma.

Introduction

Kaposi's sarcoma is a tumour originating from endothelial cells where there is a suspected infection with human herpesvirus-8 (HHV-8) [1]. It is the most common malignancy among the AIDS patients [2]. The clinical picture and the standard histology are not always sufficient for Kaposi's sarcoma to be as precise as possible distinguished from some other diseases [3]. In these cases, the conduct of an immunohistochemical study to determine the correct diagnosis is of paramount importance [4].

Five major subtypes of Kaposi's sarcoma can be differentiated: (1) classical type of predominantly older Caucasian males; (2) endemic Kaposi's sarcoma of the Sub-Sahara region, which is not HIV-associated; (3) transplantation- and immunosuppression-associated type; (4) AIDS-related type; (5) classical type in HIV-positive patients.

There is still no established golden standard in the treatment of Kaposi's sarcoma, but there are some therapeutic options that show complete or partial remissions [5].

Case Report 1

A 90-year-old man is presented at the Department of Dermatology and Dermatologic Surgery at the Medical Institute of Ministry of Interior, (MVR-Sofia), Bulgaria, who suffers from chronic venous insufficiency and benign prostatic hyperplasia. The patient was hospitalised due to the presence of multiple plaque-shaped to tumour-shaped formations in the area of the left leg. The lesions date back to approx. 2 years, due to which lesions the patient had two previous hospitalisations in other health institutions and conducted antibiotic and corticosteroid therapy without success. During the dermatological examination, in the area of both feet and the two shanks, the presence of brown-violet tumor-shaped formations was found with a diameter of 0.5 to 2.0 cm, located on an erythematous-livid base (Figure 1a, 1b and 1c).

Figure 1: a), b) and c) - Kaposi sarcoma lesions on the left and right lower extremities-presence of nodules, blisters and hyperpigmentation

The left-sided pathological changes were clinically more pronounced (Figure 1a). The available clinical picture was suspected for Kaposi's sarcoma. Samples were taken of the tumour-shaped formations, on the skin of both shanks for histological verification of the diagnosis determined.

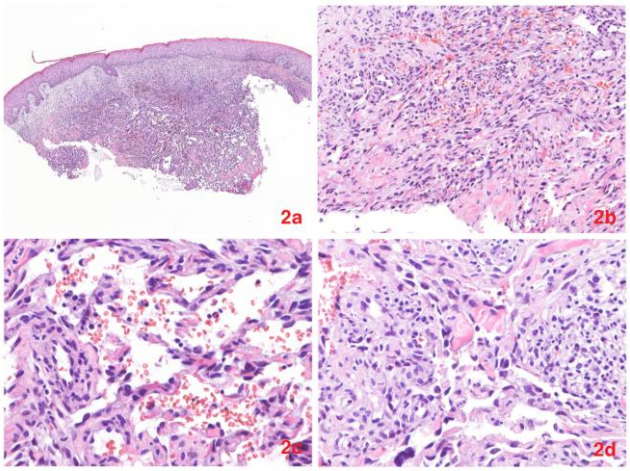

Figure 2: Kaposi sarcoma. Histopathology (H&E); a) Under a slight acanthotic epidermis vascular tumour with partially high cellularity (x40); b) Atypical spindle cells with small slit-like vascular spaces. Preexisting vessels are in part surrounding by spindle cells, this way appearing freely floating, so-called promontory sign (x100); c) Vascular lining by atypical, in part hyperchromatic spindle cells. Extravasated red blood cells (x400); d) In part additional plasma cells in the infiltrate (x400)

The histopathological examination and subsequent immunohistochemistry showed definite evidence of Kaposi's sarcoma (Figures 2a, 2b, 2c, 2d, 3a, 3b, 4a, 4b and 4c).

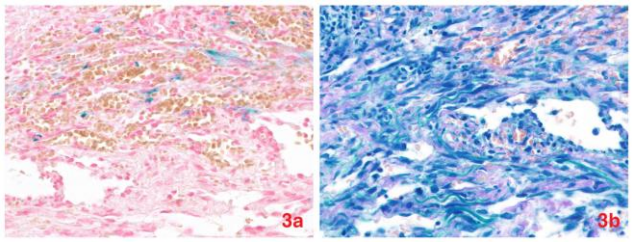

Figure 3: Kaposi sarcoma histopathology - a) among tumour cells hemosiderin deposits; b) many extravasated red blood cells

The ultrasound examination performed on the lower extremities revealed the presence of enlarged and pathologically changed lymph nodes in the left femoral triangle.

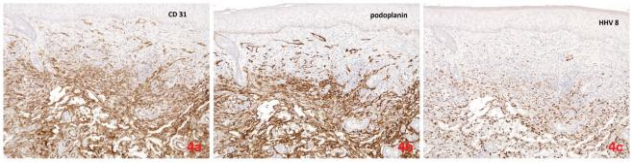

Figure 4: Kaposi sarcoma-immunohistochemistry - a), b) and c) Tumor cells are labelled with antibodies against CD31, podoplanin, and HHV8

Systemic therapy with Ceftriaxone was performed 2 g x 1/day IV for 7 days, and the local therapy included daily iodasept ointment dressings for 7 days. Nadroparin calcium was prophylactically applied 0.4 x 1/day SC for 7 days. As a result of the conducted treatment, lymph node subsiding was observed, and the patient was redirected to perform radiation therapy at a specialised oncology centre.

Case Report 2

A 76-year-old male patient presented with several nodules on his left forefoot and lower leg which developed during the last 6 months. He reported burning and pain sensations on the ulcerated lesions located on the 2nd and 3rd left toe. His medical history was remarkable for prostate cancer, nephrectomy because of a cirrhotic kidney and a first-grade atrioventricular block. On examination, we observed multiple livid or soft brownish nodules on the left lower leg and forefoot with ulcerations on the toes. Enlarged lymph nodes were palpable in both groins.

A skin biopsy was taken for histopathology. Within the dermis, bizarre formed; partly ectatic blood vessels with prominent endothelial and positive promontories sign, extravasation of red blood cells

and siderophages were noted. The vascular parts within papillary bodies showed a spindle cell type. Endothelial were positive for CD31, podoplanin, and HHV-8 and partially for CMYK by immunohistochemistry. The mitotic rate was about 10 to 20% with Ki67-staining. Diagnosis: Classical KS is shifting from patch to plaque stage.Laboratory findings: Lymphopenia of 12% (normal range: 25-45%), erythrocytes 4.29 (4.6-6.2Tpt/l), Hb 7.9 (8.6-12.1mmol/l), ß-2-microglobulin 2.6 (0.8-2.2mg/l), CD3+/CD4+-T-helper cells 79.8 (35-66%), CD3+/CD8+-T-suppressor cells 11.1 (17-46%), ratio helper/ suppressor cells 7.19 (1.0-2.3), HIV test negative. Imaging: Computerized tomography demonstrated a pulmonary nodule of 0.5cm in diameter dorsobasilar on his right side. Several Hilary and pulmonary lymph nodes with a diameter < 1 cm. Ultrasound abdomen/ groins: Tumor-like growth in the left groin and an atypical lymph node (19 mm). MRI of the head excluded any tumour spread. The patient was referred to the Department of Radiology for radiotherapy.

Figure 5: Case #2: a) Nodules on the lower left leg and foot. Left Lower leg with patches and plaques of classical KS; b) Immunostaining for CD31; c) Immunostaining for CD34; d) Immunostaining for HHV-8

Discussion

Kaposi's sarcoma (KS) was first described in 1872 in an article titled "Idiopathic multiple pigmented sarcoma of the skin" by Moritz Kaposi and to this day is named after him [6]. Tommaso de Amicis-an Italian dermatologist, confirmed his findings, albeit ten years later [7]. In 1981, Alvin Friedman-Kein changed the perceptions that KS is a rare disease affecting predominantly senior men and gives a whole new perspective by concluding that Kaposi's sarcoma can be HIV-associated [8]. The subsequent epidemiological studies in people with AIDS revealed two important features of this disease, namely that it is possible to be sexually transmitted as is HIV, on the one hand, and on the other, immunosuppression promotes the development of Kaposi's sarcoma [2].

Still, the genesis of this type of sarcoma is not fully elucidated [9]. Currently, the leading theory is that Kaposi's sarcoma is a tumour originating from the endothelium of the blood vessels that are most commonly associated with Kaposi's sarcoma herpesvirus/human herpesvirus 8 (KSHV/HHV8) infection [1] [10] [11]. It is believed that it is this virus that is the cause of a change in the differentiation and the function of the endothelial cells, resulting in the appearance of altered vascular structures to a lymphatic phenotype and determining the angioproliferative character of KS [1] [12]. A possible explanation for this mechanism is that this occurs with the involvement of VEGFR3-lymphatic endothelial-cell-specific receptor important for lymphangiogenesis [12]. It is considered that the transcription factor Its-1 activates the promoter of VEGFR3 and thus he plays a role in KSHV activation of endothelial cells during latent KSHV infection [12]. It is Its-1 that is considered to be the regulator involved in the induction of angiogenic phenotypes by KSHV [12]. As a rule, this type of sarcoma includes four types of forms: classical, African (endemic), AIDS-associated (epidemic) and iatrogenic (organ transplant-related) form [10] (Table 1). There is also another form of Kaposi-the so-called lymphadenopathic Kaposi's sarcoma, which can occur in people with AIDS but also in immunocompetent children or adults [13] [14] [15]. It affects the lymph nodes, the internal organs, the gastrointestinal tract and it can pass into a disseminated form with an aggressive course of progression [15].

Table 1: Variants of Kaposi's sarcoma

Variant	Risk Group	Median survival
Classic	Senior men of Eastern European or Mediterranean origin	Years or decades
Endemic	African children and adults	Months or years
Immunosuppression-associated, or transplantation-associated	Organ transplant recipients	Months or years
AIDS-associated	Persons infected with human immunodeficiency virus, especially homosexual or bisexual men	Weeks or months
Classical KS in HIV-positive patients	HIV-positive younger patients	Months or years

Usually, the lesions of Kaposi's sarcoma go through three stages: 1) early with the appearance of macules (patch stage), 2) followed by the appearance of plaques (plaque stage), and finally, 3) nodules (tumour stage) [10] [11] [16]. At the same time, there is also data in the literature for more specific histological variants that include anaplastic, hyperkeratotic, lymphangioma-like, bullous, telangiectatic, ecchymotic, keloidal, pyogenic granuloma-like, micronodular, intravascular, glomeruloid and pigmented KS, KS with sarcoid-like granulomas and KS with myoid nodules [10] [11].

In a historical aspect, Kaposi's sarcoma is described as a disease primarily affecting men of Mediterranean origin, as our patient, with a pre-target location, the lower limbs and slow progression [17].

Risk factors associated with KS include male gender, HLA-DR5 genetic marker, homosexuality, immunosuppression and viral agents such as Cytomegalovirus [17]. It is believed that in patients with HIV infection, KS may occur at any time, most commonly when CD4 count < 200 cells/mm^3 [14]. For this reason, their number is used as a prognostic factor for the evolution of Kaposi's sarcoma, and CD4 count > 200/mm^3 and only cutaneous involvement [18] is considered to be a good prognosis. Thus, the immunosuppression after organ transplantation or in AIDS can form the two groups of patients at highest risk and incidence of Kaposi's sarcoma [2] [19].

Histopathological, the Kaposi's sarcoma is characterised by the presence of hyaline bodies, deposits of hemosiderin, spindle cell and the formation of vascular channels between spindle cells [3] [16]. It is these features that determine the range of diseases that should be considered in a differential diagnosis-granuloma pyogenicum, leiomyoma, leiomyosarcoma or fibrosarcoma [3]. Besides these, a diagnostic error can also occur with dermatofibroma, hemangioma or scar [20]. Difficulties may also result from the localization of KS [21]. Although it is typical that the lymph nodes and the visceral organs are affected, an atypical clinical manifestation is also possible, covering other anatomical areas such as the musculoskeletal system, central and peripheral nervous system, larynx, eye, major salivary glands, endocrine organs, heart, thoracic duct, urinary system, breast, sites of previous iatrogenic trauma (wounds) and blood clots is also presented [21].

The performance of immunohistochemistry and immunohistochemical reactivity for CD31, CD34, D2-40 and FLI1 [4] [22], is of crucial importance for determining the diagnosis. The results of the immunohistochemistry and the sensitivity for these markers were found to be the same in AIDS-related and non-AIDS-related KS, as well as between the nodular-and patch/plaque-stage KS [22]. Furthermore, due to the possibility of a lymphangioma-like Kaposi's sarcoma and the existing risk of an incorrect diagnosis of lymphangioendothelioma, it is important to perform immunohistochemical staining with anti-HHV8 antibody [23]. The same also applies to conditions with chronic lymphoedema when KS can be mistaken with the Stewart-Treves Syndrome (STS) [24]. The difference between them is that KS does not necessarily require the presence of lymphedema for its development and is etiologically associated with a viral infection that is lacking in STS [25].

Difficulties in the clinical diagnosis are often also created in the cases of pseudo-Kaposi's sarcoma-acroangiodermatitis, and again only the HHV-8 study can distinguish it from KS [26].

Cutaneous angiosarcoma (CAS) is another interesting tumour that also has a vascular origin and should be distinguished from Kaposi sarcoma [27]. It is believed that programmed death-1 (PD-1) and programmed death ligand-1 (PD-L1) expression by tumour cells play a key role in the angiosarcoma pathogenesis [27]. However, there are cases in which 1) angiosarcoma can detect PD-L1 negativity in immunohistochemistry, 2) even more interesting- to be affected by PD-1 inhibitor therapy with pembrolizumab despite this negativity and 3) when it comes to lesions of vascular origin a guarantee for confirmation or exclusion of Kaposi's sarcoma diagnosis is only HHV- 8 positivity [23] [27].

Currently, a wide range of options is used for treatment of Kaposi's sarcoma that include chemotherapy, radiotherapy, immunotherapy, cytotoxic agents, liposomal anthracyclines, paclitaxel, retinoic acids, pazopanib as well as some antiangiogenic agents such as AGM 1470 (TNP 470), thalidomide and glutamine disodium (IM 862), that show promising results [5] [28]. Self-administered or in combination, the chemotherapeutic agents provide acceptable results, but recurrences of KS occur frequently, and the progression-free periods are often short [29].

For classical KS, which is radiosensitive, the radiotherapy is of key importance in the therapy of all forms. It has the greatest effect in the early stages of the disease [30] [31]. However, as it reduces the pain, oedema and ensures control of the bleeding, radiotherapy can also be used as a palliative treatment in advanced cases of KS [31] [32]. In this regard, doses of 15.2 Gy for oral lesions and 20 Gy for lesions involving conjunctiva, eyelids, lips, hands, feet, penis, and anal region provide good control of the symptoms [33]. For the other parts of the body, a dose of 30 Gy may be used, with the hypofractioning showing the best outcome regarding recurrence-free survival, the toxicity and the local control [34]. In non-AIDS associated KS (NAKS), radiotherapy provides a very good therapeutic control [34].

For patients with AIDS-related Kaposi's sarcoma, however, the first line of treatment is the high-activity antiretroviral therapy (HAART) [5]. There is evidence that even a self-administered HAART therapy may lead to spontaneous regression of KS in AIDS patients [35]. The spontaneous regression is described in the literature and discontinuation of immunosuppressive treatment in the iatrogenic form of KS after transplantation [36]. More surprising in this respect is the data from a documented partial regression in cases of non-HIV, non-iatrogenic Kaposi sarcoma [37]. The precise mechanisms under which this is done are still within the hypothetical sphere [37].

The WHO has developed international guidelines for HIV-associated KS, but there is still no standardised approach to the treatment of other types of Kaposi's sarcoma [5] [38].

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Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1694-1696.
https://doi.org/10.3889/oamjms.2018.376
eISSN: 1857-9655
Case Report

Complete Rectal Prolapse in Children: Case Report, Review of Literature, and Latest Trends in Management

Girish Gulab Meshram[1*], Neeraj Kaur[2], Kanwaljeet Singh Hura[3]

[1]Department of Pharmacology, Postgraduate Institute of Medical Education and Research and Dr Ram Manohar Lohia Hospital, New Delhi, India; [2]Department of Radiology, University of Texas Health Science Centre, San Antonio, Texas, USA; [3]Department of Paediatrics, Richmond University Medical Centre, Staten Island, New York, USA

Citation: Meshram GG, Kaur N, Hura KS. Complete Rectal Prolapse in Children: Case Report, Review of Literature, and Latest Trends in Management. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1694-1696. https://doi.org/10.3889/oamjms.2018.376

Keywords: Rectal prolapse; Procidentia; Pediatric management

***Correspondence:** Girish Gulab Meshram. Department of Pharmacology, Postgraduate Institute of Medical Education and Research and Dr Ram Manohar Lohia Hospital, New Delhi, India. E-mail: drgirish23@yahoo.co.in

Received: 28-Jun-2018; **Revised:** 22-Aug-2018; **Accepted:** 24-Aug-2018; **Online first:** 21-Sep-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Complete rectal prolapse is the circumferential descent of all the layers of the rectum through the anus. It often leads to bleeding, obstructed defecation, incarceration or fecal incontinence.

CASE REPORT: We present a rare case of a 4-year-old child with complete rectal prolapse of 12 cm in length. The prolapsed rectum was manually repositioned after reducing the oedema. The precipitating factor was identified as excessive straining while passing stools. A change in position while passing stools was advised along with a high fibre diet and a stool softener. Recurrence was not observed in the 3 month of follow-up.

CONCLUSION: Most cases of pediatric rectal prolapse are managed conservatively by addressing the associated and precipitating etiological factors. Surgical intervention may be required for recurrent or persistent cases.

Introduction

Complete rectal prolapse is a condition in which there is an extrusion of all the layers of the rectum through the anal sphincter [1]. The incidence of complete rectal prolapse is unknown in children, and there is considerable debate regarding its etiopathogenesis. Pediatric rectal prolapse occurring in the age group of 1-4 years usually resolves spontaneously or within a year of conservative management. Children not responding to conservative management may require surgical intervention [2]. We present a case of complete rectal prolapse in a 4-year-old child due to its large size and rarity. We have also reviewed the literature to discuss its up-to-date medical and surgical management.

Case report

A 4-year-old child presented to the department of pediatrics with a complaint of a red-coloured mass protruding from his anus. The parents of the child had first observed this mass while the child was passing stools, two hours earlier. No history of chronic constipation, chronic cough, weight loss, or presence of parasites in stools was present. The vitals of the child were normal. The weight of the patient was on the 42nd percentile (16 kg), and the height was on the 65th percentile (105 cm), using WHO data. Abdominal palpation showed no tenderness. On local examination, the length of the protruding rectal wall was around 12 cm with circumferential folds and slight oedema. No ulcerations were present (Figure 1). A diagnosis of complete rectal prolapse was reached based on the clinical features and examination.

Powdered sugar was sprinkled on the extruded rectal wall to reduce the oedema, and the prolapsed rectum was repositioned manually. The HIV test, sweat test, and stool culture test of the patient were negative. The blood counts and renal function tests were within the normal range. The patient was discharged the next day with anticipatory guidance to the parents. A high fibre diet and a stool softener were also prescribed. The follow-up visits of the patient in the subsequent 3 months did not show recurrence of the rectal prolapse.

Figure 1: A four-year-old child with complete rectal prolapse

Discussion

Rectal prolapse could be partial or complete. In partial rectal prolapse, a part of the rectal wall or the anal mucosa protrudes out of the anal verge, whereas incomplete rectal prolapse all the layers of rectal wall protrude out [1]. Depending on the length of the protruding rectum from the anal verge, complete rectal prolapse is classified as 1st, 2nd, and 3rd degree [3]. In our case, the complete rectal prolapse was of the 1st degree as the length of the protruding rectum was more than 5 cm and involved the mucocutaneous junction.

The peak age at which rectal prolapse is observed in children is 1-3 years without any sex predilection [4]. Although the pathogenesis of complete rectal prolapse not completely understood, the currently accepted hypothesis states that complete rectal prolapse starts as a circumferential intussusception of the rectum, which gradually progresses to complete rectal prolapse [5].

The anatomical factors which have been found to play a role in the higher susceptibility of rectal prolapse in children include vertical configuration of the sacrum, greater mobility of the sigmoid colon, loose attachment of the rectal mucosa to the underlying muscular, and poorly developed Houston's valves [6].

Predisposing factors contributing to rectal prolapse include chronic constipation, chronic cough, pertussis, cystic fibrosis, malnutrition, intestinal parasites, myiasis, diarrheal diseases, ulcerative colitis, CMV colitis, pseudomembranous colitis, rectal neoplasms, rectal polyps, ectopia vesicae, meningomyelocele, Ehlers-Danlos syndrome, Hirschsprung's disease, urinary obstruction, autism, and surgical procedures near the anus [2] [7]. In our case, we found that the precipitating factor could have been excessive straining while passing stools. Hence, the parents of the patient were advised to place the child on an adult-type of the toilet seat to reduce the strain. A high-fibre diet and a stool softener were also prescribed.

We considered ileocecal intussusception as one of the differential diagnosis. However, it was ruled out subsequently as abdominal tenderness was absent and the groove between the emerging mass and the margin of the anus, when a finger was inserted, was less than 3 cm. Partial rectal prolapse was ruled out by the size, thickness, and presence of characteristic concentric folds of the prolapsed rectum [3].

Most cases of pediatric rectal prolapse resolve spontaneously or may require manual reduction, as in our case. If the underlying conditions and precipitating factors are identified and addressed adequately, chances of persistence or recurrence are very low. Fortunately, recurrence was not observed in our patient in the 3-months of follow-up. Only around 10% of the cases do not respond to an a12-month trial of conservative management [2].

The procedure of choice for cases of persistent rectal prolapse unresponsive to conservative management is injection sclerotherapy, wherein a sclerosant (dextranomer microspheres, 98 % ethyl alcohol, 5% phenol in almond oil, 50% dextrose, 15-30% saline, etc.) is injected in the submucosal plane in the perirectal area [2] [5] [7]. This leads to an inflammatory reaction which causes adhesions between the rectal mucosa and the underlying muscles. Injection sclerotherapy has a success rate of 90-100%.

For pediatric cases not responding to injection sclerotherapy, a wide variety of surgical procedures are mentioned in the literature. However, no consensus is present over the choice of the surgical procedure due to an almost similar success rate. The guiding principles of each these surgical procedures, broadly classified as perineal or abdominal, are either narrowing/strengthening the anal sphincter, suspending the rectum, excising the redundant rectum, or restoring the weakened pelvic floor [3] [5]. New surgical techniques gaining acceptance include laparoscopic and robotic repair. The selection of the

procedure is largely based on the patient's clinical features and the expertise of the surgeon [2] [5] [7].

In conclusion, conservative management is the best approach for most cases of pediatric rectal prolapse. Correctly identifying and addressing the precipitating factors is the key for preventing recurrences. If unmanaged conservatively, injection sclerotherapy is the preferred procedure of choice. Invasive surgical interventions should be considered in recurrent and persistent cases.

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Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1697-1698.
https://doi.org/10.3889/oamjms.2018.403
eISSN: 1857-9655
Case Report

Multinodular and Vacuolating Neuronal Tumor

Charmaine Zahra*, Reuben Grech

Department of Medical Imaging, Mater Dei Hospital, Msida, Malta

Citation: Zahra C, Grech R. Multinodular and Vacuolating Neuronal Tumor. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1697-1698. https://doi.org/10.3889/oamjms.2018.403

Keywords: MVNT; DWI; FLAIR; MRI; CNS tumours

***Correspondence:** Charmaine Zahra. Department of Medical Imaging, Mater Dei Hospital, Msida, Malta. E-mail: charmainezahra@gmail.com

Received: 21-Jul-2018; **Revised:** 09-Sep-2018; **Accepted:** 10-Sep-2018; **Online first:** 24-Sep-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Multinodular and Vacuolating Neuronal Tumor (MVNT) of the cerebrum is a benign lesion described recently in the WHO CNS tumours in 2016. Although this tumour is uncommon, clinicians should be acquainted with the possible presentation and imaging findings.

CASE REPORT: We present a case of a young gentleman whose only symptom was absence seizures. Brain imaging showed lesions, compatible with this rare diagnosis.

CONCLUSION: Our description of imaging findings on MRI highlights the characteristic cystic appearances of note in the right occipital lobe, in contrast to the temporal lobe as the predominant location found in previous cases.

Introduction

Multinodular and Vacuolating Neuronal Tumor (MVNT) of the cerebrum is a rare benign distinct entity included in the WHO 2016 Central Nervous System (CNS) tumours [1].

Case report

A 33-year-old Caucasian gentleman presented with *absence* seizures. No other relevant symptoms, signs, medical, surgical nor drug history of note.

Brain MRI revealed multiple small cystic lesions, involving the cortex and subcortical white matter (Figures 1 and 2) of the right occipital lobe. The gadolinium-enhanced examination was performed which showed no pathological enhancement. A repeat MRI eight months later demonstrated no significant changes verifying stability with time. The radiological findings were compatible with multinodular and vacuolating neuronal tumour (MVNT) of the cerebrum [2].

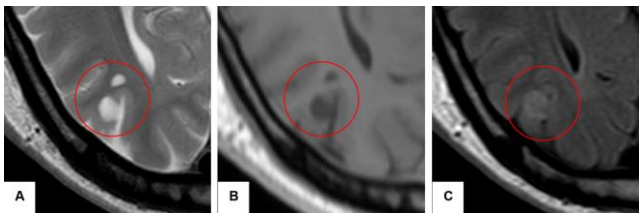

Figure 1: (A) T2-weighted (B) T1-weighted and (C) Fluid-attenuated inversion recovery (FLAIR) MRI. There are multiple well-defined cortical-based lesions. These appear hyperintense on T2-weighted and FLAIR imaging and are hypointense on T1-weighted sequences. No associated vasogenic oedema or mass effect is seen

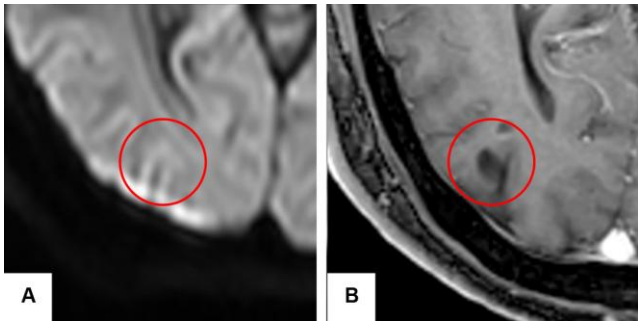

Figure 2: (A) Diffusion-weighted Imaging and (B) contrast enhanced T1 weighted sequences. The lesion does not restrict diffusion, and there is no pathological contrast uptake

This case was discussed at the neurology multidisciplinary team meeting. Only surveillance imaging was needed given its benign nature and stability over time.

Discussion

MVNT, recently included in the WHO 2016 classification of CNS tumours [1], is a rare benign, multinodular, non-neurocyte, glial/neuronal lesion with a unique membrane-bound appearance of the neuronal cytoplasm. Lesions have a round to elliptic shape, are between 1 mm to 5 mm in size and can be found as separate entities or in clusters. Due to its indeterminate pathological nature, characterisation is debatable as the entity shares features of a developmental abnormality and neoplasm, but behaves more like the former [2]. First described by Huse *et al*., these tumours do not show gender predisposition, may be associated with seizure activity of adult onset or unpredictable neurological deficiency and predominantly occur in the temporal lobe [3].

MRI is the gold-standard diagnostic test as no abnormalities are usually seen on CT scans unless the lesion is large. On MRI, the tumour appears as a non-contrast enhancing, T2/FLAIR hyperintense with the absence of restricted diffusion, no mass effect or encompassing oedema nor observable calcification. On T1 weighted imaging, lesions range from isointense to hypointense.

Only a few cases of this uncommon tumour have been published so far, all presenting with similar clinical presentation and type of localisation. This uncommon tumour of heterogeneous lineage may be confounded with other more frequent lesions including DNET and gliomas; however the former involves more cortical thickening while the latter show more cortical diffusion [2] [3].

In conclusion: (i) multinodular and vacuolating neuronal tumour (MVNT) of the cerebrum, is a new neuronal cytoplasmic pattern described in the WHO of CNS tumors 2016; (ii) lesions are benign, multinodular, well circumscribed, non-contrast enhancing, T2/FLAIR hyperintense, involving the cortex and superficial white matter, with absence of restricted diffusion and mass effect; and (iii) MVNT may or may not be symptomatic and may be found incidentally. DNET is the most common differential diagnosis.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1699-1701.
https://doi.org/10.3889/oamjms.2018.380
eISSN: 1857-9655
Brief Communication

Analysis of Concordance of Medication-Taking Behaviour in Tuberculosis Patients in Medan, Indonesia

Arlinda Sari Wahyuni[1*], Noni Novisari Soeroso[2], Dian Dwi Wahyuni[3]

[1]Department of Community Medicine, Faculty of Medicine, Universitas Sumatera Utara, Jl. Dr Mansyur No.5, Medan 20155, Indonesia; [2]Department of Pulmonary and Respiratory, Faculty of Medicine, Universitas Sumatera Utara, Jl. Dr Mansyur No.5, Medan 20155, Indonesia; [3]Department of Microbiology, Faculty of Medicine, Universitas Sumatera Utara, Jl. Dr Mansyur No.5, Medan 20155, Indonesia

Citation: Wahyuni AS, Soeroso NN, Wahyuni DD. Analysis of Concordance of Medication-Taking Behaviour in Tuberculosis Patients in Medan, Indonesia. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1699-1701. https://doi.org/10.3889/oamjms.2018.380

Keywords: Concordance; Medication-taking behaviour; Tuberculosis patients

***Correspondence:** Arlinda Sari Wahyuni. Department of Community Medicine, Faculty of Medicine, Universitas Sumatera Utara, Jl. Dr Mansyur No.5, Medan 20155, Indonesia. E-mail: arlinda@usu.ac.id

Received: 22-Aug-2018; **Revised:** 06-Sep-2018; **Accepted:** 08-Sep-2018; **Online first:** 24-Sep-2018

Funding: This research was funded by the Ministry of Research, Technology, and Higher Education in 2016. We would also like to acknowledge the Head of Health Department in Medan

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

Abstract

AIM: This study aimed to analyse tuberculosis (TB) medication-taking behaviour based on the concept of concordance between health workers and TB patients in Medan, Indonesia.

METHODS: This study was an analytical study with a cross-sectional design. A total of 100 patients undergoing TB treatment at several public health centres in Medan City participated in the study.

RESULTS: The results showed that there was a relationship between the concordance behaviour of the health workers with the attitude and medication-taking behaviour of the patients ($p < 0.05$). However, there was no relationship found between concordance and knowledge of the patients ($p > 0.05$).

CONCLUSION: The results showed that most of the concordance behaviour, knowledge, attitude, and behaviour in the sample were good.

Introduction

In the 2016 Global Tuberculosis Report, WHO estimated that there were 10.4 million new TB cases worldwide in 2015. Six countries in the world accounted for 60% of the new cases, one of which was Indonesia [1]. Therefore, the tuberculosis treatment at the public health centres has gained more attention from the Indonesian government in recent times. To date, every health centres in Indonesia, especially those in Medan, have special health workers in providing services to TB patients [2]. However, the role of the health workers in the treatment of tuberculosis was still poor. This might cause the patients to be disobedient in the advanced-phase treatment that can result in the Multi-Drug Resistance (MDR). Based on that, concordance between patients with their doctors and health workers is needed. A concordance is a form of harmonious cooperation between doctors, health workers, and patients in carrying out treatment action which focuses on consultation. In the consultation, the doctors or health workers and the patients agree to make decisions about the treatment process and combine each of their views based on the principle of partnership [3]. To date, the number of studies about the concept of concordance in TB treatment is very few. Moreover, its implementation in public health centres in Indonesia is also still limited [4].

Therefore, this study aimed to analyse the concordance behaviour of health workers with the characteristics of TB patients such as knowledge, attitude, and behaviour towards the treatment.

Methods

This research was quantitative with an explanatory survey using a cross-sectional design

aimed to explain the relationship between the independent variables and the dependent variable. The study was conducted from March to August 2016 at seven public health centres in Medan. This research has been approved by Health Research FK USU/RSUP H Adam Malik No. 305/KOMET/FK USU/2016.

The population in this study were patients with category I pulmonary TB (new patients) aged ≥ 18 years. The samples were populations who fulfilled the inclusion and exclusion criteria. The inclusion criteria were patients of category I pulmonary TB with positive smear, aged 18-60 years, willing to sign an informed consent and had undergone an intensive phase of treatment whereas the exclusion criteria were TB patients with chronic comorbidities (e.g. cancer, HIV, Diabetes Mellitus) or currently being treated with steroids.

The minimum sample size (100 people) was calculated based on the hypothesis testing formula of one population for a cross-sectional design with a value of alpha 5% and beta 20% for stage I [5]. Participants were interviewed with structured questionnaires that have been validated, namely questionnaires about knowledge on TB and its treatment, attitude toward TB treatment, behaviour or adherence to TB treatment, and interpersonal communication of the health workers based on the concordance principle. Data were analysed using SPSS 19. The analysis used was frequency distribution for descriptive analysis, chi-square test for bivariate analysis, and logistic regression.

Results

The characteristics of respondents consisted of gender, age, education, income, occupation, ethnicity, and marital status, which is given in Table 1.

Table 1: Characteristics Distribution of Patients with Pulmonary Tuberculosis in Medan

Characteristics	Total	Percentage
Gender		
Male	69	69
Female	31	31
Age		
Young (< 40 years)	45	45
Middle (40-59 years)	42	42
Old (≥ 60 years)	13	13
Education		
Low (Elementary, Middle School)	47	47
High (High School, D-III, Bachelor)	53	53
Income		
Less (<2 million)	83	83
Sufficient (>2 million)	17	17
Occupation		
Unemployed	36	36
Employed	64	64
Ethnicity		
a. Bataknese	57	57
b. Javanese	29	29
c. Others	14	14
Marital Status		
a. Married	68	68
b. Not in a marriage	32	32

The results showed that most of the respondents were male (69%) and aged < 40 years (45%). Based on their education level, 53% of respondents had a high educational background (high school and university). In general, respondents had an income of below 2 million (83%). Furthermore, 64% of respondents had a job or currently in employment. The most ethnicity found in the study was Bataknese (57%), and most of the respondents were married (68%).

The concordance results of the health workers and patients based on the knowledge, attitude and behaviour of treatment can be seen in the following Table.

Table 2: Concordance analysis between the health workers and patients Based on the medication-taking behaviour

	Concordance				*p-value**
	Good		Poor		
	n	%	n	%	
Knowledge					
Good	32	59.3	19	41.3	0.954
Poor	22	40.7	27	58.7	
Total	**54**	**100**	**46**	**100**	
Attitude					
Good	34	63	19	41.3	0.031
Poor	20	37	27	58.7	
Total	**54**		**46**	**100**	
Behaviour					
Good	37	68.5	18	39.1	0.003
Poor	17	31.5	28	60.9	
Total	**54**	**100**	**46**	**100**	

Chi-Square.

The results found that most of the good concordance behaviour of the health workers showed a good attitude (63%) and a good medication-taking behaviour (68.5%) in the TB patients. There was a relationship between the concordance of treatment with the attitude and behaviour of the patients ($p < 0.05$), but there was no relationship found with the knowledge of the patients ($p > 0.05$).

Table 3: The analysis results of the relationship closeness of concordance with attitude and behaviour

Variable	*Constant*	*B*	*p-value*	*PR*	*95% CI PR*
Concordance-Attitude	-1.413	0.882	0.032	2.416	1.078-5.408
Concordance-Action	-1.997	1.220	0.004	3.386	1.484-7.725

Logistic Regression.

The logistic regression analysis found that concordance was correlated 2.4 times to the medication-taking attitude of the patients and 3.4 times to the medication-taking behaviour of the patients.

Discussion

The study results showed that 54% of respondents had a good concordance during the TB service in the public health centres in Medan. This result is in contrast to Patriani's study (2013) which

found only 35.1% of concordance among the patients in Mataram Hospital [4]. In this study, the concordance includes the establishment of effective communication based on the principle of partnership, openness, empathy, and support between health workers and TB patients [6]. Several conditions that must be fulfilled in a concordance are a power-sharing consultation, discussion at every opportunity, adequate information, fair and balanced discussion, and adequate time [3]. After the patients hand their health problems to the health workers, the health workers evoke concordance with a partnership communication and facilitate patients to participate with clear goals [7].

This study found that there was a good concordance associated with the attitude and behaviour of the TB patients in undergoing their treatment. This result is consistent with several previous studies [4] [8] [9]. However, this study did not find any correlation between concordance and the patients' knowledge because the patients might have obtained information from various sources, not only from the health workers. Several other studies explained that the reasons patients stopped using medication were because they felt recovery and did not understand the importance of completing the treatment. In other words, there was a low level of patients' knowledge related to the concordance level [10]. A good concordance will improve 3.4 times of the medication-taking behaviour of the patients in a good direction. This will lead to higher adherence or compliance of the patients than the poor concordance behaviour. Thus, patients have the commitment, motivation, and sense of responsibility for the disease and its treatment. It is needed during the treatment of chronic diseases, such as tuberculosis. The patients should understand and be responsible for their disease. This sense of responsibility and high commitment will simultaneously prevent the surrounding environment from contracting tuberculosis, and the patients will also be prevented from MDR, a difficult treatment which requires a very long time (2 years) [11] [12].

In conclusion, the results showed that most of the concordance behaviour, knowledge, attitude, and behaviour in the sample were good. There was a relationship between concordance with the attitude and medication-taking behaviour of the TB patients. However, there was no relationship found between concordance and the knowledge of the patients about their disease and its treatment.

Acknowledgement

This research was funded by the Ministry of Research, Technology, and Higher Education in 2016. We would also like to acknowledge the Head of Health Department in Medan.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1702-1706.
https://doi.org/10.3889/oamjms.2018.331
eISSN: 1857-9655
Dental Science

3D FEA Study On Implant Threading Role on Selection of Implant and Crown Materials

Ahmad Mohamad Wazeh[1], Mohamed I. El-Anwar[2], Rami M. Galal Atia[3,4,5*], Rana Mohammed Mahjari[1], Sara Abdulelah Linga[1], Linah Mujeeb Alhaq Al-Pakistani[1], Salah Abdulazim Yousief[6,7]

[1]Alfarabi College, Jeddah, Saudi Arabia; [2]Department of Mechanical Engineering, National Research Centre, Giza, Egypt; [3]Department of Fixed and Removable Prosthodontics, Oral and Dental Division, National Research Centre, Giza, Egypt; [4]Department of Fixed Prosthodontics, Faculty of Oral and Dental Medicine, Al Nahda University, Beni Suef, Egypt; [5]Department of Fixed Prosthodontics, Faculty of Oral and Dental Medicine, Ahram Canadian University, Giza, Egypt; [6]Restorative Dentistry Dept., AL-Farabi Dental College, Jeddah, Saudi Arabia; [7]Crown & Bridge Dept., Faculty of Oral and Dental Medicine, Al Azhar University, Assuit Branch, Cairo, Egypt

Citation: Wazeh AM, El-Anwar MI, Atia RMG, Mahjari RM, Linga SA, Al-Pakistani LMA, Yousief SA. 3D FEA Study On Implant Threading Role on Selection of Implant and Crown Materials. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1702-1706. https://doi.org/10.3889/oamjms.2018.331

Keywords: Dental implant; Finite Element Analysis; PEEK; PEKK; Titanium

***Correspondence:** Rami M Galal Atia. Department of Fixed and Removable Prosthodontics, Oral and Dental Division, National Research Centre, Giza, Egypt; Department of Fixed Prosthodontics, Faculty of Oral and Dental Medicine, Al Nahda University, Beni Suef, Egypt; Department of Fixed Prosthodontics, Faculty of Oral and Dental Medicine, Ahram Canadian University, Giza, Egypt. E-mail: ramimgalal@gmail.com

Received: 30-May-2018; **Revised:** 28-Jul-2018; **Accepted:** 06-Aug-2018; **Online first:** 11-Sep-2018

Funding: This research did not receive any financial support

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

Abstract

AIM: This study deeply investigates the effect of dental implant threading and material selection on the mandibular bone under two different crown materials (Translucent Zirconia and Porcelain fused to metal).

METHODS: Two different designs of single piece dental implants were supporting dummy crown above simplified bone geometry in two finite element models. Models components were created by general-purpose CAD/CAM engineering package and then assembled inside ANSYS before meshing and assigning materials. Compressive loading of 100 N and 45º oblique loading of 50 N were tested.

RESULTS: Twenty-four case studies were analysed, and their results were compared. Micro thread reduces implant maximum Von Mises stress by about 50 to 70% than regular thread one. Oblique loading of 50 N will produce 4 to 5 times more maximum Von Mises values on implant body than 100 N vertical loading. Zero or negligible effect on the cortical bone was recorded when exchanging the tested crown material. Although titanium implant can also reduce cortical bone, Von Mises stress by 50 to 100% in comparison to reinforced PEKK (poly ether-ketone-ketone) or PEEK (poly-ether-ether-ketone).

CONCLUSIONS: Reinforced PEKK and PEEK implants can represent a good alternative to titanium implants. Zirconia crown distributes the applied load better than Porcelain fused to a metal one. Regardless of the implant material, an implant with the micro thread has superior behaviour in comparison to a regular one. Zirconia crown above titanium implant with the micro thread may represent the best option for patient bone.

Introduction

Crown and dental implant materials selection strongly affect the patient bone, especially who have osteoporosis.

Cytec Engineered Materials PEKK, composites consist of a matrix of (poly ether-ketone-ketone) polymer with aligned, continuous unidirectional fibre reinforcement. Typical fibre contents are 50-60% by volume.

PEKK composites possess outstanding flame, smoke and toxicity performance. They also have high toughness and damage tolerance. Laminates and parts can be fabricated from PEKK using a wide range of techniques including autoclave and press moulding. Prepregs are offered in various grades of PEKK polymer optimised for select manufacturing methods. Information is available upon request for additional grades of PEKK to use in injection moulding, sheet, film or fabric composites.

PEEK (poly-ether-ether-ketone), which is a

dominant member of the PAEK (poly-aryl-ether-ketone) polymer family, appeared during the 1990s as the main substitute for the metallic components and implants of high-performance thermoplastic polymers, especially in cases of orthopaedics and trauma. PEEK has first been used in aerospace industry and then in orthopaedic surgery, especially in cervical vertebra surgery. Currently, this material is also used in Dental Implantology. PEEK is a real alternative to a titanium implant thanks to its highly beneficial properties and biocompatibility while remaining affordable [1] [2].

Since PEEK showed resistance to degradation in vivo, it was offered commercially in April 1998 as a biomaterial for long-term implants (Invibio Ltd, Thornton-Cleveleys, UK) [3]. Since then, PEEK has demonstrated to be a high-performance thermoplastic polymer able to replace metallic implant components in the field of orthopaedics [4] [5] and traumatology [6] [7]. Also, calvarial reconstructions with PEEK implants were described [8]. These findings suggest that PEEK could substitute titanium as a material for dental endosseous implants.

This study aims to answer the following questions about implant material; does PEKK or PEEK could be considered as a viable alternative material for dental implants? Which implant design is preferable with or without micro thread? Which crown/implant materials combinations are recommended for patient bone?

Materials and Methods

Two finite element models were developed for two different designs of single piece dental implants. First one was for an implant with micro thread BTICV3 (BT Lock, Montecchio Maggiore, Italy), while the second one was for regularly threaded implant Zimmer (Zimmer dental Inc, USA) both have 3.7mm diameter and 13mm implant length. Additionally, dummy crown was placed on it, that two crown materials (Translucent Zirconia, Porcelain fused to metal) in combination with three implant materials (Titanium, 50% GFR-PEKK, and 30% CFR-PEEK) [3] were tested.

The dummy crown has a 1.5mm thickness in occlusal and axial dimensions. The finite element models components as the dummy crown and implants were created on “Autodesk Inventor” Version 8 (Autodesk Inc., San Rafael, CA, USA), then exported as SAT files [9] to the finite element software. In this study, the cement layer was neglected, while all materials were assumed homogeneous, isotropic, and linearly elastic and its properties are listed in Table 1.

Table 1: Material properties used in the finite element model

Material	Young's modulus [GPa]	Poisson's ratio
Crown material ... Translucent Zirconia (TZI)	210	0.35
Crown material ... Porcelain fused to metal	149.5	0.34
PEKK implant (50% GFR-PEKK)	14.0	0.41
PEEK implant (30% CFR-PEEK) [2] [10]	18.3	0.39
Titanium Implant	110	0.35
Mucosa	0.01	0.40
Cortical bone	13.7	0.30
Cancellous (spongy) bone	1.37	0.30

Simplified bone geometry was simulated as two co-axial cylinders. The inner one represents the spongy bone (diameter 13 mm & height 21 mm) that is filling the internal space of the outer cylinder (shell of 1.5 mm thickness). The outer shell represents cortical bone (diameter 16 mm & height 24 mm) [11]. The gingival thickness was assumed 2 mm that is placed above the cortical bone cylinder, where both gingiva and bone were modelled in ANSYS GUI.

The models' components were assembled in ANSYS environment (ANSYS Inc., Canonsburg, PA, USA), by performing a set of Boolean operations between the modelled components to obtain the complete model(s) assembled. The meshing of these components was done by 3D solid element “Solid-185” which has three degrees of freedom (translations in main axes directions) [12]. The resulted numbers of nodes and elements are listed in Table 2. Implants complex and cut sections of the two assembled models were presented as screenshots from ANSYS screen in Figure 1.

Table 2: Number of nodes and elements in all meshed components

	Model #1: BTICV3		Model #2: Zimmer	
Component	Nodes	Elements	Nodes	Elements
Crown	1,005	12,140	4,010	16,405
Implant complex	41,092	268,076	18,618	97,443
Mucosa	1,721	18,540	1,143	4,010
Cortical bone	1,159	18,174	1,644	5,504
Spongy bone	4,454	33,395	6,713	31,686

For each model, two loading conditions were tested as; 100 N vertical compressive load and 50 N oblique 45° load. The lowest plane of each model was considered as fixed in the three directions as a boundary condition.

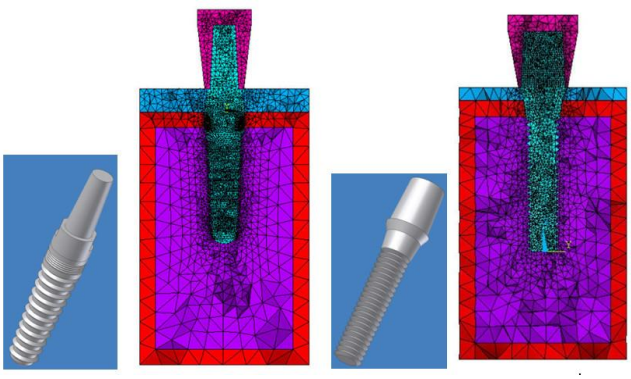

Figure 1: The implant complexes and meshed model

Linear static analysis was performed on Workstation HP ProLiant ML150, with Intel Xeon 3.2 GHz processors (with 1MB L2 cache), 10 GB RAM, using commercial multipurpose finite element software package (ANSYS version 14.0), that results of these models were verified against similar studies [13] [14] [15].

Results

Many graphical representations can be obtained from FEA; each shows the distribution of deformation, strain, and stress. Figure 2 illustrates random examples of the obtained results, while all maximum values of deformations and stresses were recorded, tabulated and compared to extract conclusions.

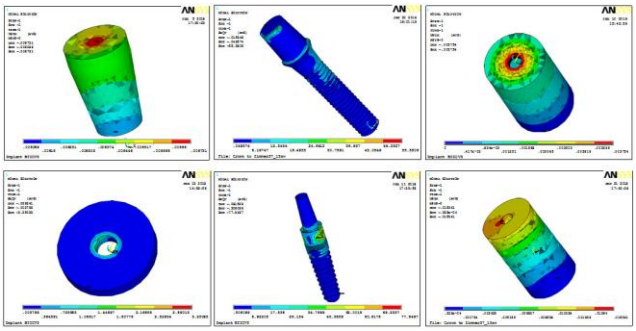

Figure 2: Sample results

Comparing maximum Von Mises stress values appeared on crowns in the twenty-four cases, as presented in Figure 3, indicated that changing crown material from Zirconia to porcelain fused to metal has a minor effect. Two to five per cent less Von Mises stress appeared on porcelain fused to metal crowns in comparison to Zirconia ones. Crowns placed above implants with micro thread received about 50% less Von Mises stress under vertical loading. On the other hand, under oblique loading, the maximum Von Mises stress exerted on these crowns were much higher. From total deformation prospective, both crown materials are equivalent that exchange crown material from Zirconia to porcelain fused to metal increase the total crown deformation by about one micron.

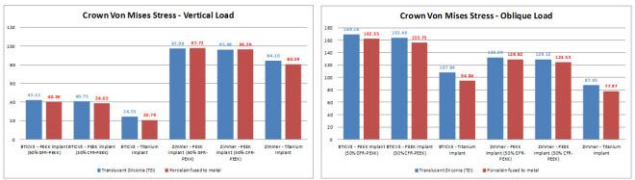

Figure 3: The crown maximum Von Mises stress comparison

Implants under more rigid crown material received less Von Mises stress, in other words lowering crown material stiffness increase implant stress. On the other hand, crown material change from Zirconia to porcelain fused to metal has a negligible effect on implant total deformation (less than 1%).

Micro thread reduces implant maximum Von Mises stress by about 50 to 70% than regular thread one. Oblique loading of 50 N will produce 4 to 5 times more maximum Von Mises values than 100 N vertical loading. Figure 4 compared all maximum Von Mises stress values obtained in this study. The total deformation of the micro-threaded implant was less by 30 to 50% than regular implants.

Reinforced PEEK and PEKK implants are equivalent to titanium one that all will receive a similar amount of stresses (within acceptable limits for each material). While it showed the double total deformation values in comparison to titanium one (within physiological limits).

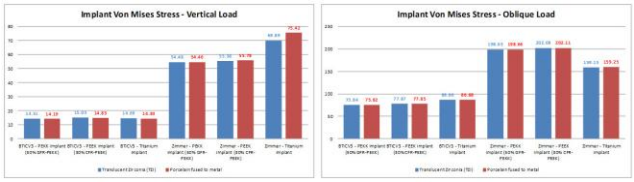

Figure 4: Implant complex maximum Von Mises stress comparison

Reducing cortical bone maximum Von Mises stress can be achieved by adding a micro thread to the implant as shown in Figure 5. The cortical bone can receive that about 50% less of the Von Mises stress if the implant body contains micro thread.

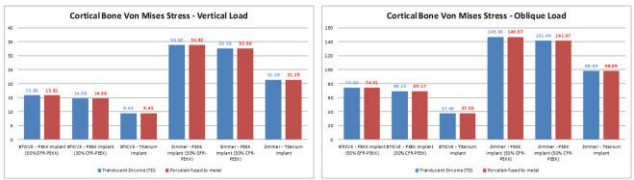

Figure 5: Cortical bone maximum Von Mises stress comparison

Zero or negligible effect was recorded when exchanging the crown material. Although titanium implant can also reduce cortical bone Von Mises stress by 50 to 100% in comparison to reinforced PEKK or PEEK, all tested materials in this study received an acceptable level of stresses (within limits). Titanium implant with micro thread showed the lowest stress on the cortical bone (of order 10 MPa under vertical load), while Regular implant thread may lead to cortical bone failure (Von Mises stress exceed 100 MPa) under 50 N oblique loading.

Discussion

Stresses exerted over the bone surrounding dental implant are an important issue [11]. There is an agreement that well and proper stress distribution on a large area of bone is preferred for the long-term success of implants. The implant material is, of course, an important factor in this point. The point of concern here is the composition of the material and how much fatigue can happen to the bone with cyclic loading (masticatory forces) [16].

In a previous study [11], it was found that the implants with threads would have more increase in the surface area about the cross-section. Many studies [11] [13] [14] [17] assessed the stress distribution and deformation of threaded and unthreaded implants and the effects on surrounding bone.

In our study, the concern was to study the micro threads versus the conventional threading, implant and cortical bone results were in total agreement with previous studies [13] [14] [15]. Where micro thread on implant reduces, the Von Mises appeared on the implant body due to increasing the ratio of side area to cross section area [15]. Also, this was augmented by clinical results as stated in the systematic review [18] that found that researches suggested less bone loss with micro-threaded neck implants.

Different prosthetic materials can be used with implant prostheses. The selection is a controversy. There is a consensus that survival of implant is not affected by the prosthesis material [19 [20]. Crown material has a negligible effect on cortical bone, which matches results of previous researches [13]. This also is augmented by results of a study [21] proving that zirconia prosthesis with proper thickness has low-stress levels. Stiffer crown material bitterly distributes the applied load on the implant that reduces implant body stresses.

In our study, the maximum stresses were below the ultimate tensile and compressive strength of bone. In an important study, [22] proving our results it was found using the finite element analysis that the crown material does not affect the bone stresses, but there was an effect from the implant material. Moreover, this will take us to the next part of our study, regarding the implant material.

Bone remodelling is controlled according to the loads directed over it. Stress shielding is the shielding of the normal loads transferred to the bone by the implant. Few studies stated that no difference is found between osseointegration around PEEK and zirconia and titanium implants [23] [24]. Also, bio inertness of PEEK, zirconia and titanium was found to be the same [25].

In our study, although titanium implant produces lower stresses than reinforced PEKK or PEEK, all of the three materials can replace each other with minor effects. Finite element analysis of carbon fibre reinforced PEEK found that they could induce fewer stresses than titanium [26], unlike our results.

There is no widespread use of the PEEK implants clinically with studied bone level effect. Moreover, another finite element study by Sarot et al., [27] said that there is no difference between stress distribution around PEEK and Titanium, which is by our study.

Reinforced PEKK or PEEK implants, can replace titanium ones in restoring the single tooth. This was in approvement with the review done on applications of polyether ketone (PEEK) in implantology and prosthodontics [28] that concluded that because of the similarity of this material ‘s physical and mechanical properties to dentin, it could be used in many dental applications including implant materials.

More clinical studies are needed to prove our results.

In conclusion, glass fibre reinforced PEKK and carbon fibre reinforced PEEK implants can represent a good alternative to titanium implants. Regardless of the implant material, an implant with the micro thread has superior behaviour in comparison to a regular one. Micro threaded implants are preferred for cortical bone and implant body, that micro thread can reduce stresses exerted on both of them. Crown material change from Zirconia to porcelain fused to metal has a negligible effect on cortical bone. Finally, Zirconia crown above the titanium implant with the micro thread may represent the best option for patient bone.

Ethical Approval

This research did not require ethical approval and followed the Helsinki declaration.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1707-1711.
https://doi.org/10.3889/oamjms.2018.343
eISSN: 1857-9655
Dental Science

Efficacy of Fiber Post Bonding To Root Dentin after Different Obturation Techniques and Cementation Timings: In Vitro Study

Rami M. Galal[1,2,3*], Nada Omar[4], Haidy Nabil[4], Yousra Aly[4]

[1]Department of Fixed and Removable Prosthodontics, National Research Centre, Cairo, Egypt; [2]Department of Fixed Prosthodontics, Al Nahda University (NUB), Beni Suef, Egypt; [3]Department of Fixed Prosthodontics, Ahram Canadian University (ACU), Giza, Egypt; [4]Department of Restorative and Dental Materials Research, National Research Centre, Cairo, Egypt

Citation: Galal RM, Omar N, Nabil H, Aly Y. Efficacy of Fiber Post Bonding To Root Dentin after Different Obturation Techniques and Cementation Timings: In Vitro Study. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1707-1711. https://doi.org/10.3889/oamjms.2018.343

Keywords: Bond strength; Fiber Post; Vertical compaction

***Correspondence:** Rami M Galal. Department of Fixed and Removable Prosthodontics, National Research Centre, Cairo, Egypt; Department of Fixed Prosthodontics, Al Nahda University (NUB), Beni Suef, Egypt; Department of Fixed Prosthodontics, Ahram Canadian University (ACU), Giza, Egypt. E-mail: ramimgalal@gmail.com

Received: 30-May-2018; **Revised:** 19-Aug-2018; **Accepted:** 30-Aug-2018; **Online first:** 21-Sep-2018

Funding: This research did not receive any financial support

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

Abstract

OBJECTIVE: The aim of this in vitro study was to assess the effect of obturation technique and cementation timings on the bonding of fibre-reinforced posts to the root canal walls.

METHODS: Twenty extracted teeth were randomly allocated to two groups according to the obturation technique and cementation timing. Central incisors with single canals were used after being decoronated. Every extracted tooth of the {vertical compaction group} (VC) group (n = 10) had been obturated using the {E & Q plus obturation system} with posts cemented in the same day; The other (CO) conventional group (n = 10) teeth were obturated using the conventional lateral compaction technique and posts cemented after one week. “SF“ Fiber posts were used after bonding and cementation using Rely X ARC resin cement with all the endodontically treated teeth. The push-out test was performed in a universal testing machine. Data were analysed by 2 way analysis of variance with Statistical significance was set to 0.05.

RESULTS: Heat softened gutta percha group showed more push out the bond strength of the bonded posts than the conventional obturation group ($p < 0.05$). In the middle region, there was no statistical significance between the two groups while there was significance in the coronal and apical thirds.

CONCLUSION: The vertical compaction technique and early cementation improved the bond strength of the resin posts in comparison to the conventional obturation technique with late cementation.

Introduction

When the dentist performs endodontic treatment for teeth, the possibility of mechanical failure and fracture increases [1]. It is well known that after endodontic treatment there is mainly: loss of tooth structure due to caries, fracture, endodontic treatment access hole, lost water content and collagen cross-linking, decreased dentin thickness in the root canal, leading to the increased possibility of fracture [2]. Posts are the suggested line of treatment to restore the coronal tooth structure when there is not enough remaining tooth structure to retain the core buildup [3] [4]. Non-metallic posts had been introduced into dentistry offering more esthetic solutions, less possibility of fracture and also more biocompatibility. Meyenberg et al., [5] in 1995 developed the strong zirconia posts. Many types of research showed that they are stronger than other post and core combinations [6] [7]. Zirconia posts were used after its use in other medical branches proving that it is strong, biocompatible and being not soluble [6] [7] [8]. Those ceramic posts have a value near that of the tooth structure and so gives enhanced esthetic results especially under all-ceramic restorations [9] [10]. But these ceramic posts are brittle, and there are many reports about their fracture

clinically [10] [11] [12]. So, there was a need for a post with less possibility of fracture and still giving good esthetic results; which is the fibre post [10] [12]. The fibre post has a modulus of elasticity similar nearly to dentin (18-22 Mpa), so any load will be uniformly distributed along the post –cement-tooth interface. So there is minimal concentrated stresses and possibility of fracture root. Therefore, we have a much better and safer line of treatment for endodontically treated teeth [13] [14] [15]. Clinical researches showed success rate from 95-99% for fibre posts without fractures during the studies periods [15] [16] [17] [18].

Retrospective clinical researches showed that the unretained post is its main problem. So the use of adhesive cement has been suggested to solve this problem. Also, the type of endodontic sealer and obturation technique may affect the retention of the post [19] [20].

Material and Methods

Twenty single rooted permanent human freshly extracted teeth were collected from the dental clinic at the National Research Centre.

The teeth were decoronated by sectioning the coronal part of each tooth using a sectioning disc (Toolouip, Germany) mounted on a low-speed handpiece accompanied with water coolant. The same operator as following carried out preparations of the teeth:

1. The root canals were instrumented with the pro taper nickel-titanium rotary system; each canal was enlarged to size F4.

2. During instrumentation, 1ml of 5.25% NaOCl was used to irrigate the canal between file sizes.

3. Canals were dried with absorbent paper points before obturation.

Samples were classified according to the obturation technique into two groups:

Group 1: Canals obturated with warm vertical compaction using (E&Q plus) and Backfill technique and AD Seal sealer (Meta Biomed, Inc.).

Group 2: Canals obturated with gutta-percha and ADSeal sealer (Meta Biomed, Inc.) using lateral condensation multiple cone technique.

In group 1, heated gutta-percha obturation involved two main steps, namely: Vertical condensation and Backfill. Vertical condensation (Down Pack): ADSeal sealer (Meta Biomed, Inc.) was applied onto the canal with a no. 20 file, following which master point, was placed 1mm short of the working length. Then, the appropriate temperature of E&Q Pen (250 degree Celsius) was set and activated by touching the spring switch on the handpiece. First, the excess of gutta-percha over the orifice was severed off using the activated E&Q Pen Tip. Then gutta-percha was warmed by inserting the pen tip 7 mm short of the working length. At this canal length, the activated pen tip was placed for 2-3 seconds and then deactivated for 8-10 seconds as the spring switch was released so that apical gutta-percha was uniformly warmed. Finally, the pen tip was again activated for two seconds so that gutta-percha in the coronal portion was retrieved. This procedure also prevented the retrieval of gutta-percha in the apical portion. The softened gutta-percha was compacted using the widest plugger, which led to perfect obturation of the apical area and accessory canals. Backfill: The gutta percha bar was placed into the activated Gun, and the Gun needle was inserted into the root canal up to the level of the already placed gutta-percha in the apical portion. The trigger was pulled slowly, and backfill was completed up to the root canal orifice. During backfill, Gun needle was pushed back simultaneously by gutta-percha being filled. The heated gutta-percha was compacted using a bigger plugger, which led to complete obturation of the root canal system.

In group 2, the root canals were filled with GP points. Master apical cone of size F4 was coated with the sealer and slowly inserted into the canal to the working length. A spreader #30/0.02 taper was used for lateral compaction. The spreader was inserted in the canal alongside the master cone. The pressure was applied apically to push the spreader in as far as possible. GP cones (#20/0.02 or #25/0.02 taper) were coated with the sealer and used as accessory cones until there was no more room in the canal for additional accessory cones. After sealing off all access cavities with Coltosol, all samples of group 2 were stored in a humid environment at 37^{o}C for one week to allow sufficient time for the sealing agents to set. While for group1, the post-drilling and cementation procedure is done on the same day.

Post insertion: Removal of gutta-percha is done using Gates Glidden drills. Then the drill of number 2 post was used then cementation of smaller number 1 post. Before cementation, the fibre posts were cleaned with 70% alcohol, silanated [34], cemented with 3mm extended outside the canal to standardise 3 mm distance from the tip of the light curing unit.

After cementation procedures using Rely X ARC cement [32], all specimens were stored in sterile saline in a sealed container for one week. Each root was sectioned perpendicular to the long axis of the root using a water-cooled precision saw to obtain 2 mm ± 0.1 thick slices. The post diameters on each surface of the post/dentin sections were measured using a digital calliper (Pachymeter, Electronic Digital Instruments, China).

Push out bond strength testing**:** The push-out test was performed by the universal testing machine (*Model 3345; Instron Industrial Products, Norwood, MA, USA*) at a crosshead speed of 1 mm/min, using a plunger of (1, 0.8 and 0.5 mm diameter) corresponding to the radicular thirds (Coronal, middle and apical) to be tested. The plunger tip was positioned to push the filling toward the larger diameter, without stressing the surrounding dentin, in apical-coronal direction.

The maximum failure load was recorded in Newton.

The bond strength was calculated from the recorded peak load divided by the computed surface area as calculated by the following formula

$$[A = (3.14x\ r_1\ X\ 3.14x\ r_2)\ L],$$

Where r_1 apical post radius, r_2 coronal post radius, $L= [(r_1-r_2)^2 + h_2]^{0.5}$ and h is the thickness of the sample slice in millimetres.

Data were collected and analysed.

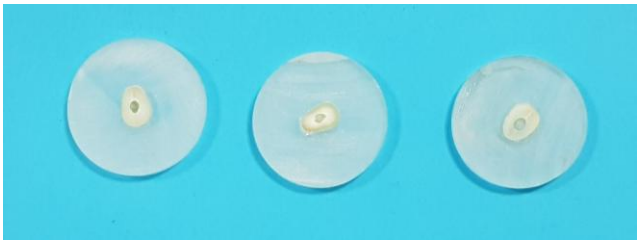

Figure 1: Sectioned root portions with cemented fibre post

Results

Two-way ANOVA showed statistically significant differences in the mean bond strength values between the conventional obturation group (CO) and the heat softened gutta percha group (HS) (P = 0.006). The higher pushout bond strength value was observed in the heat softened gutta percha group (HS) (11.465 ± 1.32 Mpa). Table 1.

Table 1: Bond strength values (Mpa) of groups

Group	N	Mean	Standard deviation
Conventional obturation	10	6.155	2.64
Vertical compaction	10	11.465	1.32

T-test was used to compare between the 2 groups regarding the different root portions, in the coronal and apical regions, there were no statistically significant differences between the two groups (p = 0.1) and (p = 0.35), while in the middle root region the bond strength values of the (HS) group were statistically higher than that of the (CO) group (p = 0.01).

Table 2: Bond strength values (Mpa) for each group in coronal, middle and apical regions

		Coronal region		Middle region		Apical region	
Obturation technique	N	mean	Standard deviation	mean	Standard deviation	mean	Standard deviation
Conventional obturation	10	5.827	6.213	8.945	6.442	3.694	2.112
Vertical compaction	10	12.748	11.07	11.545	8.635	10.104	6.344

The obturation technique affected the push out the bond strength of the cemented post Table 3.

Table 3: ANOVA results of the significant difference between the two groups

Source of Variation	SS	df	MS	F	P-value	F crit
Sample	424.0042	1	424.0042	7.936275	0.006754	4.019541
Columns	125.7363	2	62.86817	1.176732	0.316064	3.168246
Interaction	48.97633	2	24.48817	0.458356	0.63476	3.168246
Within	2885.009	54	53.42609			
Total	3483.726	59				

Discussion

The main feature of the polymerisation process of the resin cement is the polymerisation shrinkage. The resin cement used in this study has dual polymerisation property, chemical (when base and catalyst are mixed) and light (when the light interacts with the photoinitiator) which is of course affected by the light intensity. Even when using fibre posts, light cannot reach with its full intensity to the apical region that is why polymerisation is compromised in this region. There is a hypothesis that the polymerisation shrinkage due to decreased degree of convergence is reduced in the apical third [21]. Also, the presence of multiple steps increase the technique sensitivity [22], it is also hard for the removal of the excess bonding agent and its evaporation in the apical third. All these factors decrease the bond strength to the root dentin [23]; this can explain why the apical third has the lowest bond strength values in our study. The ARC cement has a great amount of diluent monomer that is why it is preferred over other viscous cement to reach the apical third [24]. Our results were opposed by a study [33] that found that the bond strength was more at the coronal third if there is proper access to the apical third.

There are different ways to detect the bond strength, like microtensile bond strength on external root dentin [25], pull out [26] and push out test [27]. It has been found that the most reliable and accurate method is the push out test [28]. The advantage of the push put to test over the pull out that it allows testing the different portions of the root, (coronal, and middle apical) [26]. The clinical situation can be mimicked by load directed on slices as done in our study [29]. It was found that the thick root sections may cause non-uniform stress distribution with subsequent wrong

results [30]. Thin sections were done in our study.

Adhesion procedures are technique sensitive, the operator's experience plays a role in it, and the experience affected the bond strength results [31]. Experienced Fixed Prosthodontist and Endodontist did the procedures in our study.

The effect of endodontic sealers on the push out the bond strength of bonded fibre posts was studied before [35]. However, very few researchers evaluated the obturation technique [36] regarding the type of material and found that there is an effect on the bond strength of fibre posts.

Our study concerned with studying the obturation technique whether the thermal filling or the conventional technique but with main concern of the possibility of earlier cementation of the fibre posts when using the thermal filling technique due to its easiness and more time-saving properties.

In a study [37] assessing the timing of post drilling and cementation after obturation either immediate or delayed after 30 minutes and after 14 days, it was found that delaying the preparation leads to more residue in the middle and apical thirds of the prepared post space with subsequent affected bonding of the post and less bond strength. These results were by our study results.

However, opposing to our study results [38], another research evaluated the influence of timing after endodontic treatment on bond strength of fibre posts with epoxy resin obturated canals. Posts also were cemented immediately and after 7 days using the same cement Rely X ARC, sectioned, push out the test was done, but it was found that the root section whether coronal, middle or apical has no significant effect. Also, the timing showed no significant effect on bond strength. This contradiction can be explained due to the difference of the obturation material used in this study and the one that we have used.

From the limitations of our study is the number of samples, number of samples to augment the results in future studies.

It was found that the early preparation of post space and cementation of fibre post is preferred over the late procedure and this can be aided by using the thermal filling vertical compaction technique.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1712-1715.
https://doi.org/10.3889/oamjms.2018.299
eISSN: 1857-9655
Dental Science

Awareness about Management of Tooth Avulsion among Dentists in Jazan, Saudi Arabia

Redhwan Abdullah Alaslami*, Fahiem M. M. Elshamy, Essamet M. Maamar, Yahya H. Ghazwani

College of Dentistry, Jazan University, Jazan, Saudi Arabia

Citation: Alaslami RA, Elshamy FMM, Maamar EM, Ghazwani YH. Awareness about Management of Tooth Avulsion among Dentists in Jazan, Saudi Arabia. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1712-1715. https://doi.org/10.3889/oamjms.2018.299

Keywords: Dental trauma; Tooth avulsion; Knowledge; Awareness; Management

***Correspondence:** Redhwan Abdullah Alaslami. College of Dentistry, Jazan University, Jazan, Saudi Arabia. E-mail: drredhwanaslami@gmail.com

Received: 31-Jul-2018; **Revised:** 09-Aug-2018; **Accepted:** 10-Aug-2018; **Online first:** 21-Sep-2018

Funding: This research did not receive any financial support

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

Abstract

AIM: This study aimed to evaluate the awareness amongst dentists working in Jazan, a city in K.S.A., about their potential role in the treatment of traumatic tooth avulsion injuries.

MATERIAL AND METHODS: A cross-sectional study was performed and all dentists in Jazan, K.S.A. were invited to participate in this study. Data was collected through a self-administrated questionnaire. A total of 200 dental practitioners answered the questionnaire. The intern/general practitioners were 75%, and the specialists were 25%. The variables comprised socio-demographic information, professional characteristics and information regarding the awareness towards the management of tooth avulsion.

RESULTS: A total of 200 dental practitioners answered the questionnaire. The intern/general practitioners were 75%, and the specialists were 25%. (44.3%) were aware of the critical time for avulsed tooth replantation while 71.8% of females did not know about the critical time for avulsed tooth replantation. 50.0% of the specialists reported that less than 30 min was needed for avulsed tooth replantation, while 59.3% of interns/general dentists advised less than 60 minutes.

CONCLUSION: A statistically significant result was observed regarding the best storage medium, a critical time for avulsed tooth replantation and tooth management before replantation.

Introduction

Majority of dental injuries occur from 8 to 11 years is due to the falling accidental [1] [2]. Twenty-five per cent of all school children experience dental trauma, and 33% of adult have experience trauma to the permanent dentition. Anterior teeth are not only important for an aesthetic reason but are also necessary for mastication, phonetics, the integrity of supporting tissues, the psychological and mental well-being of youngsters [3].

Tooth avulsion is the complete displacement of a tooth from its socket due to accidental or non-accidental injury [4]. Several studies have investigated the awareness of avulsed teeth in children among parents, and dentists, and have recommended the necessity of learning methods of management to enhance the prognosis of avulsed teeth [2, 5-7]. The prognosis of an avulsed tooth relies on three important factors; extra-oral time, storage media and root development [3].

Avulsion presents a challenge with regards to its proper emergency management [5] [6] [7] [8] [9]. Clinical outcome of the avulsed tooth is unduly compromised if adequate emergency treatment is not followed [9]. When a traumatic dental injury occurs, the patient expects competent treatment from the dental practitioners [9].

Dentists are required to manage traumatic dental injuries in children. Many studies have shown that there is insufficient knowledge regarding the immediate management of patients with traumatic dental injuries [3]. This particular study was planned to explore the awareness of the dental professionals (in Jazan region) about the management of an avulsed

tooth/teeth.

This study aimed to evaluate the awareness amongst dentists working in Jazan, a city in K.S.A., about their potential role in the treatment of traumatic tooth avulsion injuries.

Material and Methods

A cross-sectional study was performed in Jazan, a medium-sized city in the southern region of K.S.A. All dentists (interns, general practitioners and specialists) were invited to participate in this study. Data was collected through a self-administrated questionnaire (Table 1).

Table 1: Questionnaire used in the study

	Gender: Male Female Level of Specialization: Intern GP (BDS) Speciality: Oral Surgeon Prosthodontics Orthodontist Periodontist Pedodontist Restorative (Operative Endodontist) Public health Years of experiences: <5yrs 5-10 yrs. >10 yrs. Working place**: Private: Governmental:**
Q1.	What will you do if you have found patient with avulsed tooth outside the oral cavity? a. Save the tooth b. Discard the tooth c. Don't know what to do.
Q2.	Do you have information on the management of avulsed tooth? a. Yes. b. No.
Q3.	What would you do if the avulsed tooth was covered with dirt? a. Rinse with tap water. b. Would do nothing. c. Scrub with soap. d. Not sure.
Q4.	According to you which are the best storage medium for the storage of the avulsed tooth? a. Hanks balanced salt solution. b. Milk. c. Saliva. d. Saline solution.
Q5.	Would you prefer the replantation of the tooth into the socket from which it came? a. Yes. b. No.
Q6.	Which is the critical time for the replantation of the avulsed tooth? a. Less than 30 min. b. Less than 60 min. c. Less than 90 min. d. More than 90 min.
Q7.	Factors influencing the outcome of the replantation? a. Storage medium. b. Extra-alveolar period. c. Amount of the loss of periodontal ligaments. d. All of the above.
Q8.	Which method of splinting after replantation will you prefer? a. Stainless steel wire. b. Semi-rigid with nylon wire. c. Composite restorative materials. d. No splinting.
Q9.	Splinting time necessary? a. 15 days. b. 30 days. c. 60 days.
Q10.	Which type of the tooth do you think intended for replantation after avulsion? a. Deciduae. b. Permanent. c. Both.

The variables in the questionnaire comprised of socio-demographic information (gender, age and nationality), professional characteristics (years of experience, level of specialisation and working place and overseas fellowship), and information regarding the awareness about the management of avulsed tooth.

The questionnaire was distributed to the participants, and an explanation was given to each subject about the importance of their participation and the study purposes.

The statically analysis was performed using Statistical Package for Social Sciences version 21 (SPSS, Illinois, Chicago, USA). Descriptive analysis was carried out by mean of Chi-square test to evaluate association existing between time since graduation and post-graduation training. The significance level was set at $P<0.05$.

Results

All participants were well aware of the management of an avulsed tooth. Total of 200 dental practitioners had answered the questionnaires, 39% were females, and 61% were males. The mean age of the study participants was between 24–66 years, the general dentists 75% and specialists 25% (different specialities). The dentists' demographic parameters are shown in Figure 1.

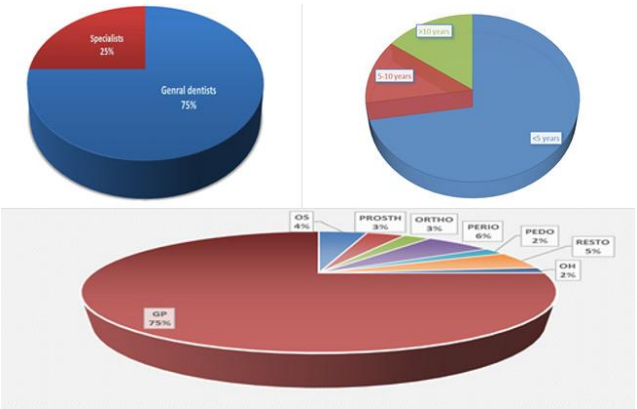

Figure 1: Demographic parameters of the sample study

There was a significant difference between the genders regarding the critical, required time for avulsed tooth replantation (44.3%) were aware of the critical time while many of female (71.8%) were not knowing about the time for avulsed tooth replantation (Figure 2).

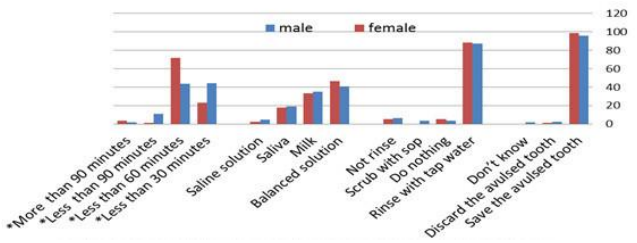

Figure 2: Comparison (Descriptive study) between male and female subjects

Half (50.0%) of specialists reported that less than 30 min is needed for the critical time for avulsed tooth replantation, while 59.3% of interns/G. Dentist advised less than 60 minutes. The difference was statistically significant regarding this point.

Regarding the best storage medium for transport of the tooth to the dentist, there was a significant difference between the specialists and G. practitioner. 50% of specialists were aware of the ideal solution to keep the avulsed tooth, Hanks balanced salt solution, but only 40% of general practitioners know that (Figure 3).

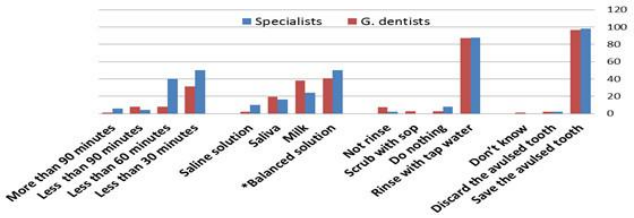

Figure 3: Comparison (Descriptive study) between specialists and G.Dentists

The percentage of specialists who think that we should hold the crown and wash with a physiological solution in tooth management before replantation was 66%, while 51.3% of general dentist think the same.

Discussion

This survey provided baseline information about the existing level of Awareness on the management of avulsed tooth among dentists working in Jazan, K.S.A.

The guidelines for the management of dental trauma published by the International Association for Dental Traumatology (IADT) and the American Academy of Pediatric Dentistry (AAPD) recommend the immediate replantation of a tooth to obtain the best prognosis. If the tooth cannot be replanted within 5minutes, it should be stored in a medium that will help maintain the vitality of the periodontal ligament fibers [2] [10] IADT and AAPD guidelines for the management of dental trauma state that the physiological transportation media for avulsed teeth include Hank's Balanced Salt Solution (tissue culture medium), saline, and cold milk [2] [11] [12].

In the present study, 50% of the dental specialist's participants suggested a balanced solution as the best transportation medium for avulsed teeth. 60% of the general dentist participants had no prior knowledge of the balanced solution. Also, the general dentist's participants preferred milk or saliva as storage media, indicating that they know milk and saliva are the most practical transport mediums for the storage of avulsed teeth because pH and osmolality of them are similar to those of extracellular fluid.

Andreason et al., [13] favour's milk as a storage medium as it maintains the vitality of periodontal ligament cells for up to 3 hours. The osmolality of milk is 232 most/l [14]. It has shown that milk can maintain the osmotic pressure of periodontal ligament cells, but cannot reconstitute cell metabolites and restore viability [8] [13] [15]. However, while milk may not be readily available at the site of trauma, storage of the avulsed tooth in milk at room temperature has been reported to preserve the viability of PDL cells for up to 60 min, whereas refrigerated milk preserves viability for an additional 45 min [16] [17].

Saliva was found to be more effective than tap water [18], and the tooth can be easily carried by the patient keeping in the buccal vestibule. But the patient's saliva, although readily available at the site of trauma, contains bacteria and their by-products [16]. The osmolarity of saliva is 60-80 most/ltr much less than the normal range (230-400 mosm/ltr) required for cell growth [8] [19]. Furthermore, several studies have reported that the vitality of PDL cells can be sustained for 30 min when immersed in the patient's saliva, but it decreases remarkably after 60 min [11] [16].

Only 10% of the specialist's participants preferred saline as storage media. Saline has been shown to be a short-term storage media because of its physiologic osmolarity. It was found that the avulsed teeth that were soaked in saline solution for 30 minutes before replantation showed less root resorption than those stored dry for 15-40 minutes [8] [20].

There was a divergent opinion among the participants regarding the ideal time for replanting the tooth. According to Andreason, teeth that are replanted within 30 minutes have a better success rate than those that were extra oral for longer periods of time before replantation [13].

Most of the participants were aware that the tooth should hold the crown and wash with physiological solution, but the other chose to have 32% of general dentist select to hold by the crown and cleaned gently by running tap water to avoid damage to the PDL cells.

In conclusion, a statistically significant result was observed regarding the best storage medium, a critical time for avulsed tooth replantation and tooth management before replantation. Majority of the dental specialists responded correctly to most of the questions according to the IADT guidelines. As general dentists form a vital link with the patient, they need to be educated on emergency management of avulsed teeth and their dilemma about some aspects of this procedure can be solved by providing required awareness and knowledge.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1716-1721.
https://doi.org/10.3889/oamjms.2018.373
eISSN: 1857-9655
Dental Science

Reasons for and Barriers to Attending Continuing Education Activities and Priorities for Different Dental Specialties

Muhammad Nazir[1*], Asim Al-Ansari[1], Mohammed Alabdulaziz[2], Yousif AlNasrallah[2], Murtada Alzain[2]

[1]Department of Preventive Dental Sciences, College of Dentistry, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia; [2]College of Dentistry, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

Citation: Nazir M, Al-Ansari A, Alabdulaziz M, AlNasrallah Y, Alzain M. Reasons for and Barriers to Attending Continuing Education Activities and Priorities for Different Dental Specialties. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1716-1721. https://doi.org/10.3889/oamjms.2018.373

Keywords: Continuing education activities; Dental specialities; Dentists

***Correspondence:** Muhammad Nazir. Department of Preventive Dental Sciences, College of Dentistry, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia. E-mail: manazir@iau.edu.sa

Received: 27-Jun-2018; **Revised:** 23-Aug-2018; **Accepted:** 24-Aug-2018; **Online first:** 22-Sep-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Continuing education (CE) activities help dentists update their knowledge and skills to ensure high standards of patient care.

AIM: This study aimed to evaluate the reasons for and barriers to attending CE activities including the priorities for different dental specialities.

METHODS: The study involves a cross-sectional research design. After statistical consultation, a questionnaire was distributed among 323 dental practitioners in the Eastern province of Saudi Arabia. The questionnaire was checked for face and content validity, and it was pilot-tested before its administration.

RESULTS: The response rate was 79.5% as 257 of 323 dentists returned the questionnaire. Most dentists reported that they attended CE activities because of personal learning needs (67.3%) and career development (66.9%). Lack of clinical experience to effectively manage patients and the practice was the least common (15.2%) reason for attending CE activities. Esthetic dentistry (77.4%), restorative dentistry (70.8%), and endodontics (70%) were the three most preferred dental specialities for CE activities. Lack of time was the most common (69.3%) barrier to attending CE activities followed by the cost (62.6%) and the distance (57.2%). More male (n = 104) than female (n = 69) dentists believed personal learning needs a reason for attending CE activities (P = 0.01). Similarly, more male (n = 104) than female dentists (n = 68) considered lack of time a barrier (P = 0.046).

CONCLUSION: Most dentists attended CE activities to fulfil their personal learning needs, and aesthetic dentistry was the most preferred dental speciality for CE activities. Lack of time and cost were important barriers to attending CE activities.

Introduction

In Dentistry, there are complex and wide-ranging oral conditions and issues, rapid flow of new scientific knowledge, development of latest technologies, advances in new drugs, ever-changing oral health care needs, and diverse demographics of the patients and their increased expectations of oral care services [1] [2] [3]. These challenges can be effectively addressed by updating the knowledge and skills of dentists and ensuring high-quality oral care throughout their career by attending continuing education (CE) activities [3]. Therefore, the understanding of dentists' opinions about their attendance of CE activities can help better plan and conduct future CE courses to improve dental practice and standards of patient care.

The distance from the place of CE activities, attendance-related expenses, type of CE courses, and instructional methods are important elements when planning CE activities [4]. The cost of continuing professional courses, the distance or the location of a course, the loss of work, and courses not relevant were the barriers to attending educational courses for general dentists [5]. It was found that increased

engagement and commitment in dental practice and the cost of the courses restricted dentists from participating in continuing professional development in Scotland [6]. The literature also pointed out the lack of motivation and interest of participants and the negative role of the employer as barriers to the participation in CE activities [7]. Evidence shows that female and rural dental practitioners had difficulty accessing CE activities in Australia [8]. Dentists working in hospitals were found to spend more time in continuing dental education [9]. Nevertheless, a mandatory statutory requirement by the professional or regulatory bodies is one of the leading reasons for the higher participation of dentists in CE meetings [10].

Al-Fouzan reported the perceived needs of general dentists for CE courses on different clinical topics in Saudi Arabia [11]. The respondents thought that they had the greatest learning needs in areas such as screening of oral carcinoma, management of medically compromised patients, and oral manifestations of systemic diseases. Chan et al. found that the dentists highly preferred cosmetic dentistry and implant dentistry courses for their continuing education [12]. Bailey et al. reported an agreement on three core components of CE activities which included medical emergencies, infection control and treatment of medically compromised patients [13]. Recently, Nayak et al. indicated that the majority of dentists preferred CE courses in esthetic dentistry and endodontics [14].

In Saudi Arathe bias, dentists are required to attend CE activities to maintain their licensures to practice dentistry, and they are offered different CE program throughout the year and across the country. However, the literature lacks data about the reasons for attending and barriers to participating in CE courses in the country. Also, information about dentists' preferences for CE activities about different dental specialities can help decision-makers better plan a CE program in the Eastern province of Saudi Arabia.

Therefore, the objective of the study is to report the reasons, the level of priorities for dental specialities, and barriers to CE activities among dentists.

Methods

This cross-sectional study was conducted on dental practitioners working in different cities of the Eastern province of Saudi Arabia. The major cities included Dammam, Al-Khobar, Dhahran, Al-Hasa while small cities/towns included Qatif, Sihat and Boqig. A sample of 323 dentists was calculated based on the confidence limit, the dentist population in the province, and the % frequency of the outcome in the population.

The instrument was developed by dental public health faculty members at the College of Dentistry Imam Abdulrahman Bin Faisal University. The content and face validity of the questionnaire were checked by faculty members during multiple meetings. The questionnaire specially designed for the present study was developed and reviewed by the faculty members. In subsequent meetings, modifications were made in the questionnaire by adding and deleting some items and improving the construction of some questions.

Demographic information included age and gender of the participants, whether they worked in the private or public sector, and whether they are general dentists, specialists or consultants. There were six items about the reasons for attending CE activities during the last one year. The questions about the level of priority for CE activities in different dental specialist used 4-point Likert response options.

The respondents were given a list of options of different dental specialities to choose from. Lack of time, the distance from participant's clinic to the place of CE activities, gender, the cost of CE activities (i.e., fee), travelling and accommodation expenses, the loss of earnings/income due to absence from the clinic, the lack of motivation and interest in CE activities and reduced availability of CE activities, and working in private and public dental hospital/clinic were the options given for barriers to attending CE activities. The respondents were asked to check all the options that apply to them. The questionnaire was pilot-tested on 30 dentists who did not participate later in the actual study.

Following the approval of the study by the Research Unit at the College of Dentistry, Iman Abdulrahman Bin Faisal University (ethics committee), the survey was distributed to the dentists working in government and private dental clinics. The self-administered questionnaire was administered by approaching the dentists in person in their clinics. The respondents who were willing to participate in the study received the hard copies of the questionnaire. The dentists who could not complete the questionnaire in the first visit were reminded in second and third visits. The second visit was performed about two-three weeks after the first visit to collect the completed questionnaires or to give a reminder.

Similarly, the dentists were revisited for the third time after two-three weeks for the collection of the questionnaire. These measures were taken to acquire satisfactory response rate and present valid results of the study. Ethical considerations were maintained during data collection as the dentists were provided with details and risks and benefits of the study. The respect for the person was ensured by asking respondents to provide verbal consent before filling out the questionnaires. The anonymous survey

maintained the confidentiality of respondents in the study.

All data were analysed using SPSS software (IBM SPSS Statistics for Windows, Version 22. Armonk, NY: IBM Corp). Descriptive analysis included calculating means and standard deviations of continuous data and percentages of categorical variables of the study. Gender differences in reasons for and barriers to attending CE activities were calculated by using Pearson's chi-square test. Statistical significance was determined using p-value < 0.05.

Results

Of 323 dentists, 257 returned completed questionnaires with a response rate of 79.5%. Mean age of study participants was 31.18 ± 12.4 years. There were 54.9% of male and 45.1% of female dentists in the study. More participants had jobs in private (65.4%) than the public sector (45.1%). The majority of participants were general dentists (65.8%), followed by specialists (31.1%) and then consultants (3.1%), Table 1.

Table 1: Demographic characteristics of study participants

Variables	N (%)
Gender:	
Male	141 (54.9)
Female	116 (45.1)
Type of job:	
Private	168 (65.4)
Public	89 (34.6)
Job Title:	
General dentist	169 (65.8)
Specialist	80 (31.1)
Consultant	8 (3.1)
Age	Mean ± SD
	31.18 ± 12.4

The personal learning need was the most commonly reported (67.3%) reason for attending CE activities. Similarly, a large majority of dentists (66.9%) reported that they attended CE activities because it was required for their career development. Almost half (48.6%) of the respondents believed that regulatory requirement by the licensing/regulatory body was the reason for their attendance. It was encouraging to see that a very small percentage of dentists (15.2%) felt the need to attend CE activities because they lacked the clinical experience to manage patients and the practice effectively.

Table 2: Reasons for attending CE activities

Variables	N (%)
Lack of clinical experience to effectively manage patients and the practice	39 (15.2)
Lack of a postgraduate degree or specialist training	62 (24.1)
Regulatory requirements by the licensing/regulatory body	125 (48.6)
Required for career development	172 (66.9)
Required for networking with dental professionals	110 (42.8)
Personal learning needs	173 (67.3)

Of the respondents, 77.4% agreed that they had a high/very high priority for CE activities about esthetic dentistry. Other highly/very highly rated dental specialities included restorative dentistry (70.8%), endodontics (70%) and prosthodontics (60.7%). Almost half the respondents gave high/very high priority for CE activities about preventive dentistry (56%), evidence-based dentistry (48.6%), periodontics (47.9%), and implant dentistry (44.7%). Among all the given specialities, 45.1% of the respondents gave low priority for CE activities about orthodontics. Thus, CE activities in orthodontics received the least importance in the study (Table 3).

Table 3: Level of priority for Continuing Education activities in different dental specialities

Specialities	Low N (%)	Medium N (%)	High N (%)	Very high N (%)
Oral Surgery	74 (28.8)	81 (31.5)	56 (21.8)	46 (17.9)
Orthodontics	116 (45.1)	74 (28.8)	35 (13.6)	32 (12.5)
Periodontics	58 (22.6)	76 (29.6)	70 (27.2)	53 (20.6)
Endodontics	37 (14.4)	40 (15.6)	87 (33.9)	93 (36.2)
Prosthodontics	47 (18.3)	54 (21.0)	76 (29.6)	80 (31.1)
Preventive Dentistry	60 (23.3)	53 (20.6)	79 (30.7)	65 (25.3)
Implants Dentistry	74 (28.8)	68 (26.5)	52 (20.2)	63 (24.5)
Restorative Dentistry	36 (14.0)	39 (15.2)	85 (33.1)	97 (37.7)
Esthetic Dentistry	33 (12.8)	25 (9.7)	78 (30.4)	121(47.1)
Evidence-based Dentistry	48 (18.7)	84 (32.7)	66 (25.7)	59 (23.0)

Lack of time, cost, and the distance to CE activities were the most commonly reported barriers to CE activities. In the study, 69.3% considered lack of time a barrier to attending CE activities. Similarly, a majority of respondents (62.6%) believed that they faced difficulties in attending CE activities due to their costs. Further, more than half (57.2%) thought that distance to the place of CE activities was an obstacle for their attendance. On the other hand, working in the public hospital/clinic was the least reported barrier (16.7%) to CE activities (Table 4).

Table 4: Barriers to Continuing Education activities

Variables	N (%)
Lack of time	178 (69.3)
Distance	147 (57.2)
Gender	15 (5.8)
Cost of attending CE activities	162 (62.6)
Travelling and accommodation expenses	135 (51.8)
Loss of earnings/income due to absence from the clinic	73 (28.0)
Lack of motivation and interest	47 (18.3)
Reduced availability of CE activities	97 (37.7)
Working in a private hospital/clinic	77 (30.0)
Working in a public hospital/clinic	43 (16.7)

Data were compared between male and female participants regarding reasons for and barriers to attending CE activities. It can be seen that more male dentists (59%) than female counterparts (41%) considered lack of time a barrier (P 0.046).

More male (n = 104) than female (n = 69) dentists thought personal learning needs a reason for attending CE activities (P = 0.01). Similarly, CE activities required for career development was recognized by more male (n = 104) than female (n = 68) dentists (P = 0.01). No other reasons and barriers significantly differed between male and female respondents (Table 5).

Table 5: Gender differences in reasons for and barriers to attending CE activities

Variables	Male N (%)	Female N (%)	p-value
Reasons for attending CE activities			
Lack of clinical experience to effectively manage patients and the practice	18 (46.2)	21 (53.8)	0.23
Lack of a postgraduate degree or specialist training	31 (50)	31(50)	0.38
Regulatory requirements by the licensing/regulatory body	68 (54.4)	57 (45.6)	0.88
Required for career development	104 (60.5)	68 (39.5)	0.01*
Required for networking with dental professionals	60 (54.5)	50 (45.5)	0.93
Personal learning needs	104 (60.1)	69 (39.9)	0.01*
Barriers to attending CE activities			
Lack of time	105 (59.0)	73 (41.0)	0.04*
Distance	76 (51.7)	71 (48.3)	0.24
Gender	7 (46.7)	8 (53.3)	0.51
Cost of attending CE activities	85 (52.5)	77 (47.5)	0.31
Travelling and accommodation expenses	68 (50.4)	67 (49.6)	0.13
Loss of earnings/income due to absence from the clinic	45 (61.6)	28 (38.4)	0.17
Lack of motivation and interest	27 (57.4)	20 (42.6)	0.69
Reduced availability of CE activities	55 (56.7)	42 (43.3)	0.64
Working in private hospital/clinic	43 (55.8)	34 (44.2)	0.84
Working in public hospital/clinic	25 (58.1)	18 (41.9)	0.64

* Statistically significant.

The respondents were asked to provide their opinions about their preferred method of delivery of CE activities. The mixed method (a combination of didactic and interactive methods) was the most preferred method (81%) of providing information in CE courses (Figure 1).

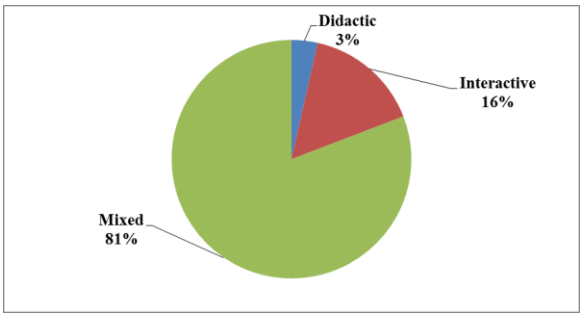

Figure 1: Preferred methods of delivery CE activities

Discussion

Our study demonstrated that the majority of the participants preferred attending CE activities to satisfy their personal learning needs. The esthetic dentistry received the highest priority for CE activities in the study. However, most of them reported lack of time as the most important barrier to the attendance of CE activities. The decision makers in dental academia should understand the current trends in dental practice and align dental curricula with ongoing demands and learning needs of dental practitioners.

There are claims about the importance of CE activities in lifelong learning and personal development [15]. It is also known that the effective organisation of CE activities can satisfy the learning needs of professionals [16]. In the present study, personal learning need was the most common reason of attending CE activities and significantly higher percentage of male than female dentists thought personal learning need a reason of their attendance (P = 0.01). A previous study indicated that improvement in knowledge was the most common reason for CE activities [17]. Our study provides evidence that CE activities can contribute to the lifelong learning and personal development of dental professionals. The role of lifelong learning is understood because of its importance in ensuring professional competency, providing optimal patient care and advancing the career [18]. It is possible that the satisfaction of personal learning needs of the participants reflects their motivation for lifelong learning.

Another explanation for high personal learning needs in our study is that most of the participants might not be knowledgeable and skilled in certain areas and felt the need to improve their knowledge and skills by attending CE activities. Whether dentists attended CE activities as lifelong learners or they were less skilled and needed to acquire new skills, in both cases the findings of this study lend credibility to the importance of CE activities in enhancing the learning process for practising dentists. Continuous emergence of new knowledge, skills and technologies in addition to the influences of political and economic forces on dental practice require a dental professional to attend CE activities to keep pace with current and future trends and demands of the dental professionals. Moreover, our findings suggest the role of CE activities in promoting career development among dentists.

In the present study, a large majority of dentists (77.4%) had a preference for CE courses in esthetic dentistry. Similar findings were reported in a previous study where most dental practitioners preferred esthetic dentistry [14]. Likewise, it was also found that 60.1% of dentists considered cosmetic dentistry as a preferred course for CE activities [12]. Implant dentistry was not the most popular preference (44.7%) of dental practitioners in the present study which is contrary to the findings of a previous study where dentists chose oral implantology as the most common preference for CE activities [12].

A high preference for CE activities related to esthetic dentistry is expected as there is public demand for dental procedures such as porcelain laminates, composite restorations, bleaching techniques etc. These dental treatments are aimed at esthetic improvement commonly requested by the patients. Therefore, dentists need to upgrade their skills to meet the aesthetic needs of their patients. In addition to esthetic dentistry, restorative dentistry and endodontics were second and third most commonly preferred dental specialities for CE activities in the present study. A previous study also reported that restorative dentistry and endodontics were the most

common subjects for CE activities [10]. In our study, high preference of participants in these disciplines reflects increased demand for learning the skills in these specialities which could be because of lack of training, and or introduction of new materials and techniques in these areas.

Significantly higher number of male than female respondents declared that lack of time was one of their barriers to CE activities in our study. These findings are conceivable as more male would choose to work fulltime than the female who opt working part-time due to family responsibilities and commitments [19]. Therefore, male dentists being more involved in dental practice are less likely to afford the time to attend CE activities.

A greater percentage of dentists (30%) judged working in private clinics as a barrier than working in public hospitals (16.7%). One plausible explanation for this phenomenon is that dentists make their earnings by providing treatment to patients and more patients they treat more money they earn in a private dental clinic. On the other hand, the dentists earn monthly salaries in the public sector. Therefore, the loss of income in private dental set up may explain why working in a private dental clinic is considered a barrier to CE activities. Also, the negative role of an employer in dental practice can account for reduced CE activities [7]. Somewhat similar findings were reported by Poyzois et al., who indicated that dental practitioners from hospital spent more time on CE activities [9]. Again, a dentist working in a big organisation like a hospital than a dental clinic may afford to spare time for CE activities due to the policies and regulations of the organisation that may encourage professional development. Opposite may be true in small private dental clinics. In line with previous studies, the cost was a common barrier to attending CE activities [6] [14] [17]. Lack of time was the most common barrier to attending CE activities and more male than female dentists found it difficult to attend CE activities due limited time in our study. These findings are in agreement with the results of a previous study [14].

There is a wide variety of CE programs in the dental profession which involves different types of instructional methods and different modes of dissemination of scientific information [20]. Interactive educational methods which include team-based learning and case-based learning have been found more effective than didactic methods in significantly improving short-term knowledge. Consequently, it resulted in high acceptance and great satisfaction among the participants and improved the indicators of their professional behaviours [21]. Khan and Coomarasamy reported that didactic teaching only increased knowledge whereas interactive educational workshops improved skills, attitudes and behaviours in clinical practice [22]. Davis et al. found that didactic CE sessions did not positively influence the clinical performance of caregivers [23]. O'Brien et al. also observed that interactive CE workshops resulted in a large change in the clinical practice whereas didactic sessions did not bring a considerable change in clinical practice [24]. In our study, a vast majority of dentists believed that mixed method which is a combination of didactic and interactive style is the most preferred methods of delivery of CE courses.

Although, the study was conducted using a calculated sample of dentists with satisfactory response rate from different cities of the Eastern province of Saudi Arabia, however, the results should be generalised to other regions of the country with caution. Also, one of the potential limitations of the self-administered questionnaire is socially desirable responses. But, the attempts were made to minimise this problem by assuring privacy and confidentiality of the participants' responses by giving them an anonymous questionnaire.

In conclusion, this study found different reasons for attending CE activities with personal learning needs and career development being the most common. CE activities related to esthetic dentistry, conservative dentistry and endodontics were highly demanded by the study participants. A majority of dentists thought that the lack of time, cost and distance were important barriers to attending CE activities. Significantly higher percentage of male than female dentists considered lack of time a barrier to CE activities.

The quantitative data analysis in the study can help the organisers of CE activities better understand different aspects of CE activities to plan and organise such programs and overcome their barriers effectively. The decision makers in dental institutions including dental faculty can utilise the findings of this study to focus on the topics of certain dental specialities where responding dentists felt the need for greater learning. This will provide an evidence base to continuously improve dental curricula to match the current market demands of the dental profession and learning needs of practising dentists with the knowledge and skills provided to dental students.

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https://doi.org/10.3889/oamjms.2018.345
eISSN: 1857-9655
Dental Science

Knowledge and Awareness towards Occupational Hazards and Preventive Measures among Students and Dentists in Jazan Dental College, Saudi Arabia

Redhwan A. Al-aslami[1*], Fahiem M. M. Elshamy[2], Essamet M. Maamar[2], Ali Y. Shannaq[2], Abdulaziz E. Dallak[2], Abdulmohssen A. Alroduni[2]

[1]Department of Conservative Dentistry, College of Dentistry, Jazan University, Jazan, Saudi Arabia; [2]College of Dentistry, Jazan University, Jazan, Saudi Arabia

Citation: Al-aslami RA, Elshamy FMM, Maamar EM, Shannaq AY, Dallak AE, Alroduni AA. Knowledge and Awareness towards Occupational Hazards and Preventive Measures among Students and Dentists in Jazan Dental College, Saudi Arabia. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1722-1726. https://doi.org/10.3889/oamjms.2018.345

Keywords: Occupational hazards; Dental professionals; Infection control

***Correspondence:** Redhwan A Al-aslami. Department of Conservative Dentistry, College of Dentistry, Jazan University, Jazan, Saudi Arabia. E-mail: drredhwanaslami@gmail.com

Received: 31-May-2018; **Revised:** 25-Aug-2018; **Accepted:** 28-Aug-2018; **Online first:** 23-Sep-2018

Funding: This research did not receive any financial support

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

Abstract

AIM: The present study was aimed to assess the level of awareness and occupational hazards of dental students and professionals who are working at dental college and hospital, Jazan University, Saudi Arabia.

MATERIAL AND METHODS: Data has been collected through a self-administrated questionnaire with closed questions. The questionnaire consisted of questions on personal information like age, gender, years of experience and awareness of occupational hazards, seminar attendance, safety measures practised, and experience of occupational hazard while in practice. Participants were asked to answer each questionnaire item as “yes” or “no”. The data were analysed with SPSS-Version and P-value was generated.

RESULTS: Out of the 200 participants, 38.5% were females, and 62% were males. Differences were found significantly high between male and female subjects regarding neck shoulder pain, hand wrist pain, backache, and anxiety and routine use of a surgical cap ($P < 0.05$). Over 33% of the participants had attended workshops or conferences about the occupational hazards. 12.85% participants reported latex allergy and 74% of the participants vaccinated against Hepatitis B. Significant differences were found in the attitude of general dentists towards the preventive measures against the occupational hazards based on years of experience.

CONCLUSION: The findings of this study are consistent with the previous studies. Specific psychological concerns related to the conditions and the environment of the practice at dental college and hospital, Jazan University were expressed because the majority of participants were students.

Introduction

Occupational hazard refers to risk or danger as a consequence of the nature of the working conditions of a particular job. The history of occupational hazards awareness can be traced back to 18th century when Bernardino Ramazzini, who is referred to as the father of occupational medicine, recognised the role of occupation in dynamics of health and diseases [1] [2] [3].

During dental work, dentists are usually exposed to some occupational hazards [4][5]. These hazards include Infectious hazards; these associated with risk of exposure to various micro-organisms during a dental procedure such as injury through a needle created while imparting anaesthesia, or an accidental biting by the patient [4] [6]. Also, an indirect infection can occur through exposure to aerosols of saliva, gingival fluid, and natural organic dust particles [4] [7] [8].

Allergic reaction from gloves containing latex is the main causes of the allergic skin irritation. Dental materials, detergents, lubricating oils, solvents, and X-ray processing chemicals represent chemical hazards

[8] [9]. Dentists are at risk of physical hazards during dental work. Eye fatigue, eye pain may occur due to poor illumination. Moreover use of suction, high-speed turbines and ultrasonic scalar results in temporary or permanent hearing loss. Musculoskeletal disorders are a common health problem among dentists [4][6]. Stress, professional burnout, anxiety and depression are common psychological hazards that can occur during dental work [10] [11].

Prevention of occupational hazards in the workplace is central to the practice of occupational health as a profession [6], Therefore, this study aimed to assess the level of awareness of the occupational hazard in addition to preventive measures undertaken by dental students working in dental clinics, college of dentistry, Jazan University in Saudi Arabia.

Material and Methods

A cross-sectional study was performed in Dental clinics at a dental college, Jazan University, K.S.A. All the students and dentists attending the clinics were invited to participate in the study. Data was collected through a self-administrated questionnaire with closed questions. To assess the level of awareness, and occupational hazards experienced.

The questionnaire used in the present study contain questions on personal information like age, gender, years of experience and awareness to occupational hazards, seminar attendance, safety measures practised, and experience of occupational hazard while in practice. Also, participants will be asked to answer each questionnaire item as “yes” or “no”. The questionnaires were delivered by hand to each participant, and an explanation will be given about the importance of their participation and the study purposes.

The statistical analysis was performed using Statistical Package for Social Sciences version 21 (SPSS, Illinois, Chicago, USA). Descriptive analysis was carried out by mean of Chi-square test to evaluate association existing between time since graduation and post-graduation training. The significance level was set at $P < 0.05$.

Results

All participants demonstrated quite good awareness and attitude towards the occupational hazards and the risk factors at work-place. Out of the 200 participants, 38.5% were females, and 62% were males. The mean age of the study participants was 21-40 years.

Gender differences were found highly significant regarding neck shoulder pain (0.003), hand wrist pain (P-value = 0.007), backache (0.007), anxiety (0.005), routine use of a surgical cap (0.000). As shown in Figure 1.

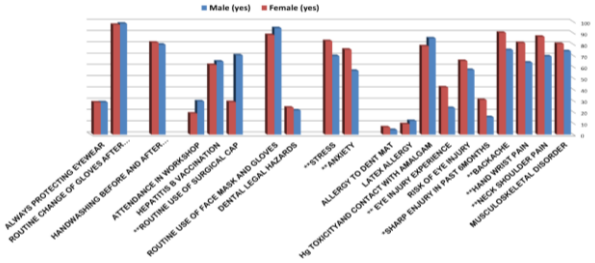

Figure 1: Frequency rates (%) of different items of occupational hazard knowledge among male and female combined subjects

The most prevalent preventive practices reported by the participants were shown in Figure 2. The use of a face mask (94.4%) and changing gloves between the patients (98.4%). However, only 33% of the participants had attended workshops or conferences on occupational hazards. It is significant to notice that 12.85% participants reported latex allergy in the current study and 74% of the participants were vaccinated against Hepatitis B.

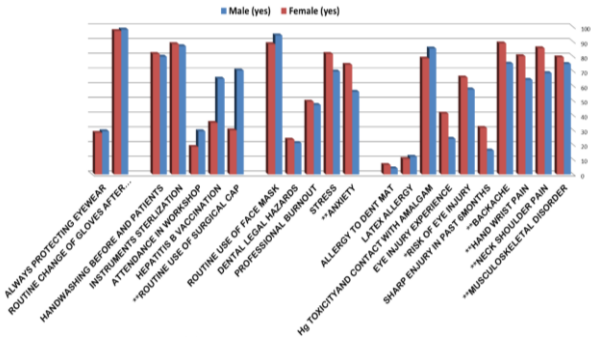

Figure 2: Frequency rates (%) of different items of occupational hazard knowledge among male and female students who said “Yes.”

By using a chi-square test, a significant *p*-value was found for musculoskeletal disorder (0.030), neck shoulder pain (0.018), hand wrist pain (0.003), backache (0.019), sharp injury in past 6months (0.009), risk of eye injury (0.046), eye injury experience (0.033), Hg toxicity and contact with amalgam (0.047), psychological hazard(0.000), professional burnout (0.000), Hepatitis B vaccination (0.000), and attendance in workshop (0.001) demonstrating high significant differences between general dentist and dental Students as shown in Figure 3.

There were significant difference items of occupational hazard knowledge according to years of experience of the general dentist for preventive

measures undertaken against occupational hazards at the workplace. It is significant to notice that only one highly significant at risk of eye injury (0.001), and the remaining not high significant stress (0.029), professional burnout (0.017) and hand washing before patients (0.021).

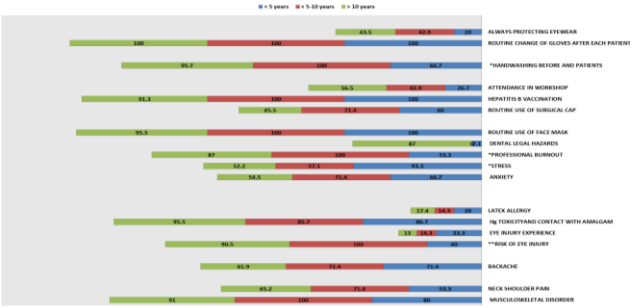

Figure 3: Frequency rates (%) of different items of occupational hazard knowledge according to the years of experience of specialists

Discussion

The present study is a cross-sectional study which was conducted among dental professionals working in dental clinics, college of dentistry, Jazan University in Saudi Arabia (K.S.A.). It was the first study to be undertaken in this place. The study examined the knowledge regarding occupational hazards. In the present study 123 male, 77 female dentists were aware of occupational hazards which show between both genders regarding occupational hazards.

In this study female was more aware of an ergonomic effect than males, this finding because highly significant of female related to neck shoulder pain, hand wrist pain and backache our study not agreement with the study of Ananya et al., [9] that find male were more aware of an ergonomic effect than females.

Khatib et al., [5] identified stress as the major occupational hazard, which coincides with the international data indicating that dentists perceive their profession as highly stressful [6] [12] [13]. Our study agrees with this finding, but the present study showed that majority of the female dentist was aware of anxiety and stress with a P value < 0.005; no other study has been conducted to support this results.

In the present study 70% males were more aware of routine use of surgical cap than 28% females which is highly statistically significant with P value 0.000, According to our findings this related to the traditions of the Kingdom community that includes wearing hijab and niqab that lead to no need to wear the surgical cap direct to the head or above to the hijab, unfortunately, no other study has been conducted to support this results.

The results of the present study have demonstrated that the majority of dentists had experienced physical health problems. A similar situation was reported in the Lithuanian and Netherlands where one out of ten dentists indicated having poor general health and three out of ten poor physical health [14] [15] [16].

Students present in this study and dentists had had a musculoskeletal disorder, neck shoulder pain, hand wrist pain and Backache. According to our findings, physical disorders and other illnesses appear early in dental careers. In the USA, more than 70 of the dental students of both sexes reported pain by their third year [17]. A study in Turkey shows a high prevalence of pain among dental male and female students: headache (34%, 22%), neck pain (67%, 43%), back pain (56%, 47%), upper limb pain (46%, 43%) and shoulder pain (78%, 58%) respectively) [16] [17]. These facts are daunting and emphasise the importance of paying attention to occupational health knowledge gained during school years, to be actively concerned about ergonomics and early diagnosis and treatment of these profession-related disorders.

In Lithuanian, neck shoulder pain, back pain and hand wrist pain disorders have previously been reported at a higher frequency that hand and wrist complaints [16]. In the Netherlands, 29% of dentists reported symptoms of peripheral neuropathy in the upper limbs or neck [18]. Regarding this conditions, back pain and neck pain were the most prevalent of all physical disorders, suggesting that the back region of dentists may be most affected by constant strain [16].

Concerning preventive measures, all of the dentists mentioned barrier techniques. Wearing protective clothing was standard procedure for all the respondents. This is in agreement with the results of a study conducted in Saudi Arabia in which only 2%-4% of dental professionals never wore gloves when treating patients [19]. The situation is almost the same in Canada: in 1994 it was found that 91.8% of dentists in Ontario, always wore gloves, 74.8% always wore masks, and 83.6% always wore eye protection [20]. A study conducted by Morris et al. showed that about 90% of dentists in Kuwait wore gloves, 75% wore masks and 52% wore eyeglasses [21]. In the Irish study, 42.0% of dentists wore gloves, 64.8% wore masks and 66.4% wore eye protection [6] [22].

The dentist's level awareness of occupational hazards was remarkably high. However, the high level of awareness was not reflected in the number of dentists that had attended workshops on occupational hazards, only 46.5% had attended workshops on occupational hazards. Education is one of the important strategies for the prevention of occupational injuries and diseases. Although the majority of the participants were aware they were at risk for exposure to injuries from sharps and hepatitis B infection, not all

of them (students) were vaccinated against hepatitis B infection despite the high risk to the entire dental team [3].

It is interesting to note 93% dentists compared with only ≈ 64% students had been vaccinated. This is probably a reflection of the attitudes of students who might feel they are at less risk of exposure. It is desirable that all the students be vaccinated properly against Hepatitis B infection because of the risk of body fluid-borne infection. This is corroborated by the fact more than a quarter of the students had experienced an injury by a sharp object in the past six months.

However, it was discovered in our study58%of the students use protective eyewear while attending to patients. The use of protective eyewear is an important means of preventing occupational injury related to the use of dental curing lights and high-speed rotary instruments, injury from splatters and projectiles including calculus and flying debris during cavity preparation is a common cause of damage to the eyes, and the use of protective eyewear should be emphasized [3] [23] [24].

Approximately 93% of the dentists and 81% of students were in regular contact with amalgam also the level of students 81% this is also high for students who have regular contact with amalgam. All dental personnel in the dental college should be alerted to the risk of mercurial poisoning [3]. It is advisable to conduct regular mercury vapour level assessments in clinical settings; receive episodic individual amalgam blood level tests; and use goggles, water spray, and suction during the removal of old amalgam restorations.

Majority of 5th years' experience dentists (93.3%) aware the effect of the stress than a 10th year and < 10th years dentists. May we agree with Al-Khatib et al., [5] who observed that stress is related to financial status. The increasing number of graduates that enter the profession each year in an area as confined as Jazan city leads to fierce competition and economic instability among dentists; this was mentioned as a further source of economic stress [6]. In the present study 53.3% of < 5 years surfing from professional burnout which is significantly Lowe, in contrast, the > 10 years' experience 87% surfing.

In conclusion, the dentists working at dental clinics, college of dentistry, Jazan University in K.S.A, is similar to the international one with a greater emphasis on the physical hazards. Specific psychological concerns related to the characteristics of the working at dental college and hospital, Jazan University were expressed because the majority of participants were students.

There is no doubt that the potential occupational health hazards in dentistry are great. Therefore, occupational hazard awareness among dentist working at dental clinics, college of dentistry enhance in motivating and planning preventive strategies at the training and teaching level. If the knowledge of preventive practices is imparted during the curriculum, it will result in the use of the effective practice of dentistry in future.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1727-1731.
https://doi.org/10.3889/oamjms.2018.368
eISSN: 1857-9655
Dental Science

Nano Hydroxyapatite & Mineral Trioxide Aggregate Efficiently Promote Odontogenic Differentiation of Dental Pulp Stem Cells

Ahmed Khaled Hanafy[1*], Souzy F. Shinaishin[2], Ghada Nour Eldeen[3,4], Riham M. Aly[5,6]

[1]Department of Oral Biology, Faculty of Dentistry, Egyptian Russian University in Cairo, Egypt; [2]Department of Oral Biology, Faculty of Dentistry, Ain Shams University, Cairo, Egypt; [3]Department of Molecular Genetics and Enzymology, National Research Centre, Cairo, Egypt; [4]Stem Cell Research Group, Medical Research Centre of Excellence, National Research Centre, Cairo, Egypt; [5]Basic Dental Science Department, Oral & Dental Research Division, National Research Centre, Cairo, Egypt; [6]Stem Cell Laboratory, Centre of Excellence for Advanced Sciences, National Research Centre, Cairo, Egypt;

Citation: Hanafy AK, Shinaishin SF, Eldeen GN, Aly RM. Nano Hydroxyapatite & Mineral Trioxide Aggregate Efficiently Promote Odontogenic Differentiation of Dental Pulp Stem Cells. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1727-1731. https://doi.org/10.3889/oamjms.2018.368

Keywords: Dental Pulp Stem Cells; Odontogenic Differentiation; Mineral trioxide aggregate; Nano hydroxy-apatite

***Correspondence:** Ahmed Khaled Hanafy. Department of Oral Biology, Faculty of Dentistry, Egyptian Russian University in Cairo, Egypt. E-mail: ahmed70884@gmail.com

Received: 20-Jun-2018; **Revised:** 18-Aug-2018; **Accepted:** 19-Aug-2018; **Online first:** 23-Sep-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: There has been an urge to shift from conventional therapies to the more promising regenerative strategy since conventional treatment relies on synthetic materials to fill defects and replace missing tissues, lacking the ability to restore the tissues' physiological architecture and function.

AIM: The present study focused on the assessment of the role of two commonly used biomaterials namely; mineral trioxide aggregate (MTA) and nano hydroxy-apatite as promoters of odontogenic differentiation of dental pulp stem cells (DPSCs).

METHODS: DPSCs were isolated, cultured in odontogenic media and divided into three groups; control group, MTA group and nanohydroxyapatite group. Odontogenic differentiation was assessed by tracing genes characteristic of different stages of odontoblasts via qRT-PCR. Calcific nodules formation was evaluated by Alizarin red staining.

RESULTS: Results demonstrated that both MTA and nanohydroxyapatite were capable of enhancing odontogenic differentiation of DPSCs.

CONCLUSION: Nano hydroxyapatite was found to have a higher promoting effect. However, in the absence of an odontogenic medium, MTA and nanohydroxyapatite could not enhance the odontogenic differentiation of DPSCs.

Introduction

Regenerative and cell-based therapies represent a promising alternative to conventional therapies to maintain pulp vitality and to avoid the more extensive treatment dictated by extraction or endodontic therapy. Mineral Trioxide Aggregate (MTA) and nano hydroxyapatite are promising materials which have wide clinical and regenerative uses. Different studies performed on MTA, showed that it plays an important role in regenerative endodontics, mainly through the activation of cementoblasts and the formation of cementum [1]. MTA was also reported to play a role in stimulation of the odontogenic differentiation of stem cells [2] and the induction of stem cell proliferation with excellent biocompatibility [3]. Another material that is widely used is nano hydroxyapatite, due to its particle size that is close to the apatite crystals present naturally in human mineralised tissue which is useful when used as a scaffold material in tissue engineering [4]. It also presents superior properties regarding enhancing stem cells proliferation and differentiation into osteoblasts resulting in bone formation [5]. Moreover, nano hydroxyapatite plays a remarkable role in guided tissue regeneration to enhance bone regeneration in the field of periodontology [6].

In this study, the role of MTA and nano hydroxyapatite as promoters of odontogenic differentiation is assessed on dental pulp stem cells.

Material and Methods

Dental pulp stem cells isolation and culture

Human dental pulp tissues were obtained from three impacted third molars. Teeth were collected from patients aged from 16–26 years. Extraction of teeth was performed in the department of Oral Surgery Department at Ain Shams University clinics under the approval of its ethics committee. The extirpated pulp tissues were minced into small pieces and digested with 2mg/mL collagenase type I (Serva) fa or 30 minutes at 37°C. Cell suspensions were cultured in 6-cm dishes in high-glucose Dulbecco modified Eagle medium (Lonza, Belgium) supplemented with 10% fetal bovine serum (FBS, Invitrogen) and antibiotics (100 U/mL penicillin and 100 mg/mL streptomycin) at 37°C in 5% CO_2. Passaging was performed when adherent cells reached 70% confluence.

Induction of odontogenic differentiation

To induce odontogenic differentiation, DPSCs from the third passage were seeded into 6-well plates at a density of $1x10^5$/well. Odontogenic differentiation was performed for 21 days by culturing DPSCs in odontogenic differentiation medium containing DMEM medium supplemented with 15% FBS, 10 mol/L b-glycerophosphate (Sigma-Aldrich), 0.2 mmol/L ascorbate-2-phosphate (Sigma-Aldrich), and 100 nmol/L dexamethasone (Sigma-Aldrich). DPSCs were divided into three groups according to the media and biomaterial used as follows; 1) Controls; positive controls were grown in odontogenic media with 15% FBS whereas negative controls were grown in DMEM with 15% FBS. 2) MTA group; which was supplied with MTA powder (Angelus, Brazil) and odontogenic medium (as above mentioned). For MTA preparation, a concentration of 0.02 mg/ml was implemented according to previous protocols [7]. Briefly, 0.02 mg MTA was dissolved in 1ml of odontogenic media and vortexed until suspended. The suspension was then left to settle for 10 minutes followed by 24 hours incubation in 37°C and 5% CO_2 to extract the bioactive contents of MTA. The resultant supernatant from this preparation was used to treat cells every other day with for 3 weeks. Finally, nano hydroxyapatite group; which comprised nano hydroxyapatite (Sigma–Aldrich, UK) at a concentration of 10 µg/mL suspended in odontogenic media [8]. For all groups media was changed three times a week for 21 days.

Real time RT-PCR analysis for odontogenic gene expression

After 3 weeks of odontogenic induction of the 3 groups of cells (nano hydroxyapatite, MTA and control groups). Total RNA was isolated using PureLink RNA Min Kit (Invitrogen, http: //www.invitrogen. com). All RNA samples were checked for purity using a ND-1000 spectrophotometer (Nano Drop Technologies, Wilmington, DE, USA). Total RNA samples were reverse transcribed into cDNA using RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher) and quantitative real time PCR was performed using 1 µg of cDNA and Maxima SYBR Green qPCR master mix (Thermo Fisher) in a LightCycler® 480 Instrument (Roche life science). Real-Time Quantitative Polymerase Chain Reaction Analysis Differential expressions of five odontogenic genes; Alkaline Phosphatase (*ALP*), Osteopontin (*OPN*), *RUNX2*, Osteocalcin (*OCN*) and *collagen1* was carried out. The primer sequences are provided in (Table 1). Samples were run twice. The raw data were then analyzed with the Relative expression software tool (REST) using the automatic cycle threshold (Ct) setting to assign baselines and thresholds for the Ct determination. Delta Ct ($\Delta\Delta$CT) values were used for this analysis. The relative expressions (REs) of the sample genes were calculated using the $\Delta\Delta$CT method and GAPDH was used as the internal control or housekeeping gene. q RT-PCR experiments were carried out at least three times. Data were presented as the average values ± SEM (standard error of the mean). Statistical significance was analyzed with paired Student t-test. Significance levels or P-values are indicated in the figure legends.

Table 1: Primer sequences for quantitative real-time PCR analysis

Gene	Forward	Reverse
Runx2	5'-AAGTGCGGTGCAAACTTTCT-3'	5'-TCTCGGTGGCTGCTAGTGA-3'
Osteocalcin	5'-TCA CAC TCC TCG CCC TAT TG-3'	5'-TCG CTG CCC TCC TGC TTG-3'
ALP	5'-AGC TGA ACA GGA ACA ACG TGA-3'	5'-CTT CAT GGT GCC CGT GGT C-3'
Collagen1	5'-ACC GCC CTC CTG ACG CAC -3'	5'- GCA GAC GCA GAT CCG GCA G-3'
Osteopontin	5'- AAGGCGCATTACAGCAAACACTCA	3'- CTCATCGGACTCCTGGCTCTTCAT
GAPDH	5'- ACCACAGTCCATGCCATCAC	3'- TCCACCACCCTGTTGCTGTA

Alizarin Red Staining

To assess in vitro mineralization, cells were washed twice with phosphate-buffered saline, fixed with 4% paraformaldehyde (Sigma- Aldrich) for 1 hour, washed with deionized H_2O, and stained with 1% Alizarin Red S (Sigma-Aldrich) for 20 minutes. They were then rinsed 3 times with deionized H_2O, the mineralized nodules were observed under inverted light microscope (Leica, 6000B-4) using Suite V3 (Leica).

Results

Isolation of human dental pulp stem cells (DPSCs)

Human dental pulp stem cells (DPSCs) were successfully isolated from pulps of extracted third molars. The cultured DPSCs were observed on a regular basis using an inverted light microscope (Figure 1). After 24 hours of isolation, DPSCs began to develop processes assuming a spindle and stellate shape which is the typical appearance of mesenchymal stem cell and attached to the bottom of the culture dish. Approximately ten days follow ing t, the initial isolation, cells reached 70% confluence. At this stage, cells were passaged to passage three where differentiation was initiated.

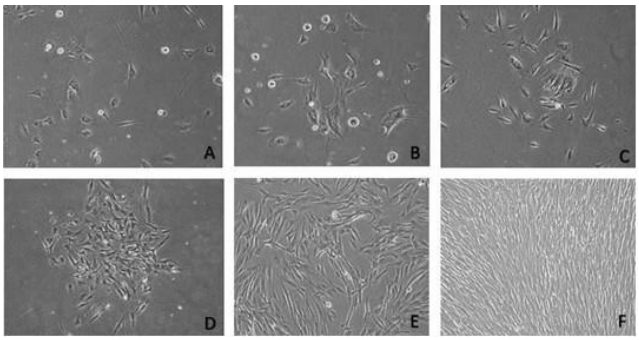

Figure 1: Isolation and morphological observation of human dental pulp stem cells (DPSCthe s) be the fore induction of differentiation by phase contrast microscopy; (A) DPSCs after 24 hours from isolation; (B) Isolated DPSCs with different morphological appearances following isolation; (C) DPSCs assuming colonies on day 3 of isolation; (D) DPSCs on day 5 of isolation showing increase in colony size with increase in cell number; (E) DPSCs a week after isolation; cells are approaching confluence; (F) DPSCs reaching 80% confluence 10 days after isolation (Magnification 100X)

Successful induction of odontogenic differentiation of DPSCs

After applying the odontogenic medium for differentiation, DPSCs were observed on a regular basis for morphological alterations. During the first week after induction, DPSCs which have reached confluency began to change their spindle-shape developing into rounded cells which gathered to form clusters. Rounded aggregates of DPSCs appeared as a result of cell migration chiefly from the periphery toward the centre of the plates (Figure 2).

On day 21, staining with Alizarin Red was done. Orange-red nodules indicating the beginning of mineralisation was demonstrated (Figure 3B).

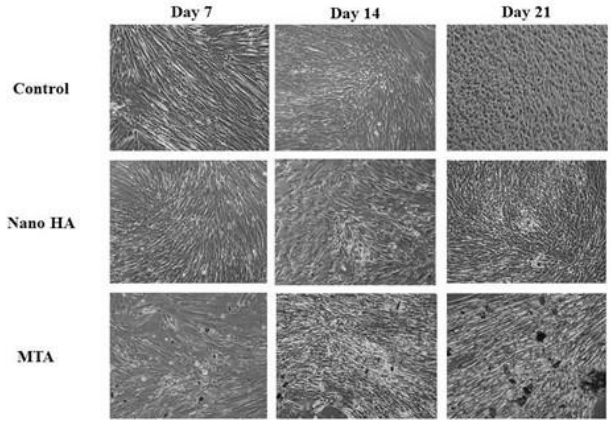

Figure 2: Morphological observation of DPSCs during odontogenic differentiation; Photomicrographs illustrating the morphological appearance of DPSCs while cultured either in odontogenic differentiation media supplemented with nano-hydroxyapatite (NanoHA) or MTA over a p,period of 21 days. DPSCs gradually transformed from spindle /stellate shape into a more rounded polygonal morphology. However, there was an apparent gradual decrease in cell number in the MTA group (Magnification 100X)

MTA and nano hydroxyapatite promoted odontogenic differentiation

Gene expression and statistical analysis were performed to assess the differential expression of five odontogenic markers in cells differentiated with either MTA or nano hydroxyapatite compared with control counterparts. The studied genes were ALP, *OPN, RUNX2, OCN,* and *collagen1* genes (Figure 3A)**.**

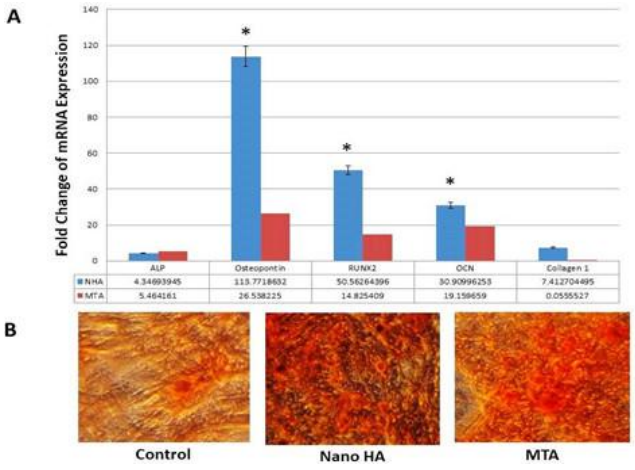

*Figure 3: Evaluation of odontogenic differentiation capacity of DPSCS cultured under different conditions; (A) Expression of genes characteristic of odontogenic differentiation; Alkaline Phosphatase (ALP), Osteopontin, RUNX2, Osteocalcin (OCN), Collagen1 in nano-hydroxyapatite (NHA) and MTA groups. GAPDH was used as housekeeping gene. The data were analysed by qRT–PCR and compared with positive control, i.e. cells cultured in odontogenic media only. Results are presented as mean ± SEM of three independent experiments (n = 3). *P < 0.05; (B) Alizarin Red staining was used to determine mineral nodule formation on day 21 in all groups*

Using q RT-PCR**,** we detected the expression of all studied genes in all groups although the quantities varied. *OPN*, *RUNX2* and *OCN* were

overexpressed in all groups with significantly higher expression in groups supplemented with nano hydroxyapatite. *Collagen1* was showed higher expression in Nano hydroxyapatite group than in MTA but with no statistical significance, whereas *ALP* was the only gene that illustrated increased expression MTA group.

It is worthy to note that both MTA and nano hydroxyapatite added to DMEM only without odontogenic supplements were not able to induce odontogenic differentiation (Supplementary Figure 1).

Discussion

In the present study, we focused on two promising biomaterials, MTA and nano hydroxy-apatite, which have been known to play an imperative role in the field of dentistry. Evaluation of the effect of the MTA and the nano hydroxyapatite on the odontogenic differentiation potential of the DPSCs was done by tracing the expression levels of genes characteristic of odontoblastic differentiation markers including *ALP, OC, OPN, RUNX2* and *Collagen 1*. Primary pulp cells are known to express type I collagen, ALP, bone sialoprotein, and osteocalcin [2]. The expression of these markers relates to different stages during odontogenic differentiation. OPN was highly expressed in both groups with a significant increase in nano hydroxyapatite group. The role of OPN in dentinogenesis is well described in the literature. During reparative dentinogenesis, OPN expression was found to play an undeniable role in the differentiation of odontoblast-like cells during pulpal healing following tooth transplantation [9].

In accordance to our study, Kuratate M. et al., suggested that pulpal responses to MTA capping involve proliferation and migration of pulp stem cells followed by their differentiation into odontoblast-like cells where osteopontin played a triggering role in the initiation of the pulpal reparative process [10]. Similarly, RUNX2; which is a key factor that is essential for odontoblastic differentiation, was highly expressed in both MTA and nanohydroxyapatite supplemented media. In accordance to our study, Matsumoto S. *et al.* proved that in the presence of MTA, the expression levels of RUNX2 in C2C12 cells (cell line obtained from RIKEN Cell Bank, Japan) were significantly increased indicating odontoblastic differentiation [11]. Furthermore, in accordance to our study, Mohamed *et al*. concluded that nano hydroxyapatite promoted odontogenic differentiation of DPSCs [12]. Also, Liu H-C et al. found that DPSCs seeded on nano-hydroxyapatite/collagen/Poly (L-lactide) could undergo odontogenic and osteogenic differentiation evident by the expression of OCN, COL 1 and ALP [13].

Next, we assessed the expression level of OCN. OCN has been known to be expressed only during the later stages of odontoblasts cytodifferentiation during tooth development. OCN was also detected in odontoblasts and their processes within the extracellular matrix at the maturation stage of enamel formation. It was shown that OCN is produced by human odontoblasts and determine the expression pattern of DSPP in human teeth [14]. Our results showed that OCN was highly expressed in both MTA and nanohydroxyapatite supplemented with odontogenic media indicating odontogenic differentiation of the DPSCs.

The role of ALP has been suggested to be implicated in early mineralisation. In our study, ALP was expressed both in MTA and nano hydroxyapatite groups. However, its expression in both groups was less than other genes studied. This finding is by Alliot-Licht B. et al., and Zhang W. et al., who revealed that ALP activity gradually increased after reaching its peak expression on the day and then declined [15] [16]. In accordance to our study, Min K-S. et al. found that DPSCs treated with MTA showed up-regulation of m-RNA expression levels of ALP and OCN which confirmed successful odontogenic differentiation of the DPSCs [17]. ALP also participates in dentin formation and was found to be highly expressed by mature odontoblasts; its absence indicates defective dentin mineralisation [18].

According to our results of genes tracing, it was noted that nano-hydroxyapatite showed higher levels of odontogenic genes expression in comparison to MTA supplemented with odontogenic medium suggesting a higher odontogenic differentiation potential of the nano hydroxyapatite. This increased differentiation potential might be attributed to the difference in their chemical composition and surface topography. Nano hydroxyapatite composition is similar to crystals present in dental hard tissues (calcium and phosphates) and has special biological and physicochemical properties [19]. Also, nanoscale of hydroxy-apatite had been shown to positively affect the adhesion and differentiation of stem cells and has excellent biological properties compared to their larger micron-structured counterparts which are attributed to increased surface reactivity [20]. Successful odontogenic differentiation of DPSCs was further confirmed by Alizarin red staining assay. Positive colorimetric changes were observed in both MTA and nano hydroxy-apatite groups as well as in the positive control group, which indicated calcific nodules formation. In accordance with our study, Jung J-Y. et al., suggested that MTA plays a role in differentiation of DPSCs into odontoblasts as evidenced by the alizarin red staining of calcified nodules [21].

Moreover, Woo S-M. et al., found that calcium ions released from MTA has a major impact on the odontoblastic differentiation of DPSCs and enhancement of mineralized nodule formation [22]. In accordance with our experiment, Liu H-C. et al., found that sporadic nodules-shaped islands after alizarin red staining as a result of the differentiation of DPSCs into

odontoblast and osteoblast when nano hydroxyapatite was used as a scaffold [13]. In our study, MTA extract was used to induce odontogenic differentiation of DPSCs after 24 hours of incubation of MTA powder in odontogenic media. By this method, we aimed to avoid direct contact of MTA with DPSCs and thus simulating the clinical conditions achieved when MTA is used for direct pulp capping. However, it was observed that MTA exerted an observable decrease in cell viability during culture, despite using a very low concentration. The concentration used was according to Hakki SS. et al., which was described as the least cytotoxic concentration capable of inducing differentiation [7]. It is worthy to mention that our results revealed that both MTA & nano hydroxyapatite were not sufficient to induce odontogenic differentiation by their selves. This was clearly evident, from the minimal expression of *ALP, OCN, OPN, RUNX2* and *collagen1* exhibited by MTA and nano hydroxyapatite added to DMEM media in the absence of odontogenic differentiation supplementation.

In conclusion, it was observed that both MTA and nano hydroxyapatite in the presence of odontogenic medium could enhance the odontogenic differentiation of the DPSCs. It was also clear that nano hydroxyapatite possess higher odontogenic differentiation potential than MTA, evidenced by higher fold increase in the expression of most of the odontogenic genes studied. These data may be useful in future studies to promote odontogenic differentiation of DPSCs and may be useful in designing regenerative therapies for dentin.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1732-1736.
https://doi.org/10.3889/oamjms.2018.326
eISSN: 1857-9655
Dental Science

The Effect of the Use of Different Types of Cement and Zirconium Post Systems on Endondontically Treated Teeth

Vesna Jurukovska-Shotarovska[1*], Biljana Kapusevska[1], Biljana Evrosimovska[2]

[1]Department of Prosthodontics, Faculty of Dental Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia; [2]Department of Oral Surgery and Implantology, University Clinical Centre Ss Pantelejmon, Skopje, Republic of Macedonia

Citation: Jurukovska-Shotarovska V, Kapusevska B, Evrosimovska B. The Effect of the Use of Different Types of Cement and Zirconium Post Systems on Endondontically Treated Teeth. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1732-1736. https://doi.org/10.3889/oamjms.2018.326

Keywords: Zirconium post systems; Multilink Automix cement; RelyX Unicem 2 Automix cement; Pull-out test

***Correspondence:** Vesna Jurukovska Shotarovska. Department of Prosthodontics, Faculty of Dental Medicine, Ss Cyril and Methodius University of Skopje, Republic of Macedonia. E-mail: v_jurukovska@yahoo.com

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

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Prefabricated zirconium upgrading systems were examined to satisfy aesthetic needs in endodontically treated teeth. Endodontically treated teeth, together with non-metallic posts and superstructure, are substructures that enable the production of prosthetic structures that will allow aesthetics, resulting from normal light transmission. To investigate and analyse the retention of zirconium post systems cemented with RelyX Unicem 2 Automix (RLX) cement with Pull-out test.

AIM: To examine the retention of zirconium post systems, cemented with Multi-Link Automix (MLA) cement and RelyX Unicem 2 Automix (RLX) cement with Pull-out test.

MATERIAL AND METHODS: In this study were used, 120 post systems of the company ZIRIX NORDIN - Switzerland, with different diameters d1 = 1.2, were used: d2 = 1.35, d3 = 1.5, and two types of resin cements: Multilink Automix-Ivoclar (MLA), and RelyX Unicem 2 Automix (RLX) - 3 M ESPE.

RESULTS: The analysis of the extraction force in newtons (N) zirconium post systems of Multilink Automix cement according to subgroups of three diameters is consequently 481.3 ± 1.9 vs 462.9 ± 4.5 vs 454.2 ± 2.2. The analysis of the extraction strength in the newtons (N) zirconium post systems of RelyX Unicem 2 Automix cement in the entire sample is 577.9 ± 6.1 N.

CONCLUSION: The largest diameter of the posts significantly increases the resistance of fractures compared to the smaller two diameters used in the experimental study.

Introduction

The new concepts of non-metallic restoration have led to the development of modern materials and methods for postendodontic treatment of endodontically treated teeth [1] [2]. Endodontically treated teeth, together with non-metallic posts and superstructure, are substructures that enable the production of prosthetic structures that will allow aesthetics, resulting from normal light transmission [3] [4].

Several new methods have been developed in aesthetic dentistry, including new composite materials, dentin adhesives and non-metallic post systems. After the definite endodontic treatment of the root canal, it is necessary to build upgrading systems for retention and support definite prosthetic restoration [5]. Paradental and endodontic status, root length, and the histological structure of devitalized teeth must be considered to achieve a successful fixed-prosthetic restoration [6] [7].

Endodontically treated roots restored by metal superstructure systems are more subjected to fractures due to the high elastic modulus compared to tooth dentine. Because of this, the aesthetic properties of these superstructure systems are limited

due to unsatisfactory aesthetics in the cervical part of the tooth [8].

Prefabricated zirconium upgrading systems were examined to satisfy aesthetic needs in endodontically treated teeth [9]. From this, it follows that the transparency of fully ceramic crowns can be successfully satisfied with the use of ceramic post systems. Zirconium post systems have good mechanical properties and provide specific protection of the tooth structure. This is due primarily to the minimal preparation and adhesive technique [10]. The purpose of this in vitro study is the adhesion of zirconium posts with two types of resin cement.

This arose the need for identification of the process of interphase extraction and resistance to dislocation of zirconium post systems with resinous materials. The objectives of this research arose from aesthetics as an important factor in everyday dental practice and the need for a long-term solution in teeth with a large coronary structure loss: to investigate and analyze the retention of zirconium post systems cemented with RelyX Unicem 2 Automix (RLX) cement and to examine the retention of zirconium post systems, cemented with Multi-Link Automix (MLA) cement.

Materials and Methods

In this study, 120 post systems of the company ZIRIX NORDIN-Switzerland, with different diameters d1 = 1.2, were used; d2 = 1.35; d3 = 1.5, and two types of resin cements: Multilink Automix-Ivoclar (MLA), and RelyX Unicem 2 Automix (RLX) - 3 M ESPE.

Three subgroups were formed:

Subgroup I: 40 zirconium post systems with diameter d1 = 1.2;

Subgroup II: 40 zirconium post systems with diameter d2 = 1.35;

Subgroup III: 40 zirconium post systems with diameter d3 = 1.5.

To perform the test, extracted incisors were used with removed paradontal tissues with coriets, and the teeth were then stored in distilled water. The coronary part of the teeth was removed using special saws and the pulp with special instruments. Then, the endodontic channels were washed using a NaOCl solution, EDTA and rinsed with water and dried. In each of the samples, zirconium post systems with different diameters were applied. Then they were cemented with two types of resinous cement (Figure 1). Smooth cement was polymerised with an ice-light for light polymerization. Then the samples were stored in the Ringer's solution for seven days. The prepared samples were placed in unified acrylate blocks (Figure 2). On the prepared samples, the so-called Pull-out test - extraction test was performed on the universal testing machine Shimadzu Universal Testing Machine. The maximum force was measured in Newtons (N).

Figure 1: Zirconium post systems, cemented in endodontically treated teeth

The pull-out test was performed at a rate of 1 mm/min. The extraction threshold is defined as the point at which the samples can no longer stand the increase of the extraction strength.

Figure 2: Zirconium post systems, cemented in acrylic blocks

Results

Zirconium post systems cemented with two types of cement RelyX Unicem 2 Automix cement and Multilink Automix, according to the three different diameters, were analysed regarding the regular/ irregular distribution of the obtained values for the pulling force expressed in newtons (N).

Analysis of zirconium post systems of RelyX Unicem 2 Automix cement in the three diameters

The analysis of the extraction strength in the newtons (N) zirconium post systems of RelyX Unicem

2 Automix cement in the entire sample is 577.9 ± 6.1 N. The minimum or maximum value of the extraction force in Newtons was 565.2 vs 590.3 N. In 50% of the post systems in the entire sample, the pulling power was less than 580.1 N Table 1.

The analysis of the extraction force in the newtons (N) of zirconium post systems cemented with RelyX Unicem 2 Automix cement according to the three subgroups of diameters is consequently 581.6 ± 4.6 vs 581.4 ± 0.7 vs 570.9 ± 6.1. In the subgroup I (d1 = 1.2 mm) zirconium post systems, the minimum, i.e. the maximum value of the pullout force is 575.2 vs 590.3 N, with 50% of the post systems with a pullout force of less than 580.8 N The zirconium post systems in Subgroup II (d2 = 1.35 mm) have a minimum or a maximum value of the pulling power of 580.1 vs 582.2N, with 50% of post systems where the pulling power was less than 581.6 N. In zirconium post systems in Subgroup III (d3 = 1.5 mm), the minimum or maximum value of the pull out force would be 565.2 v.s 576.4 N, with 50% of post systems where the pullout force was less than 571.2 N.

Table 1: Extraction force of zirconium post systems RelyX Unicem 2 Automix cement by subgroups according to the diameter

					95% Confidence Interval for Mean			
Subgroups	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
Subgroup I d = 1.2мм	20	581.59	4.62	1.03	579.43	583.75	575.25	590.34
Subgroup II d = 1.35мм	20	581.40	0.74	0.16	581.06	581.75	580.08	582.25
Subgroup III d = 1.5мм	20	570.75	3.54	0.79	569.09	572.40	565.23	576.42
Вкупно	60	577.91	6.09	0.79	576.34	579.49	565.23	590.34

One Way ANOVA: F = 67.194; df = 2; p=0.0001**significant for p < 0.05.

Analysis of zirconium post systems with three diameters of Multilink Automix cement

The analysis of the extraction force in Newton (N) zirconium post systems of Multilink Automix cement in the entire sample was 466.1 ± 11.8 N. The minimum or maximum value of the extraction force in Newtons was 451.1 vs 485.4 N. For 50% of the posts in the entire sample, the extraction force was less than 463.6 N. The table showing the analysis of the extraction strength in Newton's zirconium post systems of Multilink Automix cement is given in Table 2.

Table 2: Force extraction of zirconium post systems of Multilink Automix cement by sub-groups of diameters

					95% Confidence Interval for Mean			
Subgroup	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
subgroup I d = 1.2мм	20	481.29	1.99	0.44	480.37	482.23	478.23	485.43
subgroup II d = 1.35мм	20	462.91	4.50	1.01	460.80	465.02	455.33	470.15
subgroup III d = 1.5мм	20	454.22	2.19	0.49	453.19	455.24	451.15	458.55
Total	60	466.14	11.79	1.52	463.09	469.19	451.15	485.43

One Way ANOVA: F = 394.366; df = 2; p =0.0001* *significant for p < 0.05.

The analysis of the extraction force in newtons (N) zirconium post systems of Multilink Automix cement according to subgroups of three diameters is consequently 481.3 ± 1.9 vs 462.9 ± 4.5 vs 454.2 ± 2.2.

In the Zirconium post Systems of Multilink Automix Cement in Subgroup I (d1 = 1.2 mm), the minimum, i.e. the maximum value of the extraction force is 478.2 vs 485.4 N, with 50% of the superstructure systems where the extraction force was lower from 481.2 N.

In the zirconium post systems of Multilink Automix cement in Subgroup III (d3 = 1.5 mm), the minimum, i.e. the maximum value of the extraction force is 451.1 vs 458.5 N, with 50% of the post systems in which the pulling power was lower from 453.7 N.

Discussion

Zirconium post systems have higher resistance compared to other post systems. According to some authors, the failure rate of these post systems is only 3.2% during one to six years [11]. The authors concluded that these post systems could be used routinely only in combination with adhesive materials. It is very important to have a high potential for bonding dentine and complex resinous cement with zirconium post systems. Smooth cement showed success in some micro spaces [12] [13].

Significant average decrease in the extraction force by 18.39 (95% CI, 16.0-20.7) Newtons in measuring the Zirconium Upgrading Systems of Multilink Automix Cement in Subgroup II compared to Subgroup I (p = 0.0001).

For p < 0.05, there is a significant average decrease in the extraction force by 27.08 (95% CI, 24.7-29.4) Newtons to the Zirconium post Systems of Multilink Automix Cement in Subgroup III compared to Subgroup I (p = 0.0001).

The extraction force of Multilink Automix cement in Subgroup III compared to subgroup II for p < 0.05 is significantly lower for 8.69 (95% CI, 6.3-11.1) newtons (p = 0.0001).

Multilink Automix Cement, the extraction force of zirconium post systems is significantly reduced by increasing the diameter of post systems. A frequent recommendation among multiple authors is not to use a post with a diameter below 1.3 mm because weaker posts cannot provide sufficient stability [14]. One opinion is that the width of the post should not be greater than one-third of the width of the root in its narrowest dimension, bearing in mind that the

preservation of the remaining dentin is very important [15] [16].

The extraction force is significantly highest in Subgroup I with a significant reduction in the same for zirconium post systems from Subgroup II and Subgroup III. To determine the significance of the differences, the Tukey honest significant difference test (HSD) was applied. The differences in the values of the average extraction force of RelyX Unicem 2 Automix cement between the subgroups of zirconium post systems with three different diameters were analysed in the following combinations: Subgroup I / Subgroup II; Subgroup I / Subgroup III; and Subgroup II / Subgroup III).

In concordance to Tukey (HSD), the test for $p < 0.05$ points to a significant average reduction in pull-out force by 10.84 (95% CI, 8.2-13.4) novel when measuring the zirconium post systems of RelyX Unicem 2 Automix cement in Subgroup III compared to Subgroup I ($p = 0.0001$). The RelyX Unicem 2 Automix cement extraction force of subgroup III compared to Subgroup II is significantly lower by 10.66 (95% CI, 8.1-13.2) newtons ($p = 0.0001$). For $p > 0.05$, there is no significant reduction in the pull-out strength of zirconium post systems of RelyX Unicem 2 Automix cement between Subgroup I compared to Subgroup II, by 0.186 (95% CI, 2.4-2.8) novel ($p = 0.983$).

The extraction force of zirconium post cemented with RelyX Unicem 2 Automix cement in Newtons significantly decreases with the increase in the diameter of the post systems. The pullout force is significantly highest in Subgroup I, with its significance decreasing for Zirconium Upgrading Systems from Subgroup II and significant reduction of it for subgroup III zirconium post systems. The diameter of the post and the remaining dentin also play a major role in preventing fracture of the root. This corresponds with some In Vitro studies confirming the importance of the remaining tooth structure considering the strength and resistance of the root fracture [17] [18]. But according to some authors, when the diameter of the post increases, the surface of the tooth that is in contact with the tooth increases. According to some studies, increasing the diameter of the post does not significantly affect the retention capacities. However, it can increase the strength of the post and thus increase the risk of a root fracture [19] [20].

In conclusion, the largest diameter of the posts significantly increases the resistance of fractures compared to the smaller two diameters used in the experimental study. The extraction force of zirconium post systems that were cemented with both types of cement, the best results showed the post systems with diameter d3, compared with other diameters d1 and d2. From this, it can be concluded that the extraction force in zirconium post systems is significantly reduced by increasing the diameter of the post systems.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1737-1741.
https://doi.org/10.3889/oamjms.2018.307
eISSN: 1857-9655
Dental Science

Detection of Virus Herpes Simplex Type 1 in Patients with Chronic Periodontal Disease

Marija Ivanovska-Stojanoska[1*], Mirjana Popovska[2], Violeta Anastasovska[2], Mirjana Kocova[3], Lidita Zendeli-Bedzeti[1], Cena Dimova[4], Angela Taseva[5]

[1]University Dental Clinical Centre St. Panteleimon, Department of Oral Pathology and Periodontology, Skopje, Republic of Macedonia; [2]Ss. Cyril and Methodius University of Skopje, Faculty of Dentistry, Skopje, Republic of Macedonia; [3]University Children's Hospital, Faculty of Medicine, Ss. Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia; [4]University "Goce Delchev", Faculty of Dentistry, Shtip, Republic of Macedonia; [5]D-r Afrim, Skopje, Republic of Macedonia

Citation: Ivanovska-Stojanoska M, Popovska M, Anastasovska V, Kocova M, Zendeli-Bedzeti L, Dimova C, Taseva A. Detection of Virus Herpes Simplex Type 1 in Patients with Chronic Periodontal Disease. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1737-1741. https://doi.org/10.3889/oamjms.2018.307

Keywords: Periodontal disease; HSV-1; Multiplex PCR

***Correspondence:** Marija Ivanovska-Stojanoska, University Dental Clinical Centre St. Panteleimon, Department of Oral Pathology and Periodontology, Skopje, Republic of Macedonia. E-mail: marijaiva_stom@hotmail.com

Received: 07-Jul-2018; **Revised:** 23-Jul-2018; **Accepted:** 25-Aug-2018; **Online first:** 24-Sep-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Periodontal disease is an inflammatory-destructive condition of the supporting tissues of the teeth. Microorganisms found in the dental plaque were considered to be the primary local etiologic factor responsible for the periodontal destruction. It is also evident that herpes simplex viruses may have an impact in the etiopathogenesis of periodontal disease.

AIM: This study has been made with the aim to analyse the prevalence of herpes simplex virus type 1 (HSV-1) in the dental plaque (supra- and subgingival) of patients with the chronic periodontal disease.

MATERIAL AND METHODS: The study comprised a total of 89 patients with chronic periodontal disease divided into two groups (patients with moderate and severe periodontitis). Supragingival dental plaque samples were taken with sterile cotton (supragingival), and subgingival dental plaque samples were taken with paper absorbents. Samples were subjected to extraction of DNA and further analysis with multiplex PCR for the presence of herpes viral DNA.

RESULTS: HSV-1 virus was detected In 24.7% of all patients included in the study. HSV-1 was detected in 22.2% of patients with the moderate stage of the disease, of which in all (100%) in the supragingival plaque samples and only 16.7% in subgingival plaque samples. In two patients HSV-1 was concomitantly detected in supra and subgingival plaque samples. In patients with advanced stage of the disease, the HSV-1 virus was detected in 28.6% patients. In two of the patients, HSV-1 was concomitantly detected in supra and subgingival plaque samples. Statistically, a significant difference was found in HSV-1 positive patients with a moderate stage of disease, between the presence of the virus in subgingival (100%) and subgingival (16.7%) dental plaque samples, $p < 0.05$.

CONCLUSION: Herpes simplex viruses type 1 are present in supragingival and subgingival dental plaque.

Introduction

Periodontitis is the most common form of the oral disease in adults; this disease is an inflammatory-destructive condition of the supporting tissues of the teeth, it is considered to be a result of many factors.

For many years microorganisms found in the dental plaque were considered to be the primary local etiologic factor responsible for the periodontal destruction, so a number of putative bacteria as *Porphyromonas gingivalis, Tannerella forsythia* and *Aggregatibacter actinomycetemcomitans* is considered to be associated with the periodontal disease and are used as diagnostic markers [1] [2]. Clinical features of this commonly encountered disease are a result of interaction between microorganisms and the host immune response. It is evident that host immune responses against infection with bacteria and the subsequent production of proinflammatory cytokines are of particular importance in periodontium destruction [3] [4]. Microorganisms initiate inflammatory reactions in the periodontium, which causes in long run loose teeth, destruction of

the connective tissue, and resorption of the alveolar bone.

Some studies have shown that human herpes viruses, especially herpes simplex virus (HSV-1 and 2), cytomegalovirus (HCMV) and Epstein-Barr virus (EBV), play an important role in the pathogenesis of the periodontal disease [5] [6] [7] [8]. Association of herpes viruses with specific pathogenic bacteria can also explain the etiopathogenesis of periodontal disease [5]. Indicating that the development of periodontal disease most probably depends on the interaction between herpes viruses, specific pathogenic bacteria and destructive inflammatory mediators [9] [10].

The present study has been made with the aim to analyse the prevalence of herpes simplex virus type 1 (HSV-1) in the dental plaque (supra- and subgingival) of patients with the chronic periodontal disease, to evidence the possible association between the presence of this virus and the stage of the chronic periodontal disease.

Material and Methods

This study was made at the University Clinic of Mouth and Periodontal Diseases and Genetic Laboratory at the University Clinic for Children's Diseases- Medical Faculty in Skopje. Each patient included in the study signed informed consent for participation in the study. Institutional Ethical committee approved the study.

The study comprised a total of 89 patients divided into two groups:

- Patients who have diagnosed a moderate stage of chronic periodontal disease (clinical attachment loss of 2-5 mm) and
- Patients who were diagnosed a serve stage of chronic periodontal disease (clinical attachment level ≥ 6 mm).

Patients who were included in the examined group had to meet certain inclusion criteria.

Inclusion criteria: patients who were no-smokers, patients who did not take antiviral drugs in the previous six months and patients without systemic diseases such as renal, cardiovascular, respiratory, malignant diseases, diabetes.

Exclusion criteria: patients who take antibiotics over the last three months, patients who are on long-term treatment with drugs that influence on the periodontium (non-steroidal anti-inflammatory drugs), as well as pregnant and breastfeeding women.

In this study first was detected clinical stage of periodontal disease in patients who were included in study by determination of: plaque index-PI (Silness-Löe) [11], gingival index of gingival inflammation-GI (Löe-Silness) [12], periodontal bleeding on probing-BOP (Mühlemann-Son) [13], clinical attachment loss-CAL and measurement of periodontal pocket depth-PPD.

Clinical and laboratory examinations consisted of collecting dental plaque samples supra- and subgingivally in both clinical disease stages.

- *Supragingival dental plaque samples* were taken with a sterile cotton swab by vigorous scrubbing the tooth surface.

- *Subgingival dental plaque samples* were taken with paper absorbents, Absorbent paper points, Vericom, Eazi-Endo, Chuncheon-Sl. Korea, which was applied to the bottom of the periodontal pocket. Usually, 5-6 paper absorbents were used for one patient.

Samples contaminated with blood were not used in the examination.

After collecting the plaque samples, they were put in sterile microbiological plastic tubes-ependorfs with suspended 1ml x 1 x PBS (phosphate buffered saline) buffer (pH = 7.4) (Figure 1) and were transported to the Genetic Laboratory at the University Children's Hospital for further analysis.

Dental plaque samples were immediately subjected to the protocol for DNA digestion and extraction in the Laboratory. The test tubes with the samples were vortexed and centrifuged, after that, the supernatant was decanted and 300-350 μl buffer for digestion was added to the precipitation (0.05 M Tris, 0.001 M EDTA, 1% Tween 20, 1% Nonidet 40, 0.3 mg/ml Proteinase K), after which it was incubated in a water bath overnight at 56^{o}C. Next step was DNA extraction and precipitation by a standard method with phenol-chloroform and ethanol. All extracted DNA samples were stored at -20^{o}C for further analysis.

PCR amplification of HSV-1 virus was performed with the PCR machine-Veriti Thermal Cycler (Applied Biosystems, California, USA) according to the following protocol: an initial denaturation, followed by 40 amplification cycles and a terminal extension. Last product was A total volume of 50 μl reaction mixture contained: 1 μl of extracted DNA, 10pmol of each primer-H1P32 (5'-TGGGACACATGCCTTCTTGG-3') and H1M32 (5'-ACCCTTAGTCAGACTCTGTTACTTACCC-3'), 5 μl of 10 x reaction buffer, 0.2 mM of each (deoxynucleotide triphosphate) d NTP and 2.5 U of cloned pfu DNA polymerase enzyme (G-Biosciences, USA), previously described by Das et al., in 2012 [14].

A 10 μl of the amplified PCR product was taken and analysed with electrophoresis on a 2.5% agarose gel containing 1 mg/ml ethidium bromide in 1 x TBE (Tris/Borate/EDTA) buffer and was visualised under UV transilluminator. Presence of a fragment of

147 bp confirmed the presence of HSV-1 virus in the analysed sample. Angiotensinogen served as a control gene for monitoring the success of PCR amplification of HSV-1 virus by using the pair of primers oligo25/oligo26, resulting in PCR product of 165 bp.

The data analysis is performed in a statistical program Statistica 7.1 for Windows The following methods were used: In the analysis of the series with attribute markers (presence of the HSV1, viruses in supragingival and subgingival dental plaque in patients with moderate and advanced stage of periodontal disease), percentage percentages of the structure (%) were determined;. The difference in the frequency of detected HSV-1 virus between patients with the moderate and advanced stage of periodontal disease, the difference between the presence of the virus in supragingival and subgingival dental plaque samples in positive patients with the moderate and advanced stage of periodontal disease are performed with statistic Difference test.

Results

A total number of 89 patients were examined for the presence of HSV-1 virus. In 60.7% of these patients, a moderate clinical stage of the disease was detected, while in 39.3% an advanced clinical stage was found. In 22 (24.7%) of all 89 patients with moderate and advanced periodontal disease HSV-1 virus was detected in supra- and/or subgingival dental plaque samples (Figure 1).

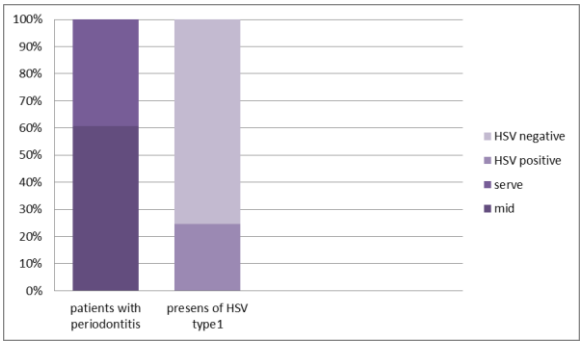

Figure 1: Presentation of patients according to the clinical stage of the disease and the detection of HSV-1 in patients with periodontal disease

Molecular analysis of HSV-1 showed the presence of the virus in 12/54 (22.2%) of patients with the moderate stage of the disease, of which in all 12/12 (100%) in the supragingival plaque samples and only 2/12 (16.7%) in subgingival plaque samples. In two patients HSV-1 was concomitantly detected in supra and subgingival plaque samples (Table 1, Figure 2).

In patients with advanced stage of the disease, the HSV-1 virus was detected in 10/35 (28.6%) patients, of which in 6/10 (60%) supra-gingival samples and 6/10 (60%) sub-gingival plaque samples. In two of the patients, HSV-1 was concomitantly detected in supra and subgingival plaque samples (Table 1, Figure 3)

Table 1: Distribution of HSV-1 in patients with the different clinical stage of the disease

Periodontal disease /	Moderate disease stage		Advanced disease stage	
HSV 1	Patients (n)	%	Patients (n)	%
Negative	42	77.8	25	71.4
Positive	12	22.2	10	28.6
Total	54	100	35	100

Statistical analysis (Difference test-which one) showed no significant difference in the frequency of detected HSV-1 virus between patients with moderate and advanced periodontal disease *(p = 0.4942)*.

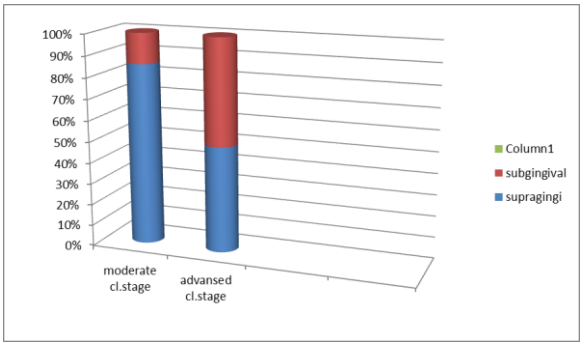

Figure 2: Distribution of HSV-1 virus in supragingival and subgingival plaque samples in virus-positive patients

On the contrary, the statistically significant difference was found in HSV-1 positive patients with a moderate disease stage, between the presence of the virus in supragingival (100%) and subgingival (16.7%) dental plaque samples, $p < 0.05$. No significant difference was observed between the presence of the virus in supragingival and subgingival plaque samples in HSV-1 positive patients with advanced disease stage, $p > 0.05$.

Discussion

Researches about the pathogenesis of periodontal diseases proved that some viruses could also have a role in the progression of periodontal disease. It is also demonstrated that a single site of active periodontal destruction as periodontal pockets may have more than a million copies of herpesvirus genomes [15]. Herpes viruses have been detected in supragingival and subgingival plaque samples, gingival biopsies, and gingival crevicular fluid (GCF) of healthy and periodontitis patients [16] [17] [18]. The gingival sulcus or periodontal pocket has also been

proposed to act as a reservoir between periods of recurrence of herpetic medical infections [19].

Herpes simplex viruses type 1, and 2 (HSV-1 and HSV-2), also known as human herpesviruses 1 and 2 (HHV-1 and HHV-2) are characterised by a short reproductive cycle and the ability to establish latency in the nerve ganglia so that might destroy the cells in the host [20]. Reactivation of herpes simplex virus can be without clinical symptoms, but about one-third of the individuals become prone to clinical recurrences [21]. HSV-1 is a common cause of oral herpes. HSV-1 is usually acquired by direct contact with an infected person, most often by contact with saliva during childhood, but it can also be transmitted vertically from a mother to a child before or during childbirth.

Viral HSV particle consists of a relatively large double-stranded DNA molecule encased within a protein cage called nucleocapsid, which is wrapped in a lipid bilayer cover. Besides lipids, the lipid cover in its structure contains glycoproteins, with a diverse role [22]. Most often these glycoproteins are membrane-bound receptors of cells that are going to be attacked.

Large part of these studies evidenced that HSV-1, EBV and CMV are detected with high prevalence of periodontal lesions, that they directly infect gingival epithelial cells and that viral loads positively correlate with disease severity [14] [23] [24] [25] [26] [27] [28] [29] [30].

It has been proved that the presence and frequency of herpesviruses in periodontal pockets increase with the increase of the periodontal pocket depth [31]. The prevalence of herpesviruses in periodontal pockets may vary according to the type of periodontal disease [7]. Herpes viruses can multiply in gingival tissue, especially in the epithelial cells and fibroblasts of clinically healthy gingiva that is easily subjected to HCV infection [32]. This implies that these cells might be a reservoir of the latent form of this virus. Herpes virus-infected cells can reduce the host defence and give rise to overgrowth of pathogenic bacteria and invade the cells more efficiently [5].

The results obtained from our study about presence of the HSV-1 virus in subgingival dental plaque samples, because of low prevalence of HSV-1 virus are not in agreement with the results of Grenier G et al., [17], Contreras A et al., [33], Sanja Matić Petrović et al., [34] that detected higher prevalence of virus Herpes simplex type 1 (HSV1) in subgingival dental plaque in patients with chronic periodontal disease. Our results are in agreement with the results of Nibaili et al., [35] which did not detect the presence of HSV-1 in the sub-gingival dental plaque.

There are findings that suggest that advanced stage of chronic periodontal disease was more commonly associated with the herpes viruses HSV1 and HSV2. Our findings are not in agreement with the observations that were made by Contreras et al., [31], in their study they detected Herpes simplex virus-1 in subgingival dental plaque in 52% of patients with advanced stage of chronic periodontal disease and herpes simplex virus-2 was detected in subgingival dental plaque in 56% of patients with severe chronic periodontitis this results are higher than that reported by Imbronito et al., [36], Greiner et al., [17],and Grande et al., [37].

In conclusion, in this study, we detected that virus Herpes simplex type 1 is present in supragingival and subgingival dental plaque. Our findings suggest that there is no significant difference in the frequency of detected HSV-1 virus between patients with the moderate and advanced stage of periodontal disease. So, we don't suggest with certainty that the presence of HSV-1 is related to the degree of periodontal tissue damage and manifestation of the different degree of periodontal destruction.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1742-1755.
https://doi.org/10.3889/oamjms.2018.378
eISSN: 1857-9655
Dental Science - Review

Contemporary Dental Ceramic Materials, A Review: Chemical Composition, Physical and Mechanical Properties, Indications for Use

Emilija Bajraktarova-Valjakova[1*], Vesna Korunoska-Stevkovska[1], Biljana Kapusevska[1], Nikola Gigovski[1], Cvetanka Bajraktarova-Misevska[1], Anita Grozdanov[2]

[1]Faculty for Dental Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia; [2]Faculty of Technology and Metallurgy, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia

Citation: Bajraktarova-Valjakova E, Korunoska-Stevkovska V, Kapusevska B, Gigovski N, Bajraktarova-Misevska C, Grozdanov A. Contemporary Dental Ceramic Materials, A Review: Chemical Composition, Physical and Mechanical Properties, Indications for Use. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1742-1755. https://doi.org/10.3889/oamjms.2018.378

Keywords: CAD/CAM; Chemical composition; Glass-ceramics; Mechanical properties; Resin-matrix ceramic; Zirconia

***Correspondence:** Emilija Bajraktarova-Valjakova. Faculty for Dental Medicine, Ss "Cyril and Methodius University" of Skopje, Skopje, Republic of Macedonia. E-mail: emibaj@yahoo.com

Received: 12-Aug-2018; **Revised:** 08-Sep-2018; **Accepted:** 09-Sep-2018; **Online first:** 24-Sep-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: The high esthetic expectations from the prosthodontic restorations have directed the qualitative development of the materials towards the all-ceramic materials that are capable of replacing porcelain-fused-to-metal systems.

AIM: This article reviews the literature covering the contemporary all-ceramic materials and systems with a focus on the chemical composition and materials' properties; also it provides clinical recommendations for their use.

RESULTS: The glass-matrix ceramics and polycrystalline ceramics are presented, as well as recently introduced machinable materials, all-zirconia and resin-matrix ceramics. The specific properties of zirconia, such as transformation toughening, stabilisation of the crystallographic structure, low-temperature degradation and factors affecting the zirconia's ageing, are emphasised.

CONCLUSION: The favourable properties of the resin-matrix ceramics, such as modulus of elasticity similar to dentin, shock-absorbing characteristics and high resilience and fracture resistance, are also covered in this article.

Introduction

Technological development in the dental industry, particularly in the field of ceramic materials, enabled the production of metal-free restorations made up of all-ceramic materials [1]. The qualitative improvements provided ceramic materials with many advantages over the porcelain-fused-to-metal system such as excellent esthetic appearance due to favourable optical properties (translucency and transparency), natural tooth color and chromatic stability, biocompatibility, chemical inertness and low thermal conductivity, optimal mechanical properties such as high flexural strength and fracture toughness, as well as wear resistance and low abrasive properties [2].

Consequently, all-ceramic materials can be used for manufacturing of all kind of single-tooth restorations [3] [4] such as veneers, inlays, onlays, crowns and posts; lithium disilicate ceramic can be used for production of 3-unit bridges (in the anterior and premolar region), whereas multi-unit bridges can be made up only by stabilized zirconia; ceramic materials with a resin matrix inside, are especially suitable for the production of crowns over the implants or tooth restorations in a region where high masticatory pressure is generated [5] [6].

This article reviews the current literature regarding the all-ceramic materials, represents the recently proposed classification system [7] based on the phase or phases present in materials' chemical composition; presents already established as well as recently developed and introduced materials, their

properties and clinical indications. Glass-ceramics have superior optical properties [8] [9] [10], stabilized zirconia, referred to as “ceramic steel” [11] not only because of the phenomenon called “tension expansion”, but including other properties such as the highest strength, high fracture toughness and Vickers hardness [12] [13] [14], whereas so-called “hybrid ceramics” appeared to have advantages in terms of fracture resistance, high resilience and shock-absorbing properties, milling efficiency, polishability and accuracy-less marginal chipping [5] [15] [16].

The purpose of this review is to help therapists take into consideration not only the patients requirements, but material features as well, when choosing a ceramic material, so all-ceramic restorations with high reliability and long-term clinical success can be made.

Dental Ceramics – Chemical Composition

For a long time, ceramic materials have been defined as compounds of metallic and non-metallic elements consisting of oxides, nitrides, carbides, and silicates [17]. Most of the ceramics used in dentistry were primarily based on silicon that usually occurs in the form of silica (silicon dioxide), due to the silicon’s high oxygen affinity or as silicates compounds [18].

The increasing use of polycrystalline ceramics (with no silicon in their composition), and the introduction of so-called ‘hybrid’ ceramics imposed the need for a new classification system [7]. According to this classification system, all-ceramic and ceramic-like restorative materials can be categorised into three groups: (1) glass-matrix ceramics, (2) polycrystalline ceramics, and (3) resin-matrix ceramics, depending on the phase/phases present in their chemical composition. “Glass-matrix ceramics” are nonmetallic inorganic ceramic materials that contain a glass phase, while “polycrystalline ceramics” are defined as nonmetallic inorganic ceramic materials that do not contain glass, but only a crystalline phase. In the third group - “resin-matrix ceramics” are included materials that have a polymer matrix, containing predominantly inorganic refractory compounds [7]. Different phases present in materials’ chemical composition affects the sensitivity of the ceramic material to the hydrofluoric acid when etching (as a surface treatment method before adhesive luting) to achieve stronger resin-ceramic bond [19].

Glass-Matrix Ceramics

The first group, glass-matrix ceramics, is further divided into three subgroups: feldspathic ceramics, synthetic ceramics, and glass-infiltrated ceramics.

Feldspathic ceramics

The traditional type of dental ceramics are feldspar-based, composed of the significant amount of feldspar ($KAlSi_3O_8$), quartz (SiO_2), and kaolin ($Al_2O_3 \cdot 2SiO_2 \cdot 2H_2O$). Feldspar is a greyish crystalline mineral that can be found in rocks rich with iron and mica. Feldspar rocks are grounded, and after using strong magnets to remove iron compounds, it is milled to obtain the purest powder. Quartz or silica (SiO_2) is the matrix component (55–65%) responsible for the translucency of the restoration. As it is not a strong material, 20–25% alumina (Al_2O_3) is added as a reinforcing component. Kaolin is a hydrated aluminium silicate that is used in a limited amount (4%) as it has opaque properties, unlike the human teeth which are translucent. It is used in the composition of dental ceramics as it binds the loosely held ceramic particles together [18] [20].

VITABLOCS® from VITA Zahnfabrik are the most used feldspar-based CAD/CAM ceramics with an average grain size of 4μm and flexural strength of 154MPa. In 1985 first inlay was produced made of VITA Mark I, whereas in 1991 VITA has promoted Mark II, a monochromatic material with improved chemical composition and physical properties. To imitate the natural colours of the tooth, VITA has introduced next generations: VITABLOCS® TriLuxe (2003) and TriLuxe forte (2007). VITABLOCS® TriLuxe includes three, while TriLuxe forte four layers of different shade intensity from the cervical to the incisal edge, especially suitable for veneers, partial and full crowns in the anterior region. Further improvement in a replica of the shade gradient of natural teeth between the dentin and the edge areas, has been succeeded with VITABLOCS® RealLife (2010), multichromatic feldspar ceramic with different colour intensity in three dimensions [21].

Numerous micropores and channels of different sizes with irregular ceramic particles can be observed on the surface of VITA Mark II after surface etching with hydrofluoric acid; such a modified surface micromorphology is suitable for capturing of a composite luting cement [19] [22].

Synthetic glass-ceramics

To remain less dependent on natural resources of raw materials, the so-called “synthetic” glass-ceramics are invented, with a greater presence of crystalline phase that reduces the possibility of crack formation, or the propagation is slowed down if it has already occurred [7]. The presence of the crystals improves the mechanical properties of the ceramic.

The microstructure of glass-ceramics comprises dispersed crystalline phase (crystals)

surrounded by a translucent glassy phase (matrix). The glassy phase possesses the usual properties of glass such as translucency, brittleness and non-directional fracture pattern. The crystalline phase improves light scattering and opacity, thereby colour adaptation of transparent glassy phase to dental hard tissues (enamel and dentin), and provides the ceramic material with strength, stability during firing and resistance to stresses that occur in the mouth [18].

The crystals are "artificially" created by controlled nucleation and crystallisation. The size and distribution of the crystals are determined by the composition and processing of the base glass and the subsequent heat treatment. This process allows “tailor-made” materials to be produced, which exhibit homogeneous structure, good optical properties, appropriate wear characteristics, as well as optimal strength [23] [24].

The final mechanical properties of the synthetic glass ceramics are determined by two groups of factors: intrinsic and extrinsic. Intrinsic factors are crystal size, number and geometry, the distribution pattern of the crystals (homogeneity), as well as thermal expansion/contraction matching between the crystal phase and glassy matrix. Long-term performance of the material also depends on the extrinsic factors such as fabrication conditions and conditions of the oral environment: humidity (stress corrosion), variations of the pH level, thermo shocks, cyclic loading and peak loads that can reach extremely high levels when hard objects are accidentally encountered during mastication [25].

Leucite-reinforced, lithium disilicate, zirconia-reinforced lithium silicate, and fluorapatite-based ceramics are representatives of synthetic glass-ceramics.

Leucite-reinforced ceramic (IPS Empress CAD, Ivoclar Vivadent) consists of leucite crystals (35–45 vol%) that are homogeneously distributed into the glassy matrix. Leucite crystals are created by the controlled firing of feldspar at 1150°C. Potassium aluminium silicate is disintegrated into leucite and two molecules of silica:

$$K_2O{\cdot}Al_2O_3{\cdot}6SiO_2 \rightarrow K_2O{\cdot}Al_2O_3{\cdot}4SiO_2 + 2SiO_2$$ [17]

The process is known as surface crystallisation, in which the crystals grow slowly along the grain boundaries towards the centre of the grain [26].

Due to the high silica content (60–65 wt%) this ceramic has improved translucency, fluorescence and opalescence, while the crystalline content is responsible for the flexural strength of 160 MPa and ability to absorb the fracture energy that results in arrested or slowed down crack propagation. The diameter of the leucite crystals ($KAlSi_2O_6$) is 1–5 μm [27] [28].

IPS Empress CAD is monochromatic ceramic, with the need for further characterisation of the milled restoration, whereas IPS Empress CAD Multi is polychromatic, with high intensity of chroma and opacity in the cervical area and high level of translucency in the incisal region [29].

As a glass-ceramic, the best surface treatment method before adhesive luting is HF-acid etching. The acid dissolves the leucite crystals more quickly than the glassy matrix resulting in a surface with honeycomb-like appearance [22] [27] [30].

Another leucite-reinforced ceramic is Paradigm C, introduced in 2006 by 3M ESPE.

Lithium disilicate ceramic (IPS e.max CAD, Ivoclar Vivadent) is comprised of approximately 70 vol% of crystalline phase incorporated in the glassy matrix. In the process of production, ceramic is cast in transparent glass ingots that contain lithium orthosilicate. The process of partial crystallisation that follows leads to the formation of 40% platelet-shaped lithium metasilicate crystals (with the average size of 0.2–1.0 μm), Li_2SiO_3, embedded in a glassy phase. It is a so-called intermediate crystalline phase or ‘blue’ state, with 130 ± 30 MPa flexural strength, in which the blocks can easily be milled in CAM unit. Milled restorations are tempered at 850°C and eventually lithium disilicate crystals - $Li_2Si_2O_5$ are formed [31] [32] [33], which impart the milled restoration with the final shade and flexural strength of 360 ± 60MPa:

Lithium orthosilicate (Li_4SiO_4) → lithium metasilicate (Li_2SiO_3) → lithium disilicate ($Li_2Si_2O_5$) [31]

Randomly-oriented, densely distributed, elongated fine-grained lithium disilicate crystals, 1.5 μm in length, scattered with shallow irregularities become visible after dissolving the glassy matrix by etching the surface with hydrofluoric acid (HF) [19] [22] [30].

Zirconia-reinforced lithium silicate is glass-ceramic material enriched with highly dispersed zirconia, developed in close cooperation between Degudent, VITA and Fraunhofer Institute for Silicate Research [34]. After breaking up the partnership, Dentsply and VITA continued with their research which, in 2013, resulted in the introduction of Celtra Duo™ (Dentsply, Degudent) and VITA Suprinity®, (VITA Zahnfabrik).

Production of the ceramic blanks and fabrication of the restoration go through three stages similar to those of lithium disilicate ceramic. In the first stage, the melted material is moulded, and the block is in the glass state. Nucleation/precrystallisation is a subsequent thermal pretreatment in which crystals start to form and grow, and the glass features ceramic properties. In this stage, the material contains only lithium metasilicate crystals and is easy to mill in the CAM unit. The final crystallisation stage is short heat treatment at 840°C for 8 min in which the restoration exhibits its final colour and physical properties. The crystalline phase consists of 25% lithium metasilicate

(Li_2SiO_3) and 11% lithium disilicate ($Li_2Si_2O_5$) crystals. During the last two stages, zirconia acts as a nucleating agent but remains dissolved in the glassy matrix [25] [35] [36].

Despite the high silica content of 55-65 wt%, these ceramics have improved flexural strength of 370-420 MPa after glazing, due to the lithia (15–21 wt%) and particularly zirconia (8–12 wt%) content (approximately ten times more zirconium dioxide than in traditional CAD/CAM glass-ceramic) [37].

The specific composition has a positive effect on the optical properties of the material; consequently, aesthetic appearance 'as a natural tooth' of the restoration (natural opalescence, fluorescence and pronounced chameleon effect) is achieved. The lithium silicate crystallites in Celtra with a size of 0.5-0.7 μm corresponds to the wavelength range of natural daylight thus mimicking the opalescence behaviour of the tooth enamel, and together with the high glass content are responsible for the fluorescence of the restoration [38].

Values for the fracture toughness (2.31 ± 0.17 MPa $m^{0.5}$), flexural strength (443.63 ± 38.90 MPa), elastic modulus (70.44 ± 1.97 GPa) and hardness (6.53 ± 0.49 GPa) of Vita Suprinity are significantly higher compared to lithium disilicate ceramic [39].

Dissolving of the glassy matrix using hydrofluoric acid before adhesive lutting of the restoration made up from zirconia-reinforced lithium silicate causes exposure of the homogeneously and densely arranged bean-like lithium silicate crystals [19] [22].

Fluorapatite glass-ceramics (IPS e.max Ceram and IPS e.max ZirPress, Ivoclar Vivadent) contain fluorapatite crystals $Ca_5(PO_4)_3F$ in various sizes embedded into the glassy matrix; the crystals, responsible for material's opalescence, are less than 300 nm in length and 100 nm in diameter, whereas crystals along the longitudinal axis are larger, 2–5 μm in length and less than 300 nm in diameter. IPS e.max Ceram is a nano-fluorapatite layering ceramic in the form of a powder, used for the production of veneers or as veneering material for glass or oxide ceramics, whereas IPS e.max ZirPress are pressable ingots suitable for the production of veneers and veneering of zirconia substructures using press-on-zirconia technique [40] [41].

The flexural strength of IPS e.max Ceram is significantly lower than IPS e.max ZirPress due to the pores present in the material, result of an air-bubbles' incorporation during mixing the ceramic powder with the mixing liquid [42]. Having high silica content of approximately 60 wt% and very low alumina content of ≈12 wt%, the flexular strength is lower (only 90-110 MPa) compared to other all-ceramic materials, which means that they can't be used for the production of the substructure of the restorations [40] [41].

Further development of glass ceramics is aiming to fulfil the protective requirements of the dental materials. ***Fluorophlogopite glass ceramic*** seems to have similar properties to other glass ceramics regarding mechanical properties, milling efficiency and etchability, but further on, this material possesses biocompatible property such as fluoride releasing. The flexural strength is approx. 120 MPa, the Weibull modulus is higher, while the Vickers hardness is lower compared to feldspar-based, lithium disilicate and resin-matrix ceramic [43].

Glass-Infiltrated ceramics

The glass-infiltrated ceramics belong to a group of ceramic-glass interpenetrating phase composites as they have at least two interpenetrating phases intertwined throughout the material [15]. The ceramic material is fabricated utilising the slip-casting or CAD/CAM technique. A slurry of densely packed ceramic particles is sintered to a refractory dye (if using the slip-casting technique) or the ceramic powder is dry pressing into a mould and compacted to produce a ceramic block (if using CAD/CAM technique) that are subsequently milled in CAM unit. The porous ceramic skeleton is infiltrated with lanthanum glass in a second firing, thus increasing the strength of the restoration [44].

Optical properties and final strength of the ceramic depends on the chemical composition of the porous core. *VITA In-Ceram™ SPINELL*, consisted of alumina and magnesia ($MgAl_2O_4$), had the lowest strength (400 MPa) but very high translucency, thus indicated for single crowns in the anterior region. The alumina content in *VITA In-Ceram™ ALUMINA* has reached 80% obtaining optimal translucency and strength (500 MPa) of the material, therefore indicated for single crowns in the anterior and posterior region, and for production of 3-unit bridges in the anterior region. Due to the alumina core strengthened with zirconia, *VITA In-Ceram™ ZIRCONIA* had highest bending strength (600 MPa) compared to other glass-infiltrated ceramics and was indicated for single crowns in posterior region and 3-unit bridges no matter of the localisation [45]. No statistically significant difference has been found in the biaxial flexural strength and fracture toughness between In-Ceram ALUMINA and In-Ceram™ ZIRCONIA [46].

The acid etching with HF acid did not change the superficial microstructure of these ceramics [30] [47] [48]. The use of this class of materials is abandoned due to the complexity and sensitivity of the manufacturing process, as well as the increased popularity of lithium disilicate ceramic and zirconia.

Polycrystalline ceramics

The main characteristic of ceramics classified in this group is a fine-grain crystalline structure without

the glassy phase. The crystals are densely arranged into regular arrays, thus reducing the crack propagation, providing the material with high strength and fracture toughness [44]. The absence of the glassy matrix is the reason for ceramics' resistance to surface etching with hydrofluoric acid [49] [50].

Alumina

Aluminium oxide (Al_2O_3) is a naturally occurring mineral (corundum, bauxite) with a high Mohs hardness of 9. It is used in engineering as an abrasive material, cutting tools, electronic substrates, whereas in medicine, due to its biocompatibility, low friction and excellent wear and corrosion resistance, is used as a bone replacement material (production of hip joint balls) [51]. For years crystalline aluminium oxide has also been used to increase the stability of dental ceramics (so-called "dispersion strengthening").

Alumina exhibits the highest resistance to hydrolysis compared to other ceramic materials, low thermal conductivity and high flexural strength (> 500 MPa). The alumina blocks (consisting of 99.5% Al_2O_3) are initially manufactured partially sintered which allows easy processing-milling. Shrinkage, which occurs during the subsequent sintering process, can be exactly calculated, so precision-fit structures are obtained. Since grinding does not cause phase transformation in the structure, restorations can be reshaped in the sintered condition, with no need of subsequent regenerative firing [52].

With an elastic modulus of 380 GPa [53], alumina is prone to bulk fractures [54]. Furthermore, the increased use of materials with improved mechanical properties, such as stabilised zirconia and its feature for transformation toughening (thus the ability for crack "repairing"), has led to a decreased use of alumina.

Procera® AllCeram from Nobel Biocare (the first fully dense polycrystalline ceramic) and In-Ceram® AL, a product of VITA Zahnfabrik, are representatives of this type of ceramic.

Zirconia

Zirconium (Zr) is a shiny silvery metal. It is relatively soft and flexible when in a highly pure form. Its most important compound is zirconium dioxide ZrO_2, chemically an oxide and technologically a ceramic material. About 0.02% of the earth crust comprises of zirconia, with the largest deposits in Brazil and South Africa as baddeleyite (monoclinic zirconia) and high proportion in Australia and India where can be found as zircon ($ZrSiO_4$) sands [55]. Zirconia was discovered by the German chemist Martin Heinrich Klaproth in 1789 [13].

Pure zirconia is a polymorphic material that occurs in three crystallographic structures depended on the material's temperature. This phenomenon is known as allotropy since different structures have the same chemical composition but a different atomic arrangement. When cooling down from the molten state, following phases can be observed: cubic (*c*) from 2680°C, the melting point, to 2370°C; tetragonal (*t*) from 2370°C to 1170°C; and monoclinic (*m*) from 1170°C to room temperature [13] [56] [57] [58]. The spontaneous transformation from the *t* phase (higher material density) to the more–stable *m* (lower material density) phase is associated with a volume increase of 3% to 5%. Occurred tension (during the cooling phase after sintering) inside of the restoration made of pure ZrO_2 results in numerous microcracks, which will eventually lead to premature failure of the restoration [59] [60].

In the distant 1929, Ruff et al., [61] [62] have discovered that the tetragonal, or even the cubic form of zirconia could be retained metastable at room temperatures by alloying pure zirconia with other cubic oxides. Since then, numerous oxygen biocompatible compounds have been proposed as a zirconia stabilizers, used to relocate the phase transformation towards lower temperatures, thus preventing the catastrophic failure of the restorations made of zirconia: MgO [63] [64] [65] CaO [66], CeO_2 [67], Al_2O_3 [68] and Y_2O_3 [66] [69].

In 1975, Hannink et al., [69], proposed that the *t–m* transformation followed by volume expansion could be used to enhance the fracture toughness of partially stabilised zirconia-based materials. In 1976, Claussen and Steeb [70] had explained this mechanism as "oriented nucleation of microcracks". When a restoration containing metastable *t*-zirconia is subject to an external source of energy, as for example in the case of a tensile stress, temperature shock, an overloading in patient with parafunction, or in contact with water at low temperatures (condition occurring over time known as "low temperature degradation" LTD) [71] [72], the cracks may occur. Zirconium oxide grains are transformed from their tetragonal to the monoclinic form accompanied by a volumetric expansion of the grains thus restricting the crack. Since this expansion is constrained by the surrounding material, the net result is compressive stress on the surfaces of the crack, which propagation is thus hindered, eventually preventing the failure of the zirconia restoration [71] [73]. This is the reason why this phenomenon is called "phase transformation toughening" [69] [70] [71] [72] [73].

As "tension expansion" is a phenomenon otherwise known only in the case of steel, zirconium oxide was also referred to as "ceramic steel" [11].

In their review paper, Lughi and Sergo [71] summarising the scientific data concluded that the main factors affecting the zirconia ageing are the stabiliser type (oxides) and its content, the grain size and the residual stress. The most appropriate stabiliser is Y_2O_3 when added between 3.5 and 8

mol%. Theoretical density of the material should be more than 99%, with the grain size less than 0.3μm and negligible monoclinic content. Residual tensile stress should be less than 300 MPa [71].

The most widely used stabiliser is Y_2O_3 in the content of 3 mol% (corresponds to 5.1% by weight) for the stabilisation of a tetragonal and 8 mol% for the stabilisation of a cubic form of Y-TZP [71]. It is well known that Y-TZP is prone to LTD in the presence of water. To date, there are several proposed mechanisms [60] [74] [75] that explain this phenomenon, but none of them is confirmed. Anyway, some facts are accepted regarding the origin and spreading of the micro-cracks: *t–m* grain transformation starts from the surface of the material and then proceeds inward, causing a surface uplift [76] [77] and creating microcracks [78], that enables water penetration below the surface. Propagation of the *t–m* grain transformation into the material [79], leads to the development of major cracks [78] that eventually ends with a catastrophic failure of the restoration. Low-temperature degradation of the Y-TZP has encouraged researchers to look for other stabilisers; aluminium trioxide, Al_2O_3, seems to have a crucial role in the ageing stability of Y-TZP ceramics and is used in very low content, with alumina particles optimally distributed within the zirconia material. It can be added independently of yttria stabiliser and its content, in the amount of 0.25 wt% having a higher degradation retarding effect to Y-TZP ceramics than 2 or 5 wt% of alumina addition, which have a comparable effect. According to Zhang et al., the apparent activation energy for the degradation process is increased by adding alumina in higher content [68].

Stabilization by cerium oxide provides better thermal stability and resistance to 'low-temperature degradation' than Y-TZP. CeO_2 gives zirconia the best properties regarding phase transformation although it is needed in larger amount comparing to Y_2O_3 to maintain the same degree of stability [80]. A Ce-TZP material with 8 mol% of ceria was characterised by less than 10% monoclinic content on the surface after 360 h water storage at 80°C [81]. When containing more than 12 mol% ceria, the system is almost non-transformable [82]. Another positive effect of adding ceria to zirconia is the pseudo-plastic behaviour of this compound (Ce-TZP can bend before fracturing) that is the most expressed feature among all other ceramic materials [83]. The major drawback of Ce-TZP ceramic materials that are commercially available is the chemical instability of Ce^{4+} that can be relatively easily reduced to Ce^{3+}, which does not have the same stabilising ability toward *t*-zirconia [84]. On the other hand, CeO_2 is yellow, affecting the colour of the final Ce-TZP restoration from light yellow to almost brownish, that may even become dark grey due to the high concentration of oxygen vacancies [84] [85].

Schmauder and Schubert [86] have shown that stress plays a critical role in the *t–m* transformation of zirconia grains as it does not occur in stress-free zones of the material, even if it contains an insufficient amount of stabiliser. As mentioned before, stress can be "applied" (tensile stress, temperature shock, an overloading in patient with parafunction), but it may also appear as a "residual stress" occurring during fabrication process of the restoration when it is fired at high temperature and then cooled down to room temperature, during sintering or veneering with materials having a different coefficient of thermal expansion (CTE) [71]. The residual stress has so-called "autocatalytic effect" that can be explained by prolonged *t–m* transformation. When some *t*-zirconia grains transforms to *m*-phase for any reason, zirconia becomes two-phase material with *m*-zirconia in compression and the remnant *t*-zirconia in tension; it is assumed that for every 10 vol% of *t–m* transformation, the tensile stress in the remnant *t*-zirconia increases by 250 MPa [87]. Because of this tensile stress, *t*-zirconia will be more prone to further transformation to *m*-zirconia. Experimentally, it was confirmed that the tensile stress initiated by *t–m* transformation as a result of the material bending, could reach a value of 700 MPa [88]. The outcome is slow increasing of the monoclinic content inside the zirconia.

Another factor that influences the stability of the tetragonal phase and LTD is an average size of the zirconia grains. Reducing the grain size (GS) has a beneficial effect on the stability of the zirconia-based materials; a reduction below a certain critical value has a potential of fully inhibiting LTD [71]. When at room temperature, the grains' critical size of pure zirconia powder is in the range of 5–10 nm, whereas in 1.5 Y-TZP powders it is about 90nm [89]. Anyway, when solid, dense zirconia material is observed, "the *t*-form can be stable even if the grains are much larger than the critical GS identified above for powders"; however, zirconia GS should be less than 300 nm to prevent LTD over a period [71]. Exact calculation of optimal GS in a solid material is complex, as the effect of different stabilisers and their content, as well as strain energy, should also be taken into consideration.

Sintering process, temperature and the dwell time influence the ageing stability of zirconia as well. Hallmann et al., [90], didn't find *t–m* phase transformation when Y-TZP has been sintered at a temperature of 1350°C. The diffusion-controlled *t–m* phase transformation initiated at 1450°C and became remarkable when the sintering temperature was increased to 1600°C [90]. Similarly, Inokoshi et al., [91] have demonstrated that increasing the sintering temperatures and elongating the dwell time (2 or 4 hours), increased the ZrO_2 grain size and decreased yttria content in the remaining tetragonal grains, which eventually lead to a higher sensitivity to LTD of Y-TZP ceramics. The best resistance to low-temperature degradation can be achieved by sintering at 1450°C with a one h dwell time [91]. According to Hjerppe et al., the bi-axial flexural strength of zirconia was not

affected by decreasing the sintering time or thermocycling, although the amount of monoclinic phase on the surface of zirconia was increased after thermocycling and water storage [92].

The effect of increased sintering temperature (from 1350°C to 1550°C) on the LTD sensitivity can be explained by the following mechanism: it causes increasing of the average size of the tetragonal ZrO_2 grains, whereas increasing content of the cubic zirconia grains that are enriched with yttrium concomitantly results in a decreased yttrium content as a stabilizer in the remaining tetragonal grains [93].

However, decreasing the sintering temperature to obtain 3Y-TZPs resistant to LTD, leads to the creation of a material with moderate mechanical properties, i.e. lower fracture toughness. To improve surface degradation resistance, Zhang et al., [94] proposed a new way of material production, incorporating stabiliser by coating yttria in the starting powder of 3Y-TZP, a method different than co-precipitation. Optimization of the mechanical properties can be achieved by adding 0.25 wt% Al_2O_3 that allows sintering at a lower temperature to obtain a higher LTD resistance without compromising the hardness and the fracture toughness. The improved LTD resistance in such material could be attributed to the segregation of Al^{3+} at the grain boundary and the heterogeneously distributed Y^{3+} stabiliser [94].

But, not only sintering conditions influence the LTD behaviour of the zirconia materials. Surface treatments (ST) of dental zirconia conducted before adhesive luting, have a great impact on the monoclinic content and thereby ageing sensitivity, i.e. degrading susceptibility. Some monoclinic grains have been observed after sandblasting the surface, with no particular influence of the abrasive particles' size on the LTD of Y-TZP [90]. Anyway, the effect of surface treatment mostly depends on the chemical composition-oxides used as stabilisers. Inokoshi et al., have shown that ST improved the ageing resistance of Y-TZP zirconia, although the highest monoclinic volume fraction was observed in Al_2O_3 sandblasted zirconia. The LTD behaviour of Ce-TZP/Al_2O_3 zirconia (with higher initial monoclinic volume fraction after ST, but stronger ageing resistance compared to the Y-TZP zirconia) was not affected by conducted treatments. Y-TZP/alumina (Y-TZP/Al_2O_3) zirconia showed a strong ageing resistance when the surface was left untreated; insignificant degradation was observed after ST [95].

All the factors mentioned above influence the crystallographic structure of the zirconia when at room temperature. The grains' transformation from tetragonal to monoclinic form is a one-way process, meaning that "once it takes place, the crack-hindering effect cannot be exploited for limiting further fractures" [2]. Restorations made of zirconia with monoclinic state of the grains are more prone to catastrophic failure. The solution for such a negative outcome has been found in the so-called "regeneration firing" that is conducted after final adjustment of the already sintered restoration. Annealing the material in the temperature range 900–1000°C for a short time, induces the reverse phase transformation from monoclinic back to tetragonal form [96].

Beside LTD, shading of the zirconia frameworks before sintering also has a negative effect on the biaxial strength and surface microhardness. Some reductions in bi-axial flexural strength, as well as dimensional changes, are seen when colouring zirconia in green-stage form. Shaded zirconia has lost less weight after sintering than un-shaded, indicating that colourant particles have been incorporated in the crystal structure of the material. The shrinkage of zirconia during sintering is also diminished by shading, which "might have clinical effects as the marginal accuracy, fit and cement thickness of the shaded is different to the un-shaded substructures" [97].

There are different types of zirconia materials depending on the zirconia's grains phase statement (composition), but only three are used in dentistry: (1) *partially stabilized zirconia (PSZ)*, a two-phase material with the tetragonal phase (transformable *t*-zirconia grains) precipitated into the cubic matrix, where stabilization is accomplished by magnesia [44] [71]; there is only one available MgO-PSZ product for the dental market, Denzir-M® by Dentronic AB, Skellefteå, Sweden, suitable for hard machining. (2) *Zirconia toughened composites (ZTC)* - a matrix with high elastic modulus embedding transformable *t*-zirconia grains; the most used matrix is alumina, so the material is known as zirconia-toughened alumina (ZTA) (this material is mostly used for the medical prostheses fabrication) [98]. (3) *Tetragonal zirconia polycrystals (TZP)* - the whole material consists of transformable *t*-zirconia grains. Stabilized with yttria as Y-TZP, has been used in dentistry for production of orthodontic ceramic brackets [99] and endodontic posts [100] since the early 1990s, as well as fixed restorations since the later 1990s [101]. PSZ and ZTC are two-phase materials, whereas TZP is a single-phase [71].

As mentioned before, zirconia blocks for the CAD/CAM technology could be used in their pre-sintered or sintered state. For better quality of the restoration, it is better if *pre-sintered* chalk-like blocks are used (in so-called *'green' stage*), with porosity in their microstructure (50% for IPS e.max ZirCAD), so the milling process is easier, the average milling time is reduced, and the milling tools can be used longer. After milling in the CAM system, enlarged crown and bridge substructures undergo sintering process (1350–1500°C). During the sintering, shrinking of the restorations (20–25%) occurs, causing the structure densification to more than 99%, so the final properties of the material are achieved [102].

Zirconia blocks for computer-aided-

manufacturing can also be obtained from the manufacturers as industrially sintered; *hot iso-static pressed 'HIP' zirconia* (known as 'white blocks'). This material is in its final high strength and is characterised by a constant grading and better homogeneity, with no need of further sintering after milling [103]. According to Stawarczyk et al., hipped zirconia has the highest flexural strength compared to several pre-sintered zirconia materials tested after sintering [104]. Anyway, milling time is longer and wear of the milling tools is higher compared to milling of pre-sintered zirconia blocks [53]. The most important thing when milling hipped zirconia is substantial amounts of surface and structural defects caused by diamond burs, which negatively impact the permanent strength and durability of the ceramic.

Zirconia-based materials have the highest strength, fracture resistance, fracture toughness and Vickers hardness among all other ceramic materials: high fracture toughness of 6–15 $MPa \cdot m^{-0.5}$, flexural strength of more than 900 MPa, high Vickers hardness of 1200–1350 HVN, high thermal expansion of more than $10 \cdot 10^{-6}$ K^{-1}, very low thermal conductivity of less than 2 W/mK (vs. 200 W/mK for gold alloy, and 40 W/mK for base metal), and a good thermo-shock resistance of ΔT=400–500°C [105].

Resin-Matrix Ceramics

In the last six years, a new category of dental materials has been promoted, hybrids, consisting of an organic matrix highly filled with ceramic particles [6] [7] [15] [106].

These materials can be included in a classification system of dental ceramics [7] as the 2013 version of the American Dental Association Code on Dental Procedures and Nomenclature [107] defines the term porcelain/ceramic as "pressed, fired, polished, or milled materials containing predominantly inorganic refractory compounds - including porcelains, glasses, ceramics, and glass-ceramics". The former version (2012) of the referred code, didn't allow materials with resin matrix to be classified as ceramic materials, as it has defined porcelain/ceramic as "non-metal, non-resin inorganic refractory compounds processed at high temperatures (600°C and above) and pressed, polished, or milled, including porcelains, glasses, and glass-ceramics". As these hybrid ceramics are composed predominantly (> 50% by weight) of inorganic compounds, it would be justified if they are taken into consideration while making systematisation of dental ceramic materials.

First, of them, Lava Ultimate from 3M ESPE has been marketed as ***'Resin Nano Ceramic'*** *(RNC)* as it contains nanoceramic particles (nanomer and nanocluster particles) bound in a highly cross-linked polymeric matrix. Lava Ultimate contains two types of nanomers that are monodispersed, nonaggregated and nonagglomerated: silica nanomers of 20 nm diameter and zirconia nanomers of 4 to 11 nm in diameter. The nanocluster particles (with the average size of 0.6–10.0 μm) are synthesized and composed of 20 nm silica nanomers and 4–11 nm zirconia nanomers. Nano-dimension of the particles, allows a high proportion of ceramic filler (approximately 80% by weight) to be incorporated into the resin. Both of them, nanomers and nanoclusters are treated with a silane coupling agent so that chemical bonds can be provided between ceramic particles and the resin matrix. The material is processed several hours in a special heat treatment process, which results in highly cured material, so there is no need of further firing after milling [108].

Specific composition and production technology resulted in a material with higher flexural strength (200 MPa), fracture and wear resistance than composite materials (provided by nanoclusters), and with significantly improved polishability and optical properties (because of the nanoparticles). The polymeric resin as a matrix contributes to some properties that composites have: the material is not brittle and is fracture resistant, with shock absorbing characteristics. Despite a high ceramic content, this material is not recommended for the production of crowns, but only for inlays, onlays and veneers [108].

Intact resin matrix with embedded ceramic nano-particles could be observed after HF etching of the material's surface [19] [22].

Taking into consideration a specific structure and composition of the spongy bone and dentin, consisted of relatively weak and brittle inorganic constituents and organic matrix that provides elasticity, a novel kind of interpenetrating phase material has been developed [15] [109] referred as ***'Polymer Infiltrated Ceramic Network'*** *(PICN)*.

VITA Enamic from VITA, have two 3-dimensional network structures interpenetrating one to another; the dominant fine-structure feldspar ceramic network (86% by weight or 75% by volume) is strengthened by a polymer network consisting of methacrylate polymer (14% by weight or 25% by volume). The blocks are manufactured in few steps: first, the ceramic powder is initially compressed into blocks followed by a sintering process to obtain a porous ceramic network. Next, a ceramic base structure is conditioned with a coupling agent. The conditioned porous inorganic network is finally infiltrated with a monomer mixture, followed by heat-induced polymerisation to create a polymer network. Both of the networks are interconnected through the chemical bonds obtained by the coupling agent [15] [110].

To reproduce the natural shade gradient, in 2017, VITA has started production of the VITA Enamic multiColor, blocks with six finely nuanced layers, from cervical to incisal area [111].

Table 1: Composition, properties and clinical indications of CAD/CAM blocks as published by representative manufacturers

Ceramic type	Product name Manufacturer	Chemical Composition mass%	Flexural strength MPa	Modulus of elasticity GPa	Clinical indications
Feldspar	VITABLOCS®, VITA Zahnfabrik: Mark I (1985) Mark II (1991) VITA TriLuxe (2003) VITA TriLuxe forte (2007) VITA RealLife (2010)	SiO_2 Al_2O_3 Na_2O K_2O CaO TiO_2 pigments	56-64 20-23 6-9 6-8 0.3-0.6 0.0-0.1 <0.1	154	45	veneers, inlays, onlays, partial crowns, anterior and posterior crowns, as a veneering CAD/CAM material for multi-unit bridge substructure made of oxide ceramic
Leucite	IPS Empress CAD, Ivoclar Vivadent (2006) IPS Empress CAD Multi	SiO_2 Al_2O_3 K_2O Na_2O BaO, CaO, CeO_2, B_2O_3, TiO_2 pigments	60.0-65.0 16.0-20.0 10.0-14.0 3.5-6.5 0.5-7.0 0.2-1.0	160	62	veneers, inlays, onlays, partial crowns, anterior and posterior crowns
Lithium-disilicate	IPS e.max CAD, Ivoclar Vivadent (2006)	SiO_2 Li_2O K_2O P_2O_5 ZrO_2 ZnO Al_2O_3 MgO pigments	57.0-80.0 11.0-19.0 < 13 < 11 < 8.0 < 8.0 < 5.0 < o 5.0 < 8	360±40	95	veneers, inlays, onlays, partial crowns, anterior and posterior crowns, 3-unit bridges (anterior and premolar), hybrid abutments, hybrid abutment crowns; 3-unit posterior bridges, as a veneering CAD/CAM material of multi-unit bridge substructure made of IPS e.max ZirCAD
Lithium-silicate	Celtra Duo, Dentsply (2013)	ZrO_2	10	370		veneers, inlays, onlays, partial crowns, anterior and posterior crowns, implant-supported crown
	VITA Suprinity®, VITA Zahnfabrik (2013)	SiO_2 Li_2O ZrO_2 P_2O_5 Al_2O_3 K_2O CeO2 pigments	56-64 15-21 8-12 3-8 1-4 1-4 0-4 0-6	420	70	veneers, inlays, onlays, partial crowns, anterior and posterior crowns, implant-supported crown
Zirconia	Vita In-Ceram® YZ, VITA Zahnfabrik (2002)	ZrO_2 Y_2O_3 HfO_2 Al_2O_3 SiO_2 Na_2O	91-94 4-6 2-4 <0.1 <0.1 <0.1	>900	210 GPa	primary telescope crowns, anterior and posterior crowns, anterior and posterior bridge (up to 2 pontics), cantilever bridges
	Lava™ Frame Zirconia, 3M ESPE (2001)	ZrO_2 Y_2O_3 Al_2O_3		>1100	210	anterior and posterior crowns, splinted crowns up to 4 units, implant abutments, crowns on implant abutments, 3-unit inlay and onlay bridges, cantilever bridges, anterior adhesive bridges, 3–4 unit bridges , long-span and curved bridges
	IPS e.max ZirCAD, Ivoclar Vivadent (2006)	ZrO_2 Y_2O_3 HfO_2 Al_2O_3 other oxides	87-95 4-6 1-5 0-1	900±50		anterior and posterior crowns, primary telescope crowns, implant superstructures, 3-unit bridges (anterior, premolar and posterior), multiple-unit bridges, inlay bridge frameworks
All Zirconia	Lava™ Plus High Translucency Zirconia, 3M ESPE (2012)	ZrO_2 Y_2O_3 Al_2O_3	>90 3 0.1	>1100	210	anterior and posterior crowns, splinted crowns up to 4 units, implant abutments, crowns on implant abutments, primary crowns, 3-unit inlay and onlay bridges, cantilever bridges, anterior adhesive bridges, 3–4 unit bridges , long-span and curved bridges
	Cercon® ht True Color, Dentsply, Degudent (2015)	ZrO_2 Y_2O_3 HfO_2 Al_2O_3 SiO_2	 5 <3 <1	1200	210	anterior and posterior crowns, primary telescope crowns, multi-unit bridges
	Zenostar® Full Contour Zirconia, Wieland Dental/Ivoclar Vivadent (2013)	ZrO_2 Y_2O_3 HfO_2 Al_2O_3	>99 4.5-6.0 <5 <1	1200±200	210	anterior and posterior crowns, primary telescope crowns, multi-unit bridges (as frameworks or full-contour)
Hybride	Lava™ Ultimate CAD/CAM Restorative, 3M ESPE (2011)	Ceramic: ZrO_2 SiO_2 Resin: Bis-GMA, UDMA, Bis-EMA, TEGDMA	⅛ ⅛	204	13	veneers, inlays, onlays
	VITA Enamic®, VITA Zahnfabrik (2013) Vita Enamic® multiColor (2017)	Ceramic network: SiO_2 Al_2O_3 Na_2O K_2O B_2O_3 CaO ZrO_2 KaO TiO_2 Polymer network: UDMA TEGDMA	wt%: 58-63 20-23 9-11 4-6 0.5-2 0.3-0.6 < 1 < 1 <0.1 66 33	150-160	30	veneers, inlays, onlays anterior and posterior crowns, implant-supported crowns
	CERASMART™, GC (2014)	Ceramic network: SiO_2 Barium glass Monomer: Bis-MEPP UDMA DMA	71 29	231		veneers, inlays, onlays anterior and posterior crowns, implant-supported crowns

Bis-GMA: bisphenol A diglycidylether methacrylate; Bis-MEPP: 2,2-Bis(4-methacryloxypolyethoxyphenyl)propane; UDMA: urethane dimethacrylate; TEGDMA: triethylene glycol dimethacrylate; Bis-EMA: ethoxylated bisphenol-A dimethacrylate; DMA: dimethacrylate.

The flexural strength of this two-phase material can reach a value of about 150–160 MPa, significantly higher than that of a porous ceramic (below 30 MPa) and polymer (135 MPa) alone [112]. The superior flexural strength of the resin-infiltrated ceramic material compared to the single components implies a reinforcement mechanism of the polymer network to the dominant ceramic network [106] [112]. Elastic modulus, hardness, and fracture toughness values are as follows: 30.14 GPa, 2.59 GPa, and 1.72 $MPa \cdot m^{-0.5}$ respectively [5], all of them between those obtained for human dentin and enamel. With the highest filler content (73.1 mass %) compared to other hybrid ceramics and composites, Vita Enamic has the highest Vickers hardness of 189.8 [113]. Moreover, VITA Enamic has similar two-body and tooth-brushing wear characteristic to natural enamel [114]. All these features, together with the possibility material to be milled very thin thus preserving the tooth structures, are in favour of using this material in patients with erosions where tooth preparation is not recommended [115].

HF etching of VITA Enamic causes dissolving of the superficial ceramic network, so that acrylic polymer network became visible with scattered irregular ceramic particles [19] [22].

The third, CERASMART™ from GC, referred to as ***"Flexible Nano Ceramic"***, is composed of relatively small and uniformly distributed particles of alumina-barium-silicate embedded in a polymer matrix [116]. The flexural strength (approx. 242 MPa) is significantly higher, whereas the flexural modulus (10.0 GPa) and Vickers hardness (approx. 64.1 HV) are significantly lower than Lava Ultimate (170.5 MPa / 14.5 GPa / 97.9 HV) and VITA Enamic (140.7 MPa / 28.5 GPa /189.8HV) respectively [113]. The modulus of resilience of 3.07 ± 0.45 MPa is the highest, and the margin edge roughness of 60 ± 16 μm is the lowest compared to other ceramics or hybrid materials [117].

All hybrids have a modulus of elasticity similar to dentin, and modulus of resilience significantly higher than feldspar-based and glass-ceramics, thus significantly higher stress can be absorbed without permanent deformation or failure. That is why hybrids are the most recommended materials for fabrication of crowns over implants, where the periodontal ligament (tissue that acts as a shock absorber) is already lost.

Milling time in the CAM unit is shorter compared to other ceramic materials, with longer lifetime on the milling burs. There is no need for sintering or crystallization firing after milling; final gloss and smoothness of the restoration can be achieved by surface polishing. Hybrids are wear resistant and "gentle" to the opposite dentition [115]. Restorations can be easily repaired in the mouth, although these materials are characterized by virtually no chipping.

Clinical Indications

Feldspar-based and leucite-reinforced ceramics according to their low flexural strength (154MPa and 160MPa respectively) are indicated for single tooth restorations such as veneers, inlays, onlays, partial crowns, and anterior and posterior crowns. Beside the listed, zirconia-reinforced lithium silicate (420 MPa) can also be used for the fabrication of implant-supported crowns. Having the high crystalline content, specific microstructure and flexural strength of 360–400 MPa, lithium disilicate ceramic can be used not only for single tooth restorations but the fabrication of hybrid abutments, hybrid abutment crowns and three-unit bridges as well (up to the second premolar as the terminal abutment). Due to the polymer content, low flexural strength (150–240 MPa) and high resilience, hybrid ceramics can be used only for single tooth restorations such as veneers, inlays and onlays (Lava Ultimate), as well as for anterior and posterior crowns and implant-supported crowns (VITA Enamic and Cerasmart).

Densely sintered, high–purity alumina can be used for primary elements of conical and telescopic crowns, crowns in the anterior and posterior area, as well as for bridges only in the anterior area with no more than 1 pontic (due to the average high flexural strength of 500 MPa).

Yttria-stabilized zirconia (with high flexural strength of more than 900 MPa) is indicated for fabrication of anterior and posterior crowns, implant abutments, implant abutments crowns, primary telescope crowns, 3-unit inlay and onlay bridges, cantilever bridges with minimum two abutment teeth and maximum of one pontic of no more than one premolar width, anterior adhesive bridges, as well as multi-unit long-span (up to 14 units) and curved bridges with a maximum of four pontics next to one another in the anterior area, and a maximum of three pontics (DC-Zircon) next to one another between abutment teeth in the posterior area. Some of them (Lava™ Plus High Translucency Zirconia, 3M ESPE) are indicated for clinical situations with limited interocclusal space, as well as when the tooth-preserving preparation is needed (minimum 0.5 mm occlusal wall thickness). CAD/CAM ceramic materials, their chemical composition, some properties and clinical indications as claimed by the respective manufacturers are presented in Table 1.

Conclusion

Although it seems that current ceramic materials could "cover" every clinical situation where

single-tooth restoration and fixed partial dentures are indicated for use, there are some limitations that should be taken seriously. All-ceramic restorations are not recommended in patients with insufficient remaining natural tooth substance (short clinical crowns), sub-gingival preparations (mainly for adhesive luting), inadequate oral hygiene, and patients diagnosed with excessive masticatory functions, in particular teeth grinders and clenchers.

The future of all-ceramic materials is bright. Further improvements in chemical composition, internal structure, grain-size decreasing to nano dimensions and improved protocols for industrial production and laboratory processing, all of them will for sure lead to a material with extraordinary features fulfilling the esthetic, mechanical and biocompatible demands.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1756-1761.
https://doi.org/10.3889/oamjms.2018.314
eISSN: 1857-9655
Public Health

The Relationship Between Lifestyle, Health Promotion Lifestyle Profile II And High Blood Pressure In University Students

Gülden Aynaci*, Ozlem Akdemir

Trakya University, Edirne, Turkey

Citation: Aynaci G, Akdemir O. The Relationship Between Lifestyle, Health Promotion Lifestyle Profile II and High Blood Pressure In University Students. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1756-1761. https://doi.org/10.3889/oamjms.2018.314

Keywords: Healthy lifestyle; Students; Young people

***Correspondence:** Gülden Aynaci. Trakya University, Edirne, Turkey. E-mail: guldenaynaci@hotmail.com

Received: 28-May-2018; **Revised:** 06-Aug-2018; **Accepted:** 07-Aug-2018; **Online first:** 18-Sep-2018

Funding: This research did not receive any financial support

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

Abstract

BACKGROUND: Identifying and controlling systemic arterial blood pressure is important in young people, and it is possible to reduce the frequency of systemic arterial hypertension by improving the lifestyle.

AIM: The aim of the study is to assess the relationship between healthy lifestyle behaviors and systemic blood pressure in university students.

MATERIALS: The study sample consisted of 200 university students from a state university in Edirne. Lifestyles and habits were evaluated with Health Promotion Lifestyle Profile II. Students' blood pressure was measured from both arms twice.

RESULTS: The mean HPLP-II score of those who frequently feel good was significantly higher than those who rarely feel good. The mean score of those who frequently wake up between 06:00-09:00 in the morning was statistically significantly higher than those who wake up outside these hours. Those who perform social or artistic activities during their leisure times had a mean scale score higher than those who don't perform. Although there wasn't a statistically significant difference according to smoking status, the mean score of non-smokers was higher than smokers. The mean scale scores were higher in frequent salt users than non-frequent users; participants with low saturated fatty acid intake had higher scores than those with high intake, and rare fast food consumers had higher scores than frequent consumers. The statistically significant difference between blood pressure values of females and males was due to higher blood pressure in male students. Those working in a part-time job had higher blood pressure values than those who weren't working. Among the students whose body mass indexes could be evaluated, there were differences in blood pressure values.

CONCLUSION: It has been observed in our study that health-related responsibilities and lifestyle behaviours increase with better leisure time activities, improved eating habits and a positive outlook on life. Turning youngs' tendencies towards healthy lifestyle behaviours to habits can make them healthier, more collective and more productive regarding physical, social and psychological well-being.

Introduction

Systemic arterial hypertension is a clinical, multifactorial disease characterised by increased blood pressure. It is generally seen together with structural and functional changes in target organs (heart, brain, kidneys) and as a result risk for cardiovascular events increases [1]. High blood pressure is the biggest contributor to the disease and death burden worldwide, and 9.4 million deaths occur each year [2]. Because it is highly dependent on changeable risk factors, the frequency of deaths can be prevented by directing lifestyle to a healthy pathway [3]. Hypertension and its complications may start at young ages [4]. Identifying and controlling systemic arterial blood pressure is important in young people, and it is possible to reduce the frequency of systemic arterial hypertension by improving the lifestyle [5].

It is accepted that the healthy lifestyle behaviours are the main way particularly to prevent chronic diseases. For this reason, regulation of lifestyle is important for protecting and improving health. A healthy lifestyle is a way of life that sustains and improves one's health and well-being. Most importantly, it involves a healthy diet, physical activities, regular life, coping with stress, interpersonal communication and health responsibility [6] [7].

In young people, a high body mass index, unhealthy eating habits, family history of hypertension, and the tendency for rising blood pressure are risk factors for hypertension. Hypertension can lead to death silently over the years [8] [9] [10] [11].

To increase the level of a healthy lifestyle, it is first necessary to evaluate behaviours. Before any intervention to improve healthy behaviours, it is very important to evaluate the way of life at present. For this purpose, the Health Promoting Lifestyle Profile which has accepted efficiency and reliability may be used [12]. This scale is widely used in the world. It may be used to evaluate the health of adolescent mothers and their families [13], elderly women and their health sustainability [14], as a preliminary test of a program for the prevention of type 2 diabetes in high-risk adolescents [15], to evaluate lifestyle behaviors after a major surgery [16], in studies that evaluate patient education and lifestyle in chronic diseases [17], and to evaluate health-promoting features in young people [18].

The aim of our study is to assess healthy lifestyle behaviors in university students and their relation to systemic blood pressure. Sociodemographic characteristics, habits, sleep quality, nutritional characteristics, healthy lifestyle behaviors and blood pressure levels were evaluated.

Materials

The study universe consisted of university students in Edirne, and the study sample consisted of 201 university students from a state university in Edirne. To evaluate the demographic information and lifestyles of the patients “Personal Information Form” was used. In the personal information form in addition to demographic features questions were asked about sleep hours, cigarette and alcohol use, eating habits, physical activities, social media use, and presence of hypertension in the family.

Lifestyles and habits were evaluated with Health Promotion Lifestyle Profile II. This scale is the revised form of Health Promotion Lifestyle Profile which was developed by Walker et al., [12]. It evaluates health-promoting behaviours related to a healthy lifestyle. It has 52 items. In the assessment of this scale, the lowest possible score is 52, and the highest possible score is 208.

Several studies conducted in various countries and with various study groups have compared this scale with various scales that assess the lifestyles of individuals. It is considered to be effective and reliable to assess healthy lifestyle behaviours [19] [20] [21].

In our study, voluntary students rested for 10 minutes before blood pressure measurement. Using a standard mercury sphygmomanometer covering two-thirds of the upper arm and having an appropriate cuff size, blood pressure was measured at sitting position from both arms twice with a 10-minute interval, and care was exercised to ensure that no cigarettes or caffeinated food were received within 30 minutes before the measurement. The higher of the two measurements was recorded.

Table 1: Definition and classification of systemic blood pressure levels*

CLASSIFICATION	Systolic blood pressure (mmHg)		Diastolic blood pressure (mmHg)
Optimal	<120	and	<80
Normal	120-129	and/or	80-84
High normal	130-139	and/or	85-89
Stage 1 hypertension	140-159	and/or	90-99
Stage 2 hypertension	160-179	and/or	100-109
Stage 3 hypertension	≥180	and	≥110

*2016 European Guidelines on cardiovascular disease prevention in clinical practice. European Heart Journal.

Approval was obtained from the Clinical Trials Ethics Committee of the University and informed consents were obtained from all participants.

All statistical analyses were performed with SPSS 20.0 Package Program. Normal distribution of the data was controlled with Shapiro-Wilk test. Two group comparisons were performed with the Student t-test. Multiple comparisons after one-way analysis of variance were evaluated with the Bonferroni test. Chi-square test was used for the relations between categorical variables. Descriptive statistics for numerical variables were given as mean and standard deviation. Descriptive statistics for categorical variables were given as percentage and frequency. The statistical significance level for all statistical analyses was defined as 5%.

Results

Table 2 shows the demographic features of the participants. This study involved university students between 18-24 years of age. The mean age of female students was 20.5 ± 1.73, and male students were 20.9 ± 1.77. Eighty per cent of the study sample was females. Some students from Health Vocational High School and Applied Sciences High School were equal. According to the place of residence during education 172 (86%) were staying at the dormitory and 26 (14%) were staying at home. 176 (88%) participants found their income level adequate, and 24 (12%) said it was low.

The mean score obtained by the students of Health Vocational High School from Health Promotion Lifestyle Profile II (HPLP II) (132.05 ± 1.70) was statistically significantly higher than the mean score obtained by the students from Applied Sciences Vocational High School (126.14 ± 1.91). No difference could be found according to income level and place of

residence during school; the score of those who didn't work in additional jobs during school was found to be statistically significantly higher than those who work.

Table 2: Demographic features and the mean HPLP-II scores of the participants in this study

	N	%	Mean Score from the Scale	Standard Deviation	P
Gender					
Male	40	20.0	126.80	2.846	0.370
Female	160	80.0	129.69	1.443	
School					
Health Vocational High School	100	50.0	132.05	1.703	0.022*
Applied Sciences High School	100	50.0	126.14	1.911	
Place of Residence					
House	26	14.0	125.6	3.004	0.312
Dormitory	172	86.0	129.5	1.424	
Family income status					
Low	24	12.0	123.8	3.718	0.151
Good	176	88.0	129.6	1.367	
Working in a part-time job					
Yes	16	9.0	116.9	4.638	0.06*
No	182	91.0	129.97	1.318	

*****indicates those with a statistically significant difference.

Table 3 evaluates lifestyle features of study participants. The mean HPLP-II score of those who frequently feel good (131.61 ± 1.47) was significantly higher than those who rarely feel good (123.36 ± 2.44). The mean score of those who frequently wake up between 06:00-09:00 in the morning (131.06 ± 1.46) was statistically significantly higher than those who wake up outside these hours. Although there wasn't a difference in sleep duration, the mean score of those that sleep 6-8 hours a day was higher than those who sleep less or more.

Table 3: Lifestyle features and the mean HPLP-II scores of the study participants

	N	%	The mean scale score	Standard deviation	P
Satisfaction with health					
Absent	6	3.0	119.67	4.410	0.187
Moderate	104	52.0	127.83	1.769	
Good	90	45.0	131.23	1.957	
How does he/she frequently feel					
Good-very good	140	70.0	131.61	1.479	0.003*
Moderate	59	29.5	123.36	2.443	
Sleep duration / in 24 hours					
< 5 hours	22	11.0	127.27	4.805	0.652
6-8 hours	160	80.0	129.70	1.394	
> 8 hours	18	9.0	126.17	4.243	
Sleeping time					
Before 12:00 pm	57	28.5	132.47	2.176	0.099
After 12:00 pm	143	71.5	127.78	1.567	
Morning waking time					
Before 06:00 o'clock	8	4.0	113.38	6.918	0.010*
Between 06:00-09:00	144	72.0	131.06	1.469	
After 09:00 o'clock	48	24.0	125.90	2.641	
Regular sports/ exercise					
Never	140	70.0	125.21	1.475	<0.001*
< 2 hours /week	10	5.0	133.70	4.412	
> 2 hours /week	50	25.0	139.14	2.426	
Breakfast					
0-3 days/ week	39	19.5	126.28	2.760	0.280
4-7 days/ week	161	80.5	129.80	1.451	
Leisure activities (hobbies, social and artistic activities)					
Absent	18	9.0	116.94	4.828	<0.001*
Moderate	140	70.0	127.79	1.384	
Frequent	42	21.0	138.74	2.920	
Quality of Life					
Bad	8	4.0	111.75	7.991	0.005*
Moderate	146	73.0	128.55	1.426	
Good	46	23.0	133.91	2.757	
Watching TV/ week					
0-1 hour	114	57.0	128.03	1.865	0.586
2-4 hours	56	28.0	131.07	2.197	
5 hours or more	30	15.0	129.60	2.597	
PC, tablet pc, laptop etc.					
0-1 hour /day	31	15.5	126.74	3.555	0.634
2-3 hours /day	74	37.5	128.69	1.830	
4 hours or more /day	95	47.5	130.22	2.000	
The frequency of social media use					
0-1 hour/day	22	11.0	130.27	4.167	0.673
2-3 hours/day	70	35.0	127.56	2.041	
>4 hours/day	108	54.0	129.89	1.801	

*****Indicates statistically significant difference.

Those who perform social or artistic activities during their leisure times had a mean scale score (138.74 ± 2.92) higher than those who don't perform such activities (127.7 ± 1.38). There wasn't any difference according to social media use and use of electronic devices such as a computer or tablet PC.

There wasn't a statistically significant difference between those who regularly exercise or perform sports activities and who don't and who have breakfast 4 times or more or less than 4 times a week but those who perform regular exercises or who have breakfast more than 4 times a week had higher scale scores than others.

Table 4 shows the mean HPLP-II scores of the participants according to risk factors. The mean score of those who use alcohol (131.04 ± 1.37) was higher than those who don't use alcohol (121.25 ± 3.31). Although there wasn't a statistically significant difference according to smoking status, the mean score of non-smokers was higher than smokers.

The mean scale scores were higher in frequent salt users than non-frequent users (133.23 ± 1.68 vs 123.98 ±1.86); participants with low saturated fatty acid intake had higher scores (132.06 ± 1.99) than those with high intake (126.89 ± 1.65), and rare fast food consumers had higher scores (130.18 ± 3.18) than frequent consumers (120.59 ± 3.29). Body mass index was evaluated in 155 students, and no significant difference could be detected. No significant difference was found in the scale score according to the stress level.

Table 4: Distribution of the participants according to risk factors and the mean HPLP-II score

	N	%	The mean scale score	Standard deviation	P
Smoking					
Yes	156	78.0	130.02	1.413	0.186
No	44	22.0	125.91	2.999	
Alcohol consumption					
No	161	80.5	131.04	1.377	0.003*
Yes	36	18.0	121.25	3.316	
Hypertension in a 1st-degree relative					
Yes	51	25.5	129.29	2.692	0.935
No	149	74.5	129.05	1.466	
Consumption of processed food					
0-1 meal/ week	84	42.0	129.36	1.964	0.922
2-3 meals/ week	86	43.0	128.57	2.116	
4 or more meals / week	30	15.0	130.00	2.671	
Salt use					
Normal	111	55.5	133.23	1.683	<0.001*
Frequent- every time	89	44.5	123.98	1.860	
Use of saturated fat- frying oil					
0-1 meal/week	86	43.0	132.06	1.999	0.047*
2 or more meals /week	114	57.0	126.89	1.657	
Fast food consumption					
Never	22	11.0	130.18	3.812	0.023*
1-3 meals/ week	149	74.5	130.62	1.473	
>3 meals/ week	29	14.5	120.59	3.299	
BMI					
Underweight	23	11.5	128.39	3.630	0.440
Normal	114	57	131.71	1.612	
Overweight	18	9.0	126.94	4.119	
Stress level					
Low	24	12.0	126.08	3.780	0.393
Moderate	106	53.0	130.71	1.774	
High	70	35.0	127.74	2.151	

*****indicates the statistically significant difference.

In Table 5 there was a significant difference between systemic arterial blood pressure and the mean scale score. The difference was mainly due to the difference between those with stage 1 hypertension and others. No case with stage 2 or 3 hypertension was detected.

Table 5: Systemic blood pressure values and the mean scale scores of the participants

Blood Pressure Values	N	The mean scale score	Standard deviation	P
Optimal	61	128.30	2.143	
Normal	107	132.44	1.658	<0.001*
High Normal	20	126.25	3.864	
Stage 1 Hypertension	12	108.42	6.612	

*****Indicates statistically significant difference.

Table 6 demonstrates the association between systemic blood pressure values and sociodemographic features. The statistically significant difference between blood pressure values of female and male students was due to higher blood pressure in male students. No significant difference in blood pressure values could be found according to the school they were attending, the place of residence, and their income status. Those working in a part-time job had higher blood pressure values than those who weren't working.

Table 6: Change in systemic blood pressure values according to sociodemographic features

	Optimal	Normal	High Normal	Stage 1	P
Gender					
Female	57 (35.6%)	84 (52.5%)	12 (7.5%)	7 (4.4%)	0.001*
Male	4 (10.0%)	23 (57.5%)	8 (20%)	5 (12.5%)	
School					
Health Vocational High School	29 (29.0%)	57 (57.0%)	11 (11.0%)	3 (3.0%)	0.293
Applied Sciences High School	31 (31.3%)	50 (50.5%)	9 (9.1%)	9 (9.1%)	
Place of residence					
Dormitory	55 (%32.0)	91 (%52.9)	16 (%9.3)	10 (%5.8)	0.653
House	6 (%21.4)	16 (%57.1)	4 (%14.3)	2 (%7.1)	
Family income status					
Low	4 (%17.4)	13 (%56.5)	3 (%13.0)	3 (%13.0)	0.273
Good	57 (%32.2)	94 (%53.1)	17 (%9.6)	9 (%5.1)	
Work in a part-time job					
Yes	3 (18.8%)	8 (50.0%)	1 (6.5%)	4 (25.0%)	0.010*
No	58 (31.9%)	97 (53.3%)	19 (10.4%)	8 (4.4%)	

*****Indicates statistically significant difference.

Table 7 demonstrates the association between lifestyle features and systemic blood pressure of the participants in this study. No difference could be detected in how they frequently feel, daily sleep duration, morning waking time, weekly breakfast frequency, and use of social media and electronic devices such as a computer, tablet pc etc.

Table 7: The association between lifestyle features of the participants and systemic blood pressure values

	Optimal	Normal	High Normal	Stage 1	P
How does he/she frequently feel					
Good- very good	44 (31.4%)	77 (55.0%)	13 (9.3%)	6 (4.3%)	0.385
Moderate	16 (27.1%)	30 (50.8%)	7 (11.9%)	6 (10.2%)	
Sleep duration/in 24 hours					
<5 hours	5 (22.7%)	13 (59.1%)	1 (4.5%)	3 (13.6%)	0.205
6-8 hours	51 (31.9%)	87 (54.4%)	15 (9.4%)	7 (4.4%)	
> 8 hours	5 (27. 8%)	7 (38.9%)	4 (22.2%)	2 (11.1%)	
Sleep time					
Before 24:00 at night	12 (21.1%)	34 (59. 6%)	9 (15.8%)	2 (3.5%)	0.094
After 24:00	49 (34.3%)	73 (51.0%)	11 (7.7%)	10 (7.0%)	
Breakfast					
0-3 days/ week	11 (28.2%)	21 (53.8%)	5 (12.8%)	2 (5.1%)	0.912
4-7 days/ week	50 (31.1%)	86 (53.4%)	15 (9.3%)	10 (6.2%)	
Computer, tablet pc, laptop etc.					
0-1 hour/day	9 (29.0%)	19 (61.3%)	3 (9.7%)	0 (0%)	0.227
2-3 hours /day	23 (31.1%)	42 (56.8%)	3 (4.1%)	6 (8.1%)	
4 hours or more /day	29 (30.5%)	46 (48.4%)	14 (14.7%)	6 (6.3%)	
The frequency of social media use					
0-1 hour/day	5 (22.7%)	13 (59.1%)	2 (9.1%)	2 (9.1%)	0.458
2-3 hours/day	25 (35.7%)	38 (54.3%)	3 (4.3%)	4 (5.7%)	
>4 hours/day	31 (28.7%)	56 (51.9%)	15 (13.9%)	6 (5.6%)	

*****Indicates statistically significant difference.

No statistically significant difference could be found according to whether or not they perform social and artistic activities in their leisure times and whether or not they regularly exercise.

The associations between the risk factors of the study participants and systemic blood pressure values are demonstrated in Table 8. There was a significant difference in systemic blood pressure values between those who use alcohol or not; no difference could be detected according to smoking status, the frequency of salt use, the frequency of consumption of foods including fatty acids, processed food and fast food. Among the students whose body mass indexes could be evaluated, there were differences in blood pressure values. No significant difference could be found according to the stress level.

Table 8: The association between risk factors of the participants and systemic blood pressure values

	Optimal	Normal	High Normal	Stage 1	P
Smoking					
No	49 (31.4%)	87 (55.8%)	11 (7.1%)	9 (5.8%)	0.069
Yes	12 (27.3%)	20 (45.5%)	9 (20.5%)	3 (6.8%)	
Alcohol use					
No	53 (32.9%)	88 (54.7%)	14 (8.7%)	6 (3.7%)	0.016*
Yes	8 (22.2%)	17 (47.2%)	5 (13.9%)	6 (16.7%)	
Salt consumption					
Normal	30 (27.0%)	63 (56.8%)	13 (11.7%)	5 (4.5%)	0.370
Frequent- Every time	31 (34.8%)	44 (49.4%)	7 (7.9%)	7 (7.9%)	
Consumption of saturated fat- frying oil					
0-1 meal/week	23 (26.7%)	52 (60.5%)	9 (10.5%)	2 (2.3%)	0.139
2 or more meals /week	38 (33.3%)	55 (48.2%)	11 (9.6%)	10 (8.8%)	
Fast food consumption					
Never	7 (31.8%)	10 (45.5%)	4 (18. 2%)	1 (4.5%)	0.445
1-3 meals/ week	45 (30.2%)	83 (55.7%)	14 (9.4%)	7 (4.7%)	
>3 meals/ week	9 (31.0%)	14 (48.3%)	2 (6.9%)	4 (13.8%)	
BMI					
Underweight	12 (%52.2)	10 (%43.5)	0 (%.0)	1 (%4.3)	<0.001*
Normal	31 (%27.2)	69 (%8.8)	10 (%8.8)	4 (%3.5)	
Overweight	4 (%22.2)	5 (%27.8)	6 (%33.3)	3 (%16.7)	
Stress level					
Low	9 (%37.5)	9 (%37.5)	2 (%8.3)	4 (%16.7)	0.242
Moderate	33 (%31.1)	56 (%52.8)	12 (%11.3)	5 (%4.7)	
High	19 (%27.1)	42 (%60.0)	6 (%8.6)	3 (%4.3)	

*****Indicates statistically significant difference.

Discussion

Prevalence of hypertension is increasing worldwide, and research has shown that young age group is affected increasingly more especially in the last 20 years. Hypertension increases morbidity and mortality. Prevalence of hypertension in the young age group is important because of the serious consequences of hypertension and the probability of secondary hypertension in this age group. Hypertension is an important preventable risk factor for cardiovascular diseases [22] [23].

Due to changing conditions in every aspect of life in our age, a more passive lifestyle which is not compatible with people's natural structure has become widespread. In every day, school or business

life, stressful and unfavourable conditions can trigger unexpected physical problems as well as some psycho-social disadvantages. Improving physical activity, supporting healthy eating habits, and improving the ability to cope with stress play an important role in maintaining both physical and psychosocial well-being.

In our study, the rate of high-normal systemic arterial pressure in university students was 8.1%, and the rate of hypertension was 5.6%. Results of our study are consistent with studies that found 7.4% hypertension in Ethiopia and 7% hypertension in Kuwait [24]. In Saudi Arabia, 7.5% of the students were hypertensive [25]. These results are consistent with the study that reported 9.3% hypertension prevalence in medical students in Jeddah [26]. In a study conducted at King Fahd University in Dammam, blood pressure was reported to be 13.8% among male students [27]. But this finding is low compared with reported hypertension rates in Nigeria (19.3%), Tunisia (35.1%), and Gambia (38%) [28]. The differences may be due to data collection methods, the socioeconomic status of the evaluated population, and differences in healthy lifestyle behaviours.

As the number of positive health behaviours increases, the score of Health Promotion Lifestyle Profile II also increases [12]. The mean scale score in our study was detected to be 129.69 ± 1.44 in females and 126.80 ± 2.84 in males.

A study published by the American Pediatric Academy reported that young people are more likely to reduce their intake of food than to increase physical activity in healthy lifestyle behaviours to maintain their physical appearance [29]. It has been observed in our study that health-related responsibilities and lifestyle behaviours increase with better leisure time activities, improved eating habits and a positive outlook on life. As the ability of individuals to feel healthy grows, the healthy lifestyle they have acquired will become a habit.

Turning young people's tendencies towards healthy lifestyle behaviours to habits can make them healthier, more collective and more productive regarding physical, social and psychological well-being. Supporting healthy lifestyle behaviours in educational institutions will help to protect youth from chronic diseases such as hypertension as well as contribute to the social development of young people. Thus, healthy and dynamic young people, who are exemplified in the society, will lead the way of making healthy lifestyles attitudes in the whole society.

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https://doi.org/10.3889/oamjms.2018.363
eISSN: 1857-9655
Public Health

The Model of Self Care Behaviour and the Relationship with Quality Of Life, Metabolic Control and Lipid Control of Type 2 Diabetes Mellitus Patients in Binjai City, Indonesia

Rina Amelia*

Department of Community Medicine/Public Health, Faculty of Medicine, Universitas Sumatera Utara, Jl. dr. Mansyur No.5 Kampus USU Medan 20155, Indonesia

Citation: Amelia R. The Model of Self Care Behaviour and the Relationship with Quality Of Life, Metabolic Control and Lipid Control of Type 2 Diabetes Mellitus Patients in Binjai City, Indonesia. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1762-1767. https://doi.org/10.3889/oamjms.2018.363

Keywords: A model of self-care behaviour; Quality of life; Metabolic control; Lipid control

***Correspondence:** Rina Amelia. Department of Community Medicine/Public Health, Faculty of Medicine, Universitas Sumatera Utara, Jl. Dr Mansyur No.5 Kampus USU Medan 20155, Indonesia. E-mail: rina2@usu.id

Received: 08-Aug-2018; **Revised:** 10-Sep-2018; **Accepted:** 11-Sep-2018; **Online first:** 21-Sep-2018

Funding: This study is funded by Directorate of Research and Community Service, General Directorate of Research Strengthening and Development, Ministry of Research, Technology, and High Education by Research Contract Number: 003/SP2H/LT/DRPM/IV/2017 dated April 20, 2017

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

Abstract

BACKGROUND: Diabetes is a type of chronic disease with exceptional medical care for a patient's lifetime, which ultimately requires lifestyle and behavioural adjustments to prevent complications to death. Patients with good self-care behaviour will cause diabetes to be controlled to avoid complications to death and make patients have a better quality of life.

AIM: This study aims (1) to determine the model of self-care behaviour in Type 2 diabetes patients in Binjai City (2) to analyse the effect of self-care behaviour on quality of life, metabolic control and lipid control of Type 2 diabetes patients in Binjai City.

METHODS: This type of research is survey-based and explanatory using a cross-sectional approach. The study population was Type 2 Diabetes Mellitus (T2DM) patients who remained patients in 8 primary health centres in Binjai City. The consecutive sampling yielded a sample size of 115 people. Data analysis method uses descriptive statistics and Structural Equation Modeling (SEM) using SPSS and Amos 16.0.

RESULTS: The results showed that all factors that build T2DM patient self-care behaviour were able to be predictors that shape the patient's self-care behaviour. The self-care behaviour model consists of knowledge, attitudes, communication, financing, family support, motivation, and self-efficacy. Motivation is the most significant predictor of its contribution to the self-care behaviour of Type 2 diabetes patients. Self-care behaviour was also known to be significantly related to the quality of life, metabolic control and lipid control of T2DM patients ($p < 0.05$).

CONCLUSION: Self-care behaviour in T2DM patients can have a substantial and significant impact on quality of life, metabolic control and lipid control possessed by Type 2 Diabetes patients.

Introduction

Population in Indonesia will be dominated by people with diabetes, which is predicted to increase continues to increase from 8.4 million patients in 2000 to 21.3 million in 2030 [1]. Indonesia is also the seventh-ranked country with 8.5 million diabetes patients after China, India and the United States, Brazil, Russia, Mexico [2]. Even today Indonesia's position has moved up, from the seventh rank to fifth as a country with the world's most significant number of people with diabetes. The prevalence of diabetics in North Sumatra in 2013 was 1.8% higher than the national rate, and the results of the previous Indonesian Basic Health Research were 0.8% and 2.3%, the prevalence of diabetes diagnosed by doctors based on symptom interviews was also higher than the national figure (2.1%) [3].

American Association of Diabetes (ADA) states diabetic patients in desperate need of behavioural self-care to care for them to improve their

quality of life and reduce the complications of diabetes [4]. Self-care is the ability of the patient with the family, and the community to promote health, prevent illness, maintain health, and deal with disease and disability with or without the help of health care providers [5]. There are 98% of diabetes care is self-care behaviour [6]. There are 7 main behaviors in self-care, namely: healthy eating (healthy diet), being active (adequate physical activity), monitoring (blood sugar control), taking medicine (consumption of anti-diabetic or insulin drugs, problem solving (problem-solving), healthy coping (healthy coping) and reducing risk (reducing risk) [7].

All of these self-care activities are positively related to the control of the patient's blood sugar levels, reducing complications and improving the quality of life of diabetic patients. Based on several studies it is known that the factors that influence the level of self-care are categorized (1) elements derived from the patient's self, namely knowledge, attitudes, beliefs, concern for their health, low adherence, social, economic, demographic and cultural support (2) factors come from doctors, namely: effective doctor-patient communication, less pleasant doctor-patient relationships, less knowledge about diabetes (3) factors related to healthcare facilities, namely: access to health services, health financing expensive, uneven distribution of health workers [8].

The quality of life of patients with T2DM is influenced by age, gender, social demography, complications, duration of illness, BGL control, psychosocial factors (social support), and therapeutic regimens [9]. Assessment of the quality of life of patients with type 2 diabetes mellitus, using instruments that have been developed, namely the WHOQOL-BREF questionnaire. Besides, the quality assessment uses an examination of the patient's laboratory results by looking at parameters such as HbA1C and fat profile as an indicator that diabetes has been well controlled, to improve the quality of life of people with diabetes [10].

The city of Binjai has now become a city with the rapid economic growth of its population and has an impact on changes in people's lifestyles, as evidenced by increasing the number of Type 2 Diabetes Mellitus patients every year [11]. There have been eight primary health centres in the city of Binjai in recent years, experiencing an increase in the number of people with type 2 diabetes. Data in 2015 states that Type 2 diabetes is ranked as the 10th most disease with 712 cases, while data in 2016 indicates that diabetes has an increase in rank. Seventh with the highest number of illnesses with 1,419 cases [12]. This is in line with the research conducted by [13] in one of the most prominent modern shopping centers (malls) in Binjai city, which shows that out of 1,554 visitors who participated in this study there were 1,238 people (79.7%) who were obese and at risk of developing metabolic disorders such as Type 2 diabetes mellitus. Therefore this condition describes the health of the Binjai community which is related to consumption patterns, eating habits which resulted in an increased prevalence of obesity and diabetes.

This study aims to design a self-care behaviour model for T2DM patients and analyse the effect of the self-care behaviour model on quality of life, metabolic control, and lipid control of T2DM patients in Binjai city. This model is expected to be a reference to improve diabetes patient's self-care behaviour, especially at the level of primary care, and become input to the health services to find out and fulfil the indicators of self-care behaviour so that all diabetic patients have good self-care behaviour.

Methods

This research is analytical research using a cross-sectional approach, consists of 2 steps, i.e. (1) designing a model of self-care behaviour; and (2) analyze the influence of self-care behavior model that has been formulated by research results by the quality of life (QoL) and metabolic control (BGL and HbA1C) and lipid control (total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides) of T2DM patients. The research was conducted in eight main Primary Health Center (PHC) in Binjai for one month. The study population consists of T2DM patients WHO routinely receive treatment at eight main Primary health centre in Binjai. They are specified based on the following criteria: (1) diabetic patients recorded on eight PHC in Binjai, (2) the patient come themselves without the help of others (3) patients aged 40-65 years, (4) patients are willing and committed to participate in the research. However, there was exceptions, (1) diabetes in pregnant women (2) patients with complications and interfere with physical activity, mental and emotional (3) patients can't work well together for the research. (1) Diabetic patients recorded on eight PHC in Binjai (aged 40-65 years) and had attended diabetes service programs regularly, and (2) patients are willing and committed to participate in the research. However, there were exceptions, (1) diabetes in pregnant women, and (2) patients with complications and interfere with physical activity, mental and emotional (from anamneses and medical record). The technique of sampling with continuous sampling, with the number of samples of research, is the 115 people. The technique of sampling with continuous sampling, with the number of samples of research, is the 115 people. Before the research study, the research study protocol was approved by the Research Ethics Committee of the Faculty of Medicine, Universitas Sumatera Utara, and all patients participated voluntarily and signed the informed consent.

The primary data was collected through interviews and direct blood tests. The formation of a

self-care behaviour model uses a questionnaire containing seven aspects of self-care forming that have been designed beforehand and have been tested for their validity and reliability. Laboratory tests include glycemic control and lipid control. The glycemic control was consisting of BGL and HbA1C by taking venous blood and examined with a Colorimeter + Full Spectrophotometer Automatic method and HbA1 examination using Doronad affinity + Modified HPLC method examination. Assessment of lipid control (total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides) was carried out by direct examination of venous blood (after 10 hours fasting) and then examined using the full automatic colourimeter + full-colour spectrophotometer method using a Pentra 400 device.

The technique of data analysis was descriptive statistics analysis and Structural Equation Models (SEM) with Software of SPSS and Amos. The researchers get Ethical Clearance from The Commission On The Ethics Of Health Research Faculty Of Medicine, Universitas Sumatera Utara and research is funded entirely by the researchers and not burdening the respondent.

Results

Binjai City has eight main PHC located in five sub-districts, namely: Binjai Estate PHC, Rambung PHC, Binjai Kota PHC, Tanah Tinggi PHC, Kebun Lada PHC, Jati Makmur PHC, H.A.H. Hasan PHC, and Bandar Senembah PHC. The characteristics of the studied T2DM patients in Binjai City are summarised in Table 1 below.

Table 1: Basic Characteristics of Diabetes Melitus Type 2 patients in Binjai City (n = 115)

Characteristics	Frequency (person)	Percentage (%)
Age Group		
Early adolescent (26-35 years old))	3	2,6
Late adolescent (36-45 years old)	9	7,8
Early Elderly (46-55 years old)	39	33,9
End Elderly (56-65 years old)	64	55,7
Gender		
Man	30	26,1
Woman	85	73,9
Level of education		
Illiterate	7	6,0
Primary	22	19,0
Secondary	28	24,3
High school	37	32,1
Graduate school	26	22,6
Occupation		
Haphazard workers	58	50,4
Laborers	4	3,4
Farmers	5	3,5
Private workers	9	7,8
Government workers	20	17,4
Others	19	16,5
Monthly Income		
Below Regional Income Rate	58	50,4
Within Regional Income Rate	36	31,3
Above Regional Income Rate	21	18,3
Marital Status		
Married	89	77,4
Single/Divorced	26	22,6
Ethnic		
Javanese	51	44,4
Bataknese	9	7,8
Melayunese	11	9,6
Padangnese	10	8,7
Banjarnese	3	2,6
Karonese	8	6,9
Others	23	20

Table 1 shows the basic characteristics of the study population. As presented in Table 1, a total of 115 participants with diabetes type 2 participated in the study. The majority were over 56-65 years old (55.7%), female (73.9%) and married (77.4%), high school graduate (32.1%), haphazard workers (50.4%), and with ethnic of Javanese ethnicity (44.4%).

Self-care characteristics and Self-care Forming Dimension of Type 2 Diabetes Mellitus Patients in Binjai City

Seven indicators form the dimension of self-care behaviour with 28 questions. The seven forming indicators are then categorised into two, namely good and poor (Table 2)

Table 2: Distribution of Self Care Behavior Dimensions of Diabetes patient

Dimensions	Good		Poor	
Self-Care Behavior	n	%	N	%
Knowledge	63	55	52	45
Attitude	67	58	48	42
Communication	58	50	57	50
Financing	62	54	53	46
Family Support	78	68	37	32
Motivation	82	71	33	29
Self-efficacy	58	50	56	49

According to Table 2, the best dimension of self-care is the dimension of patient motivation in treatment (71%), while the small aspect is self-efficacy and communication (50%).

The level of Quality of Life and Dimensions of Quality of Life for Type 2 Diabetes Mellitus Patients in Binjai City

The Quality of life assessments was made using the World Health Organization Quality of Life (WHOQoL questionnaire, which assesses quality that consists of the physical health domain, psychological domain, and social health domain (Table 3).

Table 3: Distribution of Quality Life of Diabetes Patients

Quality of Life	Frequency (person)	Percentage (%)
Good	4	3,5
Enough	110	95,7
Poor	1	0,9

Table 3 shows the majority of T2DM patients in Binjai City have enough/sufficient quality of life that there are 110 people (95.7%), and only one person (0.9%) who have a poor quality of life. The World Health Organisation Quality of Life assesses the quality of life based on four domains, namely the quality of life of diabetic patients in an adequate category, as many as 88 people (76.5%) for the physical area, as many as 63 people (54.8%) for the psychological domain, as many as 97 people (84, 3%) for the social field and 79 people (68.7%) for the environmental area.

Metabolic Control of Type 2 DM Patients in Binjai City

The metabolic controls examined in this study included BGL random (momentary), HbA1C levels and examination of fat profiles (Total Cholesterol, HDL Cholesterol, LDL Cholesterol, and Triglycerides).

Table 4: Distribution of Metabolic Control Parameters for Diabetes Patients

Control of Metabolic	Mean	SD	Value Minimum	Value Maximum
BGL (mg/dL)	267.5	103.2	95.0	600.0
HbA1C (%)	9.9	2.3	4.9	15.5
Total Cholesterol (mg/dL)	219.5	42.8	115.0	385.0
LDL Cholesterol (mg/dL)	132.5	37.4	53.0	257.0
HDL Cholesterol (mg/dL)	45.7	12.4	25.0	91.0
Triglyceride (mg/dL)	207.6	113.9	54.0	753.0

Table 4 shows the average value of BGL is 267.5 mg/dL, HbA1C is 9.9%, Total Cholesterol is 219.5 mg/dL (hypercholesterolemia), LDL: 132.5 mg/dL HDL: 45.7 mg/dL TG: 207.6 mg/dL.

From the results of the data above, then a self-care behaviour model is formulated by the available data.

The analysis scheme for this research model can be seen in Figure 1 below:

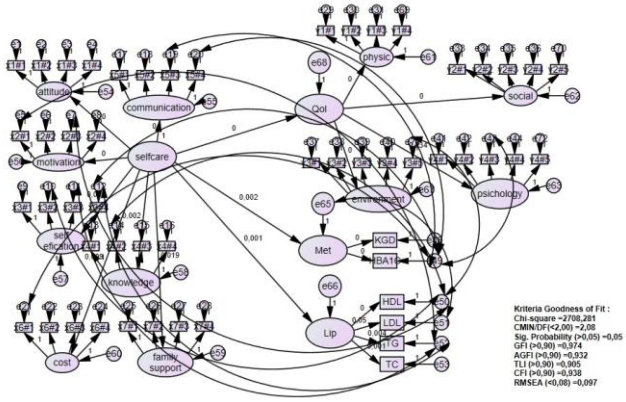

Figure 1: Full Model Research Analysis

Based on Figure 1, an explanation is obtained for the feasibility test of the following models, namely:

Table 5: Feasibility Testing Index for SEM Models

The goodness of Fit Index	Cut-off Value	Result of Analysis	Model Evaluation Analysis
χ2 - Chi-square	Diharapkan kecil	1301	Marginal
Probability	≥ 0,05	0.001	Marginal
RMSEA	≤ 0,08	0,097	Marginal
GFI	≥ 0,90	0,974	Good
AGFI	≥ 0,90	0,932	Good
TLI	≥ 0,90	0,905	Good
CFI	≥ 0,90	0,938	Good

From Table 5, it is known that this research has been included in the fit or feasible category so that it is continued in the next analysis to test the research hypothesis. The test results to find out the truth of the hypothesis are the following:

Table 6: Regression Weight Test Results

Relationship Between Variables			Est.	P	Information	Hypothesis
Self-care	→	Quality of life (Qol)	0,879	0,000	Significant	Ha = Accepted
Self-care	←	Communication	0,976	0,000	Significant	Ha = Accepted
Self-care	←	Attitude	0,150	0,000	Significant	Ha = Accepted
Self-care	←	Motivation	1,013	0,000	Significant	Ha = Accepted
Self-care	←	Self-efficacy	0,974	0,003	Significant	Ha = Accepted
Self-care	←	Knowledge	0,961	0,002	Significant	Ha = Accepted
Self-care	←	Financing	0,182	0,009	Significant	Ha = Accepted
Self-care	←	Family support	0,041	0,019	Significant	Ha = Accepted
Self-care	→	Metabolic control	0,413	0,002	Significant	Ha = Accepted
Self-care	→	Lipid control	0,301	0,001	Significant	Ha = Accepted

The results suggest that:

1. Self-care has seven factors capable of measuring or forming a self-care variable for a patient with Type 2 diabetes in Binjai City.

2. The self-care variable has a positive and significant effect on quality of life with a p-value (0.0001), and the magnitude of the effect is 0.879 (87.9%).

3. All domains of quality of life factors have a significant impact on changes in the quality of life of patients suffering from Type 2 diabetes mellitus in Binjai City.

4. The self-care variable has a positive effect and significant to metabolic variables with a p-value (0.002), and the magnitude of the effect is 0.413 (41.3%).

5. The Blood Glucose level and HbA1C have a significant role in the metabolic control of patients with a significance value of BGL (p = 0.0001), and the amount of the estimated effect on the patient's metabolic control is 0.842. Whereas HbA1c had a significant role or impact on the metabolic control of patients with a significance value of HbA1c (p = 0.0001) and the estimated value of its effect on the patient's metabolic control was 0.788.

Discussion

Actions in self-care diabetes are the same as self-management that must be carried out and become a responsibility during the patient's life [14]. Self-care is done to control blood sugar levels and control diabetes to treat and prevent complications [15].

Researchers succeeded in directing T2DM patients in Binjai City to form self-care behaviours namely: knowledge, attitude, communication,

financing, family/social support, motivation, and self-efficacy. The average self-care behaviour of T2DM patients in Binjai City is 359.8 (SD 29.5). From these results, it is known that self-care behavior of Type 2 DM patients in Binjai City can be categorized as good (66.4%). Good self-care behavior in Type 2 DM patients can be seen from the seven forming indicators of self-care behaviors that have been formulated at the beginning of the study, in general, are in a proper category. The quality of life of diabetic patients in Binjai City is in the sufficient category (95.7%). Based on the four domains, all domains are in an adequate category.

The result of this study shows that only a few T2DM patients experience deterioration in the quality of life due to DM. This may be due to good self-care behaviour and low rates of complications in diabetic patients. Psychological domains and environmental domains are domains that have a better quality of life than other domains that are 44.3% for psychological and 31.3% for the environment. The psychological domain of diabetic patients in Binjai City is known to be better than domains because there are powerful family and religious support. This relates to research that states family support related to the psychological health of patients is better for the disease [16]. However, keep in mind the patient's perception of the quality of life is different in each country [17] [18].

The results also showed that there was a significant relationship between self-care behavior with HbA1C levels and BGL of Type 2 DM patients in Binjai City where the better the self-care behavior, the more controlled HbA1c levels of diabetic patients [9] [10] [11] [12] [13] [14] [15] [16] [17] [18] [19] [20] [21]. HbA1C is the best indicator for the risk of future complications so that the HbA1C examination is better than fasting blood sugar examination. The higher the HbA1C value, the higher the patient is at risk for complications. Every 1% reduction in HbA1C levels can reduce the risk of microvascular vascular disorders by 35%, reduce other complications by 21% and reduce the risk of death by 21%. This research is supported by the opinion that there is a significant effect between the level of self-care with HbA1C levels in patients who came to Hasan Sadikin General Hospital Bandung Endocrine polyclinic [22]. It is even known that there is a relationship between self-care, self-efficacy, and social support with HbA1C levels at the Public Health Centers in Boyolali in Java Province [19] [23] [24]. Patients with appropriate lifestyle strategies and self-care are critical elements in the prevention of diabetes. Cause more severe complications [25]. Healthy behaviour leads to better treatment adherence than patients who carry out therapy [26]. Control measures for DM are essential, primarily by trying to get the blood sugar level as close to normal as possible, is one of the best prevention efforts against the possibility of developing complications in the long term. The criteria for stating good control include: no or minimal glucosuria, no ketonuria, no ketoacidosis, rarely occurs. Hypoglycemia, normal pp glucose, and normal HbA1c (glycated haemoglobin or glycosylated haemoglobin). HbA1c examination results are a very accurate single examination to assess long-term glycemic status and are useful for all types of people with diabetes, especially patients in Binjai City.

Fat profiles are also known to be one of the parameters assessed as glycemic control of diabetic patients. The results of the analysis stated that there was an influence between self-care behaviour and fat profile of Type 2 DM patients in Binjai City. In uncontrolled Type 2 DM, triglyceride and chylomicron levels and plasma FFA increase due to decreased transport of triglycerides into fat depots. The decrease in lipoprotein lipase activity also plays a role in this decrease in traffic [23]. Based on the above concept, it is known that the tendency to increase the big profile in diabetic patients [27] [28] [29].

Self-care can affect the quality of life of diabetic patients where there is a feeling of satisfaction and happiness to live their daily lives as they should. Some aspects of diabetes that affect the quality of life is the existence of special needs that are sustainable in the treatment of DM, such as diet regulation, limitation of physical activity, controlling blood sugar levels, any symptoms that may arise when blood sugar levels are unstable, complications that can occur as a result of diabetes and sexual dysfunction. All patients who have chronic diseases are involved in behaviours that influence their decisions and health, namely disease control and the results depend on the significant degree of self-management effectiveness.

In conclusion, self-care behaviour has a positive and significant effect on quality of life, metabolic levels and lipid control in Type 2 DM patients in Binjai City. The results of this study are input to all primary services to improve aspects improve diabetes patient self-care behaviour in Binjai City and throughout Indonesia. Patients need education and enhance the role of families to support Type 2 DM patients in Binjai City, especially in controlling periodic metabolic and lipid control.

Acknowledgements

This study is funded by Directorate of Research and Community Service, General Directorate of Research Strengthening and Development, Ministry of Research, Technology, and High Education by Research Contract Number: 003/SP2H/LT/DRPM/IV/2017 dated April 20, 2017.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1768-1772.
https://doi.org/10.3889/oamjms.2018.324
eISSN: 1857-9655
Public Health

The Intervention of Community Role for Improving Health Status of Pregnant Women Suffering HIV-AIDS in Medan

Siti Saidah Nasution*, Erniyati Erniyati

Department of Maternity and Pediatric Nursing, Faculty of Nursing, University of Sumatera Utara, Jl. Prof. Maas, Kampus USU, Medan 20155, Indonesia

Citation: Nasution SS, Erniyati E. The Intervention of Community Role for Improving Health Status of Pregnant Women Suffering HIV-AIDS in Medan. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1768-1772. https://doi.org/10.3889/oamjms.2018.324

Keywords: Pregnant Mother; HIV-AIDS; Intervention; Community Role

***Correspondence:** Siti Saidah Nasution. Department of Maternity and Pediatric Nursing, Faculty of Nursing, University of Sumatera Utara, Jl. Prof. Maas, Kampus USU Medan 20155, Indonesia. E-mail: m.mohamed@ut.edu.sa

Received: 24-Apr-2018; **Revised:** 23-Jul-2018; **Accepted:** 27-Jul-2018; **Online first:** 24-Sep-2018

Funding: This research was financially supported by Research Institution of the University of Sumatera Utara via TALENTA Scheme 2017

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

Abstract

AIM: This research was aimed to analyse the influence of the intervention towards the pregnancy health status of the HIV-AIDS patients.

METHODS: The type of the experiment is a quasi-experiment, and statistically analysed with SPSS 17. The data collection was held by spreading questionnaires and conducting interviews. The sample of the research consists of 39 pregnant mothers with HIV-AIDS in Medan, Indonesia. The intervention provided counselling about the treatment for expectant mothers with HIV-AIDS involving the community.

RESULTS: The result of the research generally shows that intervention potentially increase the knowledge and the attitude of the pregnant mother. Intervention has a significant result which is $p < 0.05$, high-risk detection, ANC, selection of birth assistance and healthcare service provider.

CONCLUSION: Intervention has an impact in increasing the knowledge, attitude and health status of the pregnant mothers. The involvement of the society takes an important role in the healthcare of the expectant mothers with HIV-AIDS.

Introduction

The acceleration of the decline of maternal mortality rate (MMR) and infant mortality rate (IMR) has to involve all aspects, such as medical aspect, health service management and socio-cultural aspect of the society [1]. The health status is defined by the mother's condition starting from the pregnancy, labouring, to the postpartum period [2]. The treatment that is conducted with the mother, as well as the infant, is related to the perception and the daily habits of the family and society. The factor of the high rate of MMR and IMR that is related to the role of health officers can be resolved by upgrading the quality and scope of the health service, however the socio-cultural-related problems are sensitive issues and difficult to be intervened if the involvement of local community is not included [3] [4]. Women with HIV/AIDS have higher morbidity and mortality rate [5] [6]. Pregnant mothers with HIV-AIDS and family will encounter several problems in reproduction health that requires comprehension and adaptation towards changes on herself and family as well as the society that will affect the life quality [7] [8] [9].

Based on the WHOQOL – BREF concept, the life quality can be measured from the physical health aspect, psychological well-being, social relationship and power society [10]. Psychological changes generally happen to pregnant mothers with HIV/AIDS. Besides the anxiety of contagion to the fetus, they have various types of complications that are possible to happen to the fetus as well as the mother herself. The complications are the rupture during labour, a baby born with disabilities, low birth weight (LBW), preterm and fetus infected with HIV/AIDS [11]. These results in psychological changes, such as ambivalence, feelings of doubt about pregnancy,

depression, excessive concern for the fetus, and even post-partum blues. The social aspect of HIV/AIDS patients happen to the social life such as stigmatisation and isolation may influence the psychological condition of the mother [12]. Health status is generally influenced by habit or culture adopted by the community because the act of treatment to be performed by the knowledge and perception of a person [13] [14]. Health status is a dynamic and individual circumstance that is influenced by personal and environmental factors.

According to Green (2005), there are 4 (four) factors that influence one's health status that is hereditary, health service, environment and behaviour [15]. Of the four factors, the ones that play a bolder role in one's health status are environmental factors (45%) and behavioural factors (30%). Delay in decision-making at the family level can be avoided if the mother and the family learn the signs of jeopardy on the pregnancy and childbirth and understand the appropriate action to take to overcome the problem to avoid the high risk to both mother and baby [16]. Based on the above assumptions and phenomena, an intervention is needed to improve the health status of pregnant women who have HIV/AIDS through empowerment and community involvement programs. The intervention conducted is counselling about health care for pregnant women suffering from HIV-AIDS delivered by the community and health cadres. The application of the theory with community empowerment in preventing and decreasing the morbidity and mortality rate in mother and baby [17].

Based on Mikkelsen (2011), several concepts are frequently used as a basis for differentiation of culture, character, basic personality, perception, time concept, thinking, language, non-verbal communication, values, behaviour (norms, rules, nature) and grouping as well as social relations [18]. Most human actions and behaviour are based on the values of what is considered as bad, good and neutral in people's lives [19].

Methods

The method was the quasi-experiment type, with the pretest-posttest design. Intervention is done in two stages: the first phase of forming community team consisting of health professionals to be involved in health care counselling. The team was trained and educated with materials that are related to the care of pregnancy, childbirth, and postpartum and infants of mothers with HIV/AIDS. Then the team conducted counselling on pregnant women who have HIV/AIDS. Intervention by giving counselling to pregnant women was performed with media leaflets and posters. After intervention for 6 months, the measurement of the behaviour of pregnant women on the variance of knowledge and attitudes has conducted. Maternal health status is measured from the record of high-risk detection, the implementation of antenatal care, the selection of birth attendants and the selection of pregnancy and health checkpoints. Data analysis was performed with the frequency distribution and presentation, T-test dependent and regression.

Results

Based on Table 1, the characteristics of mothers are at high risk, where the age of pregnant women is still much over 35 years and less than 20 years, the number of children more than 2, the level of education is still a lot of elementary and family income is below than enough and the character of pregnant women in general categorized under high-risk conditions.

Table 1: Frequency distribution of characteristic data of pregnant women respondents (n = 37)

Variables	Intervention	
	N	%
Age		
a. <20 years old	6	16.21
b.20-35 years old	12	32.43
c. >35 years old	19	51.35
Number of Children		
a. Primi	5	13.51%
b. 1-2	24	64.86%
c. >2	8	21.62%
Education		
a.No school	6	16.21
b. Elementary	10	27.02
c. Junior High School	6	16.21
d.Senior High School	6	16.21
e. Higher Education (D3. S1)	9	24.32
Occupation		
a. Farmer/Laborer	6	16.21
b. Entrepreneur	15	40.54
c. Public/Private Company Employees	2	5.40
d. Others	14	37.83
Earnings		
a. <Rp.700.000	6	16.21
b. Rp.700.000-2.000.000	11	29.72
c. >Rp. 2.000.000	20	54.05
Religion		
a.Islam	22	59.45
b. Christian	8	21.62
c. Hindu	2	5.40
d. Buddhism	5	13.51
Total	37	100.0

Table 2 displays that there is a difference of knowledge score before and after knowledge on pregnant women, in which the value of $p < 0.05$ ($p = 0.031$).

Table 2: The different score of knowledge pre-test & post-test on pregnant women (n = 28)

Knowledge	Case				*P*
	Before		After		
	N	%	n	%	
Good	13	35.13	30	81.08	0.031
Insufficient	24	64.86	7	18.91	

From Table 3, it can be seen that there is a difference of attitude before and after (knowledge) on

the team members who care about public health in which the value $p < 0.05$ ($p = 0.002$).

Table 3: The difference between the score of attitude pretest & posttest on pregnant women (n = 28)

Knowledge	Case				*P*
	Before		After		
	N	%	n	%	
Good	16	43.24	32	86.48	0.002
Insufficient	11	29.72	5	13.51	

From Table 4, it is shown that there is the difference in the health status score before and after the intervention, in which the value $p < 0.05$ for several variables that are high-risk detection, ANC treatment, choice of labour assistance and choice of health care centre.

Table 4: Health status of pregnant women before and after intervention (n = 39)

Variables	Before	After	*P*
High-Risk Detection			
a. Has record	10	20	0.046
b. No record	29	19	
ANC			
a. Fit	9	14	0.006
b. Not fit	30	25	
Choice of Labor Assistance			
a. Health Officer	8	21	0.007
b. Non-Health Officer	31	18	
Health Care Place			
a. Health Care Centre	8	17	0.005
b. Non-Health Care Centre	31	22	
Total	39	39	

Discussion

The result of the study generally indicates the influence of interventions in improving the health status of pregnant women who have HIV/AIDS. Interventions are conducted through community empowerment by community empowerment by the realisation of potential capabilities in the care of pregnancy, childbirth, post-partum and newborns by taking into account socio-cultural community, involving community leaders and local tradition leaders [20]. Nursing care in pregnant women with HIV/AIDS involves community leaders and local customary leaders. Intervention is done by activating the role of society that plays an important role in influencing beliefs, traditions and customs. This is consistent with studies suggesting that HIV patients were given attention-based and cognitive-minded interventions experienced a reduction in stress and depression by 8 weeks and 6 months after the intervention [21].

Community empowerment is an effort to automate the community through the realisation of the potential ability possessed [18]. One of the effective strategies to improve health status through community empowerment application is by the educative approach, i.e. a series of activities that are implemented systematically planned and directed with the active participation of individuals, groups and communities aimed at solving problems by considering social, economic and cultural aspects [22] [23].

This opinion is in conjunction with Mikkelsen B. (2011) who says that the logic model underlying the participatory strategy or the participation of the community in the effort of community empowerment to achieve the development goal can be achieved harmoniously and the possibility of conflict among social groups, but this can be mitigated through the local democracy pattern [18] [24] [25].

Community participation has a positive impact on development, and it is an effective tool for mobilising local resources such as people and nature with the aim of implementing specific development programs. The counselling activity is a form of education with the aim to equip health and community cadres with good and true knowledge and positive attitude in the health care of pregnant women suffering from HIV-AIDS [3] [7]. The implementation of the intervention is one of the cognitive approaches that can help optimise the role of the community in improving the behaviour of pregnant women in maternal and infant health care [26]. This is consistent with the theory which states that knowledge can improve emotional control, increase client's self-reliance, increase self-esteem, increase endurance and can help clients to adapt to problems or diseases that can ultimately improve health status [15].

The formed behaviour begins with the cognitive domain which further raises the inner response in the form of attitudes toward the object, and this response ultimately will be in the form of acts or skills. Information sharing is a cognitive approach to psychosocial interventions designed to analyse and change the wrong beliefs or values held by clients and help clients learn to use effective coping strategies [15]. According to Notoatmodjo (2003) who said education, experience, culture, belief, are the factors that influence one's knowledge and attitude. Moreover, knowledge affects the consciousness level of solving health problems, healing treatments, disease anticipation and healthcare [13]. Behavioural factors that can influence the health status of the community cannot be separated from the culture and habits of families and the environment of society on a daily basis, including the stigma attached to HIV/AIDS patients [27].

Community leaders such as traditional leaders, village officials and religious leaders are the people who are considered to play a role and influence people's decisions on health care related to culture. Public health status improvement program will not run well by the objectives if it does not study the environmental and cultural factors of surrounding communities and involve the community in the program. Implementation of the interventions conducted in this study involved community leaders

directly, who are role models for communities in their region [26].

The right strategy in changing the behaviour and perceptions of the people related to culture is very appropriate to involve community leaders. This is by the opinion that the social condition is a condition or position that is purposely arranged socially, thus places a person in a certain position in the social structure of society [21]. Ones who occupy the highest position in society and are role models in everyday life are people who understand knowledge of religion, knowledge of healing disease/shaman, understanding the customary law and local culture more, have a lineage of kings or come from the family of society leaders in the past [19].

Based on this, the implementation of intervention by involving the community and observing the social culture of local communities in improving the health status of pregnant women suffering from HIV-AIDS through the care of pregnancy, childbirth, post-partum and infant is an appropriate and effective action in supporting more optimal achievement of public health status program [12]. In accordance with the role and function of health personnel that are facilitating the involvement of all family members and the community in helping individuals and families in decision-making, helping families to get positive experiences aligning with expectations, overcoming problems in maternal and infant care, as well as interactions among them through education programs, referred to like the approach of family, centred maternity care [20].

Human behaviour is influenced by its environment, both physical and socio-cultural environments [15]. The approach to health behaviour change in society should be initiated with the ability of health workers to master the various socio-cultural backgrounds of the related community.

The social and economic backgrounds have relations to public health behaviour. The social and economic backgrounds have relations to public health behaviour, especially pregnant women who suffer from HIV-AIDS. According to research factors that can affect the stigma felt by HIV-AIDS patients is the level of education and the process of disease [27]. Related to the behaviour of factors that can affect the health status of the community, especially mothers and babies, but in general, can not be separated from the culture and habits of families and the environment of everyday society [16]. This is in line with the notion that the indirect causes of maternal and neonatal deaths are due to societal conditions such as education, socio-economic and cultural [25].

As a health officer, understanding of community-related behaviour is important in influencing the behaviour of pregnant women and families. Research conducted by Caetano et al. (2006) showed that there was an effective decline in HIV infection about sexual behaviour and the transmission of sexual infections using behavioural therapy approaches [5]. HIV-risk behaviours can be reduced in targeted populations through interventions that provide risk reduction counselling, emphasising cognitive approaches to problem-solving and behaviour change, and helping individuals to build the skills they need to reduce HIV risk [7].

In conclusion, the implementation of Interventions through community involvement in the health care of pregnant women who have HIV/AIDS has an impact in optimising the knowledge and the maternal health status. The result of data analysis shows that there is a change in knowledge and attitude with p-value < 0.05 (p = 0.0001). Maternal behaviours related to health status that have a significant effect are high-risk detection, ANC implementation, selection of birth assistance and selection of treatment sites.

Acknowledgement

Authors would like to acknowledge Research Institution of the University of Sumatera Utara for research funding support via TALENTA Scheme 2017. Also, Thanks to society, especially expectant and family who supported this research sampling.

Ethical Aspects

Authors state that the research follows the ethical aspect as regulated by University of Sumatera Utara, Indonesia.

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ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2018 Sep 25; 6(9):1773-1778.
https://doi.org/10.3889/oamjms.2018.335
eISSN: 1857-9655
Review Article

Pain: The Neglect Issue in Old People's Life

Maryam Noroozian[1], Shima Raeesi[2], Rezvan Hashemi[2], Leila Khedmat[3], Zahra Vahabi[4*]

[1]Memory and Behavioral Neurology Division, Department of Psychiatry, Roozbeh Hospital, Tehran University of Medical Sciences, 606 South Kargar Avenue, Tehran 1333795914, Iran; [2]Department of Geriatric Medicine, Ziaeian Hospital, Tehran University of Medical Sciences, Tehran, Iran; [3]Department of Community Medicine, School of Medicine, Tehran University of Medical Science,Tehran, Iran; Health Management Research Center and Department of Community Medicine, Faculty of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran; [4]Neurology Division, Department of Geriatric Medicine, Ziaeian Hospital, Tehran University of Medical Sciences, Aboozar Avenue, Tehran 1366736511, Iran

Citation: Noroozian M, Raeesi S, Hashemi R, Khedmat L, Vahabi Z.. Pain: The Neglect Issue in Old People's Life. Open Access Maced J Med Sci. 2018 Sep 25; 6(9):1773-1778. https://doi.org/10.3889/oamjms.2018.335

Keywords: Pain; Geriatrics; Dementia; Depression; Pain assessment; Pain management

***Correspondence:** Zahra Vahabi. Neurology Division, Department of Geriatric Medicine, Ziaeian Hospital, Tehran University of Medical Sciences, Aboozar Avenue, Tehran 1366736511, Iran. E-mail: vahab1357@gmail.com

Received: 23-Jul-2018; **Revised:** 20-Aug-2018; **Accepted:** 28-Aug-2018; **Online first:** 20-Sep-2018

Funding: This study is supported by a grant provided by Tehran University of Medical Sciences

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

Abstract

BACKGROUND: As the elderly population increases dramatically, the chronic age-related disease should be noted. In the elderly, chronic pain is a common health problem.

METHODS: This search was performed in 3 databases (PubMed, Google Scholar, Embase). We have reviewed articles related to pain management in the elderly.

RESULT: The prevalence of pain in people aged above 60 is twice that in younger people. Pain is estimated to be 45-85 per cent in the elderly. Pain is not a part of the ageing process, but many older people can experience it. Perception of pain can be affected by environmental, emotional, cultural and cognitive factors. Pain in the elderly often remains untreated and misdiagnosed.

CONCLUSION: Pain management in elderly needs different approach because of unreported pain in this population, and usually they have multiple problems and comorbidities that complicate evaluation and treatment.

Introduction

In 2012, an estimated 8 per cent of the world's populations were aged 65 or older, with the expectation to nearly double (about 16 per cent) in 2050 (Figure 1). Considering the rapid increase in the number and percentage of older people, especially in less developed countries, it is imperative to adapt quickly to this new reality. The importance of chronic diseases such as chronic obstructive pulmonary disease, cardiovascular disorders, Alzheimer disease, ALS, diabetes mellitus, osteoarthritis and cancer should be considered with this epidemiologic transition [1].

Pain is the most common reason people visit doctors and can result in disability and suffering [2]. It is reported that more than one hundred million Americans are affected by pain annually, and they spend about 600 billion dollars for pain relief which is more than payment for diabetes, cancer and heart diseases [3] [4].

In the elderly, chronic pain is a common health problem (Table 1) [5]. The prevalence of pain in people aged above 60 is twice that in younger people [6]. Uncontrolled pain in the elderly has a great financial burden on health system [7]. According to some studies, nearly 20% of the elderly have received many forms of analgesic during the past 6 months because of chronic pain [8] [9] [10]. It should be

considered that 75% of patients with pain do not receive pain control and 45 to 80% of others receive inadequate pain control [10].

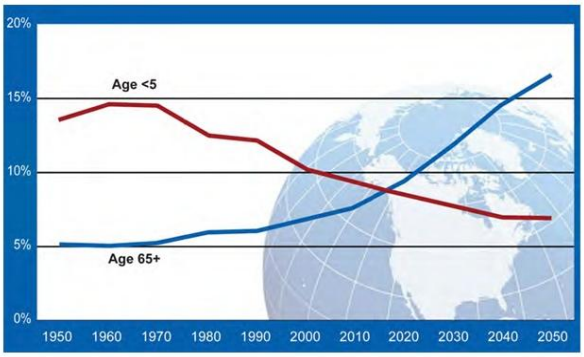

Figure 1: Young children and older people as a percentage of the global population: 1950-2050

In adults, there is female predominance in pain complaints, the severity of pain and longer duration of pain. However, the reason is not clear. It could be due to different biological mechanisms or the effect of psychological and social factors [11] [12]. Prevalence of pain is increased from childhood to adulthood. Therefore pain is most common in the elderly [13] [14]. It is estimated to be 45-85 per cent in the elderly [15] [16].

Table 1: Pain syndromes associated with ageing

Cancer pain
Osteoarthritis
Angina
Rheumatic disease(such as Rheumatic fever)
Postherpetic Neuralgia/trigeminal Neuralgic
Visceral pain(Irritable bowel syndrome, Peptic ulcer, gastritis, dyspepsia)
Alcohol Abuse
Malnutrition
Pain associated with atherosclerosis and diabetic neuropathy
Temporal arthritis
Discopathy
A headache

Pain is defined as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described regarding such damage” [8]. Pain is classified in different ways, but chronological classification is used more commonly.

Acute pain is associated with an injury or illness. The location and cause of this type of pain can be identified easily, and it usually has a predictable course with the expectation to diminish as the injury heals. It lasts less than 3 months without long-term effects on the patient’s quality of life. The key roles of this pain are warning and protecting against tissue injuries [16]. Pain due to surgery and trauma are examples of acute pain.

Inadequately treated acute pain can cause tachycardia, tachypnea, widening of pulse pressure and increased sympathetic nervous system activity [8] [16]. Also, untreated pain can lead to gait impairment, which may cause injuries from falls and accidents [17].

Persistent pain that lasts more than 3 months is called chronic pain. There are different risk factors for developing chronic pain, including female sex, increasing age, environmental factors, previous pain experience and physiological factors like anxiety or depression [8].

Another important cause of chronic pain is cancer. Pain in cancer patients can be caused by the disease itself, treatment, or autoimmune antibodies associated with the malignancy [18] [19]. Anatomically, chronic pain can be classified into receptive (nociceptive) and non-receptive (neuropathic) subgroups. The first one results from stimulation of somatic or visceral pain receptors. Pains resulting from a bone fracture, muscle spasm or joint disease are examples of somatic pain. Damage to an organ, system or tissue-like pleura or peritoneum can cause visceral pain (Figure 2).

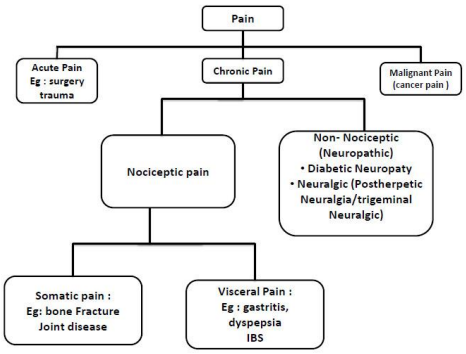

Figure 2: Classification of the pain

Nerve damage during surgery, radiotherapy, medicine or advanced diseases may result in neuropathic pain. It can be described as burning, tingling or pins and needles [8].

There are four basic stages during nociception: transduction, transmission, modulation and perception. In the elderly, there are some differences that are mentioned briefly in the article.

When nociceptors (free nerve endings, located in different tissues) are exposed to a sufficient quantity of stimuli, transduction begins. A variety of chemical agents like histamine, serotonin, bradykinin and prostaglandin and neuropeptides including neurokinin A, substance P and calcitonin gene-related peptide (CGRP) are released which sensitise nociceptors. Following this step, the permeability of neuronal cells increases and depolarisation occurs [20] [21]. Thermal and mechanical pain threshold is higher in elderly than younger people [22] [23], but pain tolerance decreases with ageing [22] [23] [24]. Pain threshold is not different in demented patients. Nevertheless, the tolerance threshold is higher in non-demented ones [25] [26].

Moreover, it has been shown that somatosensory threshold for non-noxious stimuli

increases with age, whereas pressure pain threshold decreases and heat pain threshold can have no changes [27]. It is hypothesized that sensory-discriminative aspect of pain can be intact in dementia, while the effective emotional part of pain process would be compromised. This finding is supported by the fact that some brain regions such as the lateral thalamus and sensory cortex are relatively preserved during pathologic processes resulting in dementia.

On the other hand, the limbic system and prefrontal cortex functions are usually impaired during degenerative changes in Alzheimer disease [25] [26]. After stimulation of nociceptors, the message is transmitted to the spinal cord by type A or type C fibres. Contrary to C fibres, type A fibres are myelinated and have rapid conduction velocity. The smaller C fibres transmit thermal and chemical stimuli more slowly [22] [23].

Descending inhibitory signals from the thalamus, brainstem and interneurons play a modulatory role in dorsal horn which activates inhibitory neurons by releasing neurotransmitters [28]. These neurotransmitters including norepinephrine, serotonin, GABA, glycine, endorphin and encephalin can block substance P and other excitatory neurotransmitter activity. Perception of pain is the result of this complex process. The nociceptive process, in addition to physiological and emotional responses, contributes to the sensation of pain that is experienced by the person [29].

Greater involvement of structures from medical pain system (e.g. anterior cingulate cortex, medial thalamus and anterior insula) can result in more pain complaints in vascular dementia compared to Alzheimer disease [30]. It has been shown in a study that there is a correlation between vascular lesion extent in the subcortical white matter, especially dorsolateral prefrontal cortex, and intensity of the effective component of pain [30]. Different factors like hereditary and genetics, concomitant disease, level of intellectual complicity or ability, and stress from daily activities or trauma can have a role in age-induced changes in the central nervous system [31]. Moreover, it has been shown that age-related decrease in neuroendocrine functions can lead to different types of neurological disorders such as Alzheimer and Parkinson diseases [32].

Being old, exposes patients to several conditions such as cerebrovascular disorders, cancers and falls, increasing the risk of chronic pain in this population [33]. Pain is not a part of the ageing process, but many older people can experience it. Many elderlies believe that pain is a normal event in ageing. Therefore they don't report it. The same happens in cancerous patients and pain is not reported because of fear of disease progression. Some older people want to show they are compliant and they don't report pain, especially in nursing homes [34] [35]. Perception of pain can be affected by environmental, emotional, cultural and cognitive factors. Because of health system managers' assumption, pain in the elderly often remains untreated and misdiagnosed [34].

Dementia is a condition characterised by progressive deterioration of cognition [36]. Alzheimer disease is the most common type of dementia that affects 5 per cent of individuals aged over 65 and one-third of those over 85 [15]. Some significant barriers to pain assessment are seen in dementia due to memory loss, personality changes and loss of other functions such as judgement, abstract thinking and language skills [37]. Moreover, some aggressive or withdrawal behaviours in these patients are related to pain, but these behaviours could be mistaken for a dementia symptom. As a result, evaluation and assessment of pain in dementia may be difficult [37] [38].

A higher prevalence of pain in adults with dementia is reported in numerous studies. In comparison to younger individuals, it has been shown that the presence of pain is twice as common in people over 60 years. In a study, about 66 % of elderly nursing home residents experienced chronic pain, but only 34% of them were detected [39].

Undetected and untreated pain in demented patients, can cause cognitive (decreased attention and concentration) and behavioural (aggression and depression) problems which cause an increase in care demand, caregiver dependency and the need for health system [37].

Somatic pain is a common complaint among depressed patients [40]. Depression has seen in 5 to 85% of patients with chronic pain syndromes, and 65% of depressed people have at least one pain complaint [41].

Depression is often under-recognised and thus undertreated in patients with pain syndrome. 75% of patients with depression present with physical complaints like pain, but depression as a cause is not diagnosed [42] [43]. Moreover, evaluation for depression should be considered in case of unexplained pain or unexplained exacerbation of stable chronic pain. On the other hand, depression complicates management of pain syndromes, decrease response to treatment and induces poor prognosis [41].

Because of the subjective nature of pain, it is difficult to measure it quantitatively. On the other hand, a thorough assessment is essential for selecting suitable treatment modalities. According to patient's ability to communicate, pain intensity is measured by using appropriate pain scale. To achieve this goal, having a complete history and examination considering both physical and psychological aspects is necessary [44].

Pain assessment is usually inadequate in the elderly patients with dementia; therefore treatment is

not enough in many cases. In this population, pain assessment could be done by considering three main issues including self-report (as gold standard), behavioural or physiological measures. Many different types of self-reporting scales are available to be used among older adults with dementia [20] [45] [46].

Pain intensity is one of the important components of pain management which can be assessed by using different tools including Visual Analog Scale (VAS), Verbal Rating Scale (VRS), Numerical Rating Scale (NRS) and Facial Pain Scale (FPS). Increasing age can lead to difficulty thinking and impaired communicating skills in older adults which can result in difficulty using these tools in geriatrics [46]. It seems that other tools considering behavioural with combination physical aspects are more useful in elderly. Observation of changes in behaviour and function, involving sleep, appetite, physical activity, motility, facial and body language alongside physiological indicators (e.g. heart rate and blood pressure) can provide valuable information, especially in demented patients [37].

There are some examples of behavioural pain scales which are mentioned below:

This tool was established by worry et al. in 1992. Ten items like sleep, verbal reaction and problems of behaviour in 3 dimensions are assessed. Based on pain intensity, each of 10 items can be described in a rating from zero to three. Scores ≥ 5 out of 30 confirm pain existence. This scale can reflect the progression of pain but does not represent pain at the specific moment. On the other hand, the value of this test is limited because the nurses completing this scale should know the patient [47] [48] [49].

This scale was designed by Baculon et al. for detecting changes in behaviour in the elderly with or without communicating impairment. It consists of 10 items such as sleep, verbal reactions and interaction with the environment that are assessed during and aftercare. The reliability and validity of this tool are not tested. This scale is modified from the University of Alabama Birmingham Pain Behavior Scale that was designed for assessing chronic pain. It consists of 6 items scored during movement and rest. Restlessness, rubbing and vocal complaints are 3 of the items. Each present item scores 1, and if not observed, the score is 0. Scores 1-2 show mild, 3-4 moderate and 5-6 refer to severe pain. However, more modification of the scale and psychometric testing is needed.

It is established by Fuchs et al. to assess chronic pain in the elderly patients with dementia. This tool has a long list consisting of 60 items in four subclasses including facial experience, activity/body movement, social/personal mood & physiological/eating/sleeping and vocal features.

This scale is designed by Warden et al. for pain assessment in advanced dementia. It consists of 10 items. As an example, facial experience can be scored as following: Smiling = 0, sad = 1, frightened = 3. A greater score indicates more pain. This tool needs more testing.

This scale is developed by Villanueva et al. for pain assessment in advanced dementia. It has 24 items covering facial experience, the activity of daily living and caregiver's judgment of pain. Because of the different scoring of methods, it could be problematic to calculate cutoff scores.

It is consisting of 18 items covering behavioural, emotional, autonomic and postural dimensions developed by Sign and Orrell for pain rating in dementia. Each items can have scores from 0 (absent) to 3 (severe).

This tool consists of 6 items (like physiological and physical changes) rating from 0-3 for pain assessment in end-stage dementia. The classification of pain intensity is as follow> 3: mild pain, 8-13 moderate and > 14: severe pain. It needs more investigation for reliability and validity.

According to a systematic review, SOLO PLUS 2 and PACSLAC are the most appropriate scales which are currently available [37].

Because of the subjective nature, it is difficult to measure pain quantitatively. On the other hand, a thorough assessment is essential for selecting suitable treatment modalities. To achieve this goal, having a complete history and examination considering both physical and psychological aspects is necessary [44]. Pain intensity determination is an important component of pain management which can be assessed using different tools including Visual Analog Scale (VAS), Verbal Rating Scale (VRS), Numerical Rating Scale (NRS) and Facial Pain Scale (FPS). Increasing age can lead to difficulty in thinking and impaired communicating skills in older adults which can make use of these tools in geriatrics difficult [46].

Pharmacodynamics and pharmacokinetic changes in the elderly should be considered in selecting treatments. Efficacy, safety and cost of pharmacological modalities should be considered for appropriate choice of the treatment regimen. Comorbid disease and polypharmacy are important as well [50]. When treating pain, a careful review of all patients' medication is essential to eliminate any unnecessary drugs. It can help to prevent probable drug interactions [50] [51] [52].

In conclusion, pain management in elderly needs different approach because of unreported pain in this population, and usually they have multiple problems and comorbidities that complicate evaluation and treatment. On the other hand, they have a higher incidence of medication side effects and potential for adverse effect and complication secondary to treatment.

Authors' Contributions

All authors reviewed the manuscript, and they read and approved the final manuscript.

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